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Evaluation of a portable MOS electronic nose to detect root rots in shade tree species

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ABSTRACT

The early detection of wood decays in high-value standing trees is very important in urban areas because mitigating control measures must be implemented long before tree failures result in property damage or injuries to citizens. Adverse urban environments increase physiological stresses in trees, causing greater susceptibility to attacks by pathogenic decay fungi. The detection of fungal root rots in urban trees is particularly difficult because conventional detection tools, currently used for diagnosis of wood decays, are not feasible below ground level. Portable electronic olfactory systems or electronic noses (e-noses), currently used in many different scientific fields and industries, previously have been tested for the early diagnosis of wood decay fungi and wood rots. We evaluated the accuracy and effectiveness of the portable PEN3 electronic nose to discriminate between healthy and decayed root segments of five shade trees species, artificially inoculated separately with three species of root-rot fungi and incubated in different soil types under laboratory conditions. The PEN3 e-nose discriminated between healthy and inoculated root fragments and between different decay fungi in different soil types for most host-fungus combinations, but the discrimination power of this e-nose varied depending on tree species and strain of root-rot fungus analyzed. We provide explanations for the ineffectiveness of the e-nose to detect low levels of decay for certain host-fungus combinations. The advantages of e-nose detection over conventional wood decay detection tools also are discussed.

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1. Introduction

The urban environment, unlike natural forested environments, is particularly adverse for trees that frequently must endure high levels of air, soil and water pollution (Škrbić et al., 2012) as well as soil compaction that causes severe biochemical dysfunctions (Kozlowski, 1999; Edmondson et al., 2011). In addition, limited soil volume for root development leads to permanent water stress, poor mineral nutrition, and inadequate support of above-ground plant parts (Randrup, 1996; Day et al., 2000). These environmental factors dramatically increase physiological stresses that decrease urban tree fitness and increase their susceptibility to attack by pathogenic agents (Luley, 2005). The most threatening pathogenic agents of urban trees are wood decay fungi that degrade the chemical and structural composition of woody components, reducing their structural stability that leads to limb, bole or root failures (breaks) especially during severe weather events (Lonsdale, 1999; Mantheny and Clark, 1994; Weber and Mattheck, 2003). Root rots are even more detrimental because they are more difficult to investigate and detect due to their less accessible position below ground level (Anselmi and Mazzaglia, 2003).

The early detection and diagnosis of fungal root rots is particularly important in the urban environment because trees become highly susceptible to structural failures long before observable signs of wood decay fungi (fruit bodies) appear near the root collar or on the lower bole. Trees that become structurally-compromised by root rots are very hazardous to the community because structural failures can cause catastrophic losses to both human life and personal property. Thus, early detection of tree decay is essential to mitigate damage associated with failing trees.

Trunk-and root-rot diagnoses of standing trees are currently performed primarily by electrical conductivity meters, constant feed drills, single pulse sonic and ultrasonic techniques, breaking core samples, computerized tomography (Johnstone et al., 2010) or via molecular identification of decay fungi (Schmidt et al., 2011). Applications of the electronic nose as a diagnostic tool previously have been developed and tested for use in plant pathology (Wilson et al., 2004), with good diagnostic results subsequently reported for detecting postharvest fungal diseases of blueberry,

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cucumber, pepper and tomato (Laothawornkitkul et al., 2008; Li et al., 2009), for monitoring crop-health status in the greenhouse and under field conditions (Spinelli et al., 2006; Jansen et al., 2010), and for detecting basal stem rot of palm trees (Markom et al., 2009). Three different commercially available electronic-nose devices have provided good results demonstrating the early detection of incipient stem decay of artificially-inoculated sapwood segments collected from the boles (trunks) of harvested landscape trees (Baietto et al., 2010).

2. Objectives

The objectives of this study were to test the feasibility, accuracy and effectiveness of a commercially available metal oxide semiconductor (MOS) gas sensor type electronic-nose device (the PEN3 e-nose) to detect and discriminate between healthy and decayed root segments of selected hardwoods and conifers, previously inoculated separately with three important species of pathogenic root-rot fungi common to urban environments, based on differences in sensor array responses to mixtures of volatile organic compounds (VOCs) present in the headspace (analytes) derived from different root-sample types. The experiment was conducted *in vitro*, but healthy and inoculated roots were incubated in different types of soil substrata in order to obtain data under conditions that more closely approximated normal, edaphic field conditions from which test roots segments were obtained.

3. Materials and methods

3.1. Fungal strains

Five strains of three major root-rot fungal pathogens, including one strain of *Armillaria mellea* (Vahl) P. Kumm, two strains of *Ganoderma lucidum* (Curtis) P. Karst, and one strain of *Heterobasidion annosum* (Fr.) Bref., were selected as test fungi because these species are among the most important root-rot pathogens that frequently decay roots of urban tree species. One strain each of *A. mellea* and *G. lucidum* (accession numbers It01 and It04, respectively) were obtained from the Department of Plant Production, University of Milan fungal collection. One strain of *G. lucidum* (accession number It02) was acquired from the Southern Hardwoods Laboratory wood decay fungi collection of Dr. A.D. Wilson (Stoneville, MS). One strain each of *G. lucidum* and *H. annosum* (accessions It03 and It05, respectively) was isolated from tissues of freshly collected basidiomes.

Fungal strains (accessions It01, It02 and It04) were previously preserved in pure cultures with sterile distilled H_2O covering mycelial plugs within 1.8 ml cryotubes (Nunc A/S, Roskilde, Denmark) stored at 5 °C (Burdsall and Dorworth, 1994). Accessions IT03 and IT05 of *H. annosum* were obtained from fresh basidiomes collected from old, decayed standing live trees to assure virulence of these strains. Basidiomes were placed into clean paper bags and brought back to the laboratory within 1 h. A 2-mm portion of contextual tissue was taken from a fresh fruiting body and plated on the surface of 4.5% sterile Malt agar (MA) medium (SigmaM9802, Sigma–Aldrich, St. Louis, MO) containing 0.1% streptomycin sulfate (Sigma S9137) in order to obtain pure cultures. Several mycelial plugs were subsequently transferred at least two times on the same substrate in sterile Petri dishes to assure culture purity.

3.2. Fungal growth and inoculum preparation

Two plugs of mycelium were transferred from cryotubes in storage to sterile Petri plates on 4.5% Malt Agar (Sigma M9802) substrate. The substrate was previously sterilized for 40 min in an autoclave at 121 °C and 15 psi, poured into 10-cm plastic Petri plates and cooled down to room temperature on a sterile surface within a laminar flow hood (Nuare Laminar Flow Products, Plymouth, MN). The two mycelial plugs were placed far apart on the same Petri dish in order to obtain the widest spatial distance between fungal colonies. Every Petri dish was firmly sealed with Parafilm "M" (Pechiney Plastic Packaging, Chicago, IL). All isolates were transferred a second time to sterile dishes on 4.5% Malt Agar substrates to assure that cultures were pure and to prevent bacterial proliferation. Eight mycelial plugs from each Petri dish culture were transferred into a 250 ml PYREX® sterile glass flask containing 150 ml of 3% Malt Extract (ME) sterile broth. The flasks were plugged with sterile cotton and gently shaken in order to distribute the plugs in the flasks. After 1–3 weeks, a large mass of mycelium was formed in the flasks. A hand-held stainless steel mixer (T10 basic ULTRA-TURRAX®, IKA® Werke GmbH & Co KG, Staufen, Germany), previously sterilized by autoclaving, was used to macerate and disperse the mycelial mass within the liquid culture.

3.3. Root sampling and segments preparation

Small root segments (1–3 cm in diameter and 2–10 cm length) were collected from one adult tree of each of the following species: *Aesculus hippocastanum* L., *Cedrus deodara* Roxb. ex (D. Don) G. Don, *Platanus* × *acerifolia* (Aiton) Willd., *Quercus robur* L., and *Liquidambar styraciflua* L. All trees had previously been planted in a private garden in Besana Brianza, Italy, except for *L. styraciflua* whose root portions were collected in a plant nursery in Erba, Italy. The trees were in very good state of health as were their roots at the time the root segments were harvested. Only root segments from roots with good cambial tissue, devoid of any abnormal growth, were collected for experimentation. The collected root segments were then transferred into clean and labeled paper bags that were brought to the laboratory within 24 h.

Root segments were rinsed with tap water to remove every visible traces of soil, blotted on tissue paper and cut by means of a scalpel into small fragments (0.5–1 cm length). All small lateral roots were removed in order to eliminate the presence of any possible mycorrhizal fungi. The root fragments were put into 500 ml Pyrex® glass flasks and sterilized for 15 min in an autoclave at 121 °C and 15 psi, and then sterilized again 2 days later to prevent any fungal or bacterial contamination. To test the sterility of the roots, some fragments were transferred onto sterile MA substrate and incubated at 25 °C in the dark for 1 week and examined for contaminating microbial growth. All sterile roots fragments were then stored in a sterile area at 20 °C ± 0.5 for a short time until inoculation.

3.4. Soil sampling and preparation

Two different kinds of soil substrata were selected for this experiment. Samples of urban soil (us) were collected near a central-city street with moderate to high vehicular traffic (via Celoria, Milan, Italy). This kind of soil was extremely compacted, rich in hard organic and inorganic debris, almost totally non-structured and deeply altered due to heavy impact by anthropic activities. The soil core samples were collected randomly by means of a soil corer that was manually inserted to a depth of 10, 15 and 20 cm. Soil cores were placed in moisture-permeable paper bags and brought back to the laboratory within 1 h. Samples of a professional soil (ps) were obtained from a floriculture nursery (Vulcan, Terflor, Capriolo, Italy). Soil samples were collected in 25-l packets, mixed, transferred to paper bags and brought back to the laboratory within 1 h.

Soil samples of accession SU01 were first dry sieved to discard all solid fragments greater than 2 mm diameter. No chemical or physical analysis was performed on the soil samples before sterilizing in an autoclave at 121 °C for 15 min. Both soil types were dried in an oven at 105 °C for several hours until a steady-state weight was obtained. Bulk samples of each soil type were divided into 100 smaller aliquots (50 g each), put into 250 ml sterile dark glass bottles, firmly sealed and labeled, and stored in a sterile area at 20 °C ± 0.5.

3.5. Root-segment inoculations within sterile soil substrates

Most of the five fungal strains of the three root-rot pathogens had attained good growth in ME liquid culture after about 20 days of incubation. However, strains ADW-992601 of *G. lucidum* and strains PATO and JNB-6Z302 of *A. mellea* required about 30 additional days to reach the same amount of growth. The preparation of inoculated root samples of five tree species (including *A. hippocastanum*, *C. deodara*, *P.* × *acerifolia*, *Q. robur*, and *L. stiraciflua*) were prepared in all possible combinations with five strains of three fungal root-rot pathogens and two soil substrata types (compacted urban soil and floricultural professional soil mix), resulting in 50 total root type-fungal strain-soil substrate combinations that were prepared for later analysis of headspace volatiles using the PEN3 e-nose following incubation and root decay. These 50 combinations were derived from permutations of (root species + fungal strains + soil substrate) sample variables. In addition, uninoculated (healthy) root samples of each of the five tree species were prepared as controls with the two types of soil substrata, resulting in a total of 10 control samples.

The experimental protocol used to inoculate root segments within aliquots of sterile soil substrata followed a modified procedure adapted from the methods utilized by Chaves and Costa, 1999, Pandey and Pandey, 2005, Shashi and Vishwa, 2005, and Garcês de Araújo et al., 2007. Five grams of root segments of each root type were added to the soil in separate 50 ml glass incubation bottles to which 25 ml of macerated fungal liquid culture of each strain was added along with 6 ml of sterile water. This maintained a relative humidity of 60% inside of the bottle which is the most favorable moisture level for fungal growth and decay of root woods (Misra et al., 2007; Revankar et al., 2007). Every bottle was labeled with the inoculum date, the soil type, the fungal strain and the root fragments origin. After 6 months, 3 ml of sterile water were added to each glass bottle to maintain the correct humidity level.

3.6. The PEN3 electronic nose instrument

All e-nose analyses were performed using an Airsense (Schwerin, Germany) PEN3 electronic nose. This MOS gas-sensing device is a very compact (92 × 190 × 270 mm), light weight (2.1 kg) and portable olfactory system. The complete system consists of a gas-sampling unit and sensor array, requiring a personal computer for data analysis. The sensor array is composed of 10 different metal-oxide gas sensors positioned in a very small chamber with a volume of 1.8 ml. Table 1 lists all the MOS sensors in the sensor array used in the experiment and indicates individual sensor sensitivities and detection limits for specific organic and inorganic gases. The metal-oxide sensors consist of a ceramic support tube containing a platinum heater coil. Each tin-dioxide sensor surface is coated with specific catalytic additives and positioned outside of the ceramic support tube.

The PEN3 e-nose operates with filtered, ambient air as a carrier-gas with a flow rate of 10–400 ml min^{-1}, sample-chamber temperature of 0–45 °C, and sensor-array operating temperature of 200–500 °C. The sensing reaction is based on an oxygen exchange between the volatile gas analyte molecules and the metal coating material. Electrons are attracted to the loaded oxygen and result in decreases in electrical conductivity, detectable by a transducer element (electrode) attached to each sensor when gases are adsorbed and react on the sensing film of the sensor surface. Instrument sensitivity to various VOC gas analytes ranged from 0.1 to 5.0 ppm (Baietto et al., 2010).

Typical PEN3 sensor output graphs for representative healthy and decayed root segments are presented in Fig. 1a and b. The graphs show comparisons between the sensors signals of one healthy (non-inoculated) and one decayed (inoculated) root sample of *P.* × *acerifolia* after 1 year from the inoculum with the wood decay fungus *H. annosum.* Comparisons of normalized sensor outputs expressed as histograms are presented in Fig. 1c. The highest variability between sensors responses to volatiles was noticeable in sensors number 2 and 6.

3.7. Pre-run procedures, data collection and statistical analyses

The PEN3 e-nose instrument was pre-warmed for 10 min prior to each run session, as recommended from the Manufacturer. A standardized run schedule was used for all samples based on the following two-stage run cycle: sampling run time, enough for sensors to reach a stable value, 80 s; sensors cleaning, 300 s, to allow re-establishment of the instrument base-line (Baietto et al., 2010). Data from the sensor array were collected at 1 s intervals: a 4-s

Table 1
Sensor sensitivities and detection limits for the PEN3 sensor array.

Sensor number	Sensor name[a]	Sensor description and sensitivities	Detection limits[b]
1	W1C	Aromatic organic compounds	Toluene, 10 mg kg^{-1}
2	W5S	Very sensitive, broad range sensitivity, reacts to nitrogen oxides, very sensitive with negative signal	NO_2, 1 mg kg^{-1}
3	W3C	Ammonia, also used as sensor for aromatic compounds	Benzene, 10 mg kg^{-1}
4	W6S	Detects mainly hydrogen gas	H_2, 0.1 mg kg^{-1}
5	W5C	Alkanes, aromatic compounds, and nonpolar organic compounds	Propane, 1 mg kg^{-1}
6	W1S	Sensitive to methane. Broad range of organic compounds detected	CH_3, 100 mg kg^{-1}
7	W1W	Detects inorganic sulfur compounds, e.g. H_2S. Also sensitive to many terpenes and sulfur-containing organic compounds	H_2S, 1 mg kg^{-1}
8	W2S	Detects alcohol, partially sensitive to aromatic compounds, broad range	CO, 100 mg kg^{-1}
9	W2W	Aromatic compounds, inorganic sulfur and organic compounds	H_2S, 1 mg kg^{-1}
10	W3S	Reacts to high concentrations (>100 mg/kg) of methane and aliphatic organic compounds	n.d.

[a] As reported in the "sensors options" of the e-nose software (Winmuster 1.6.2.5, Airsense Analytics GmbH, Schwerin, Germany).
[b] From a previous work of Gomez et al., 2007.

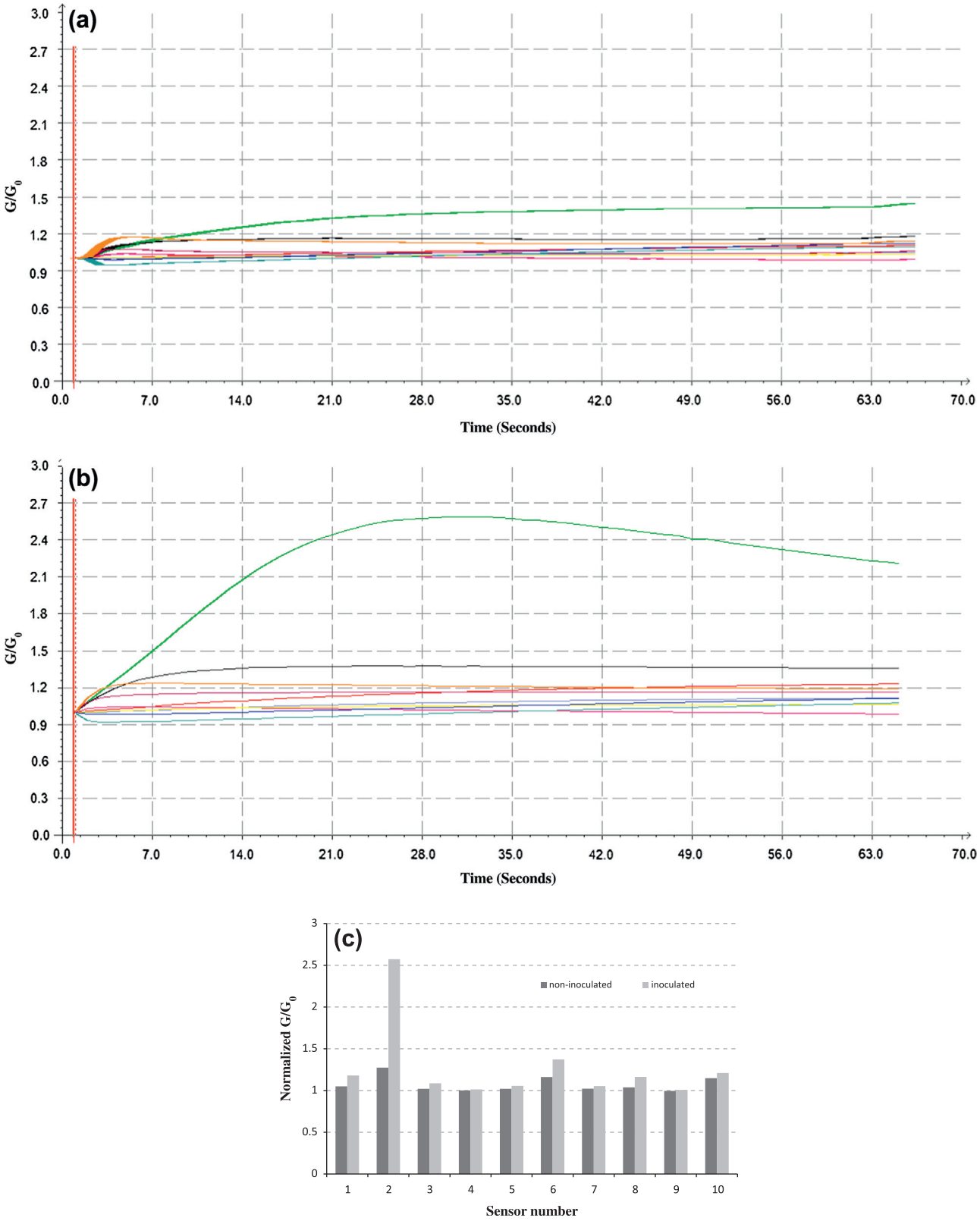

Fig. 1. Typical sensor-array response outputs from representative samples of healthy (control) and inoculated (decayed) root segments. Sensory array output from: (a) a healthy root sample of *Platanus* × *acerifolia*, and (b) a decayed root segment of *P. acerifolia* after inoculation with the wood decay fungus *Heterobasidion annosum*. Histogram of normalized intensity responses of individual sensors in the sensor array to headspace volatiles released from the samples are reported in (c). G/G0 is the ratio of the electrical conductivity response of the sensors to the sample gas (G) relative to the carrier gas (G0) over time. The following data were adjusted relative to the carrier gas (air) baseline.

sampling interval between 47 and 50 s into the run was utilized, and all data were averaged from three replications per sample.

A luer-lock needle (Terumo Italia srl, Rome, Italy), connected to a 3 mm Teflon tube (Fisher Scientific, Illkirch Cedex, France) was used to perforate the wrap of each black glass bottle to aspirate an sample air with accumulated headspace volatiles derived from each analyte. The headspace gas sample was pumped over the sensors at a carrier gas flow rate of 200 mL min^{-1} and the run cycle was controlled by Winmuster 1.6.2.14 software (WMA Airsense Analytics GmbH, Schwerin Deutschland).

The sampling chamber temperature was set at 30 °C, controlled by a thermostatic bath, and filtered atmospheric air was used as the carrier gas. Reference air was preconditioned by passing room air sequentially through an active-carbon filter (Whatman plc, Maidstone, UK) to remove organic compounds, moisture, particulates and microbes. Because sensor conductivity drifted as the sample gas passed over the array, the data were adjusted based on the changing ratio of conductivity between G and G0 (i.e., the electrical conductivity response of the sensors to the sample gas relative to the carrier gas or baseline signal over time).

All samples were analyzed with the PEN 3 e-nose at least two times and the mean values between averaged sensor values, as well as the peak values of all sensors, were used for statistical analyses. Principal component analysis (PCA) and linear discriminant analysis (LDA) were performed by Winmuster software to discriminate between the different classes of samples. PCA allowed the extraction of useful information (discrimination of sample types) from the data and to explore their structure, including correlation between variables and the relationship between subjects (Beebe et al., 1998). LDA was used to maximize the variance between sample categories (aroma classes) and minimized the variance between measurements recorded from samples within each sample category (Meloun et al., 1992). PCA analyses were done using a [k-Nearest Neighbor] (kNN) discrimination setting level 3 (highest level of confidence) that provides a Euclidean distance measure of statistical significance differences between healthy and decayed PCA data plots.

4. Results and discussion

The PEN3 sensor-array response of individual sensors to VOC mixtures released from *P.* × *acerifolia* root samples inoculated with *H. annosum*, *A. mellea*, and healthy controls are presented in Fig. 2a. Sensors 2, 6, 8 and 10 showed the strongest responses to VOCs and the greatest differences in responses to the three sample types, while the other six sensors had relatively weak responses and smaller differences. Sensor 2, having a broad range of sensitivity to VOC gas analytes, exhibited the widest range of responses and most marked differences between sample types. The response of sensor 2 was significantly higher for *H. annosum* volatiles than for *A. mellea* or the control. Similarly, sensor responses to headspace VOCs derived from healthy root samples of *L. styraciflua* and root samples inoculated with *H. annosum* and *A. mellea* showed the greatest differences for sensor 2 and slightly lower responses and differences between sensors 6, 7, 8 and 9 (Fig. 2b). The sensor 2 response to volatiles from health control roots of *L. styraciflua* was much higher than the response to volatiles from *H. annosum*- and *A. mellea*-inoculated roots.

4.1. Linear discriminant analysis

Linear discriminant analysis (LDA) was performed on e-nose output data of different root sample types to evaluate the capability of the PEN3 e-nose to discriminate between headspace VOC mixtures derived from different root samples types. The operating hypothesis (tested in the current study) was that woods of different tree species release different aroma mixtures of VOCs as previously reported by Baietto et al. (2010). The first test was to evaluate the capability of the PEN3 e-nose to discriminate between healthy and decayed roots of different plant species independently of the type of soil substrate used for incubating the root segments. The graphical outputs (aroma maps) of LDA results comparing healthy and inoculated root segments of *A. hippocastanum*, *C. deodara*, *L. styraciflua* and *Q. robur*, are presented in Fig. 3. The variance in the data explained by linear discriminant components LD-1 (*x*-axis) and LD-2 (*y*-axis) along with total variance of these two components (LD-1 + LD-2) are presented in Table 2 with corresponding values of statistical differences between LDA aroma data plots (groups) of healthy to inoculated roots for each host root type.

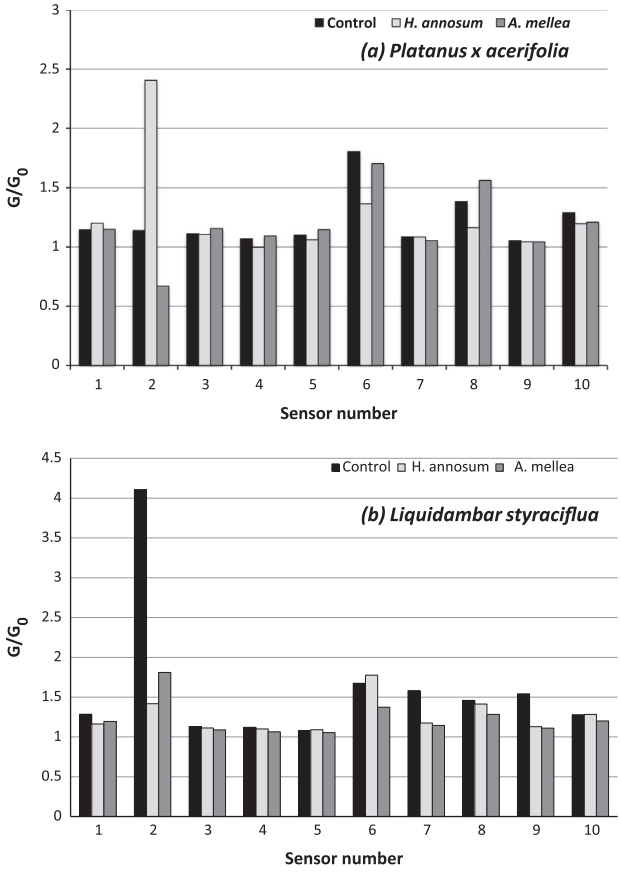

Fig. 2. Sensors responses of the PEN3 e-nose to headspace VOCs released from samples of (a) *Platanus* × *acerifolia* and (b) *Liquidambar styraciflua* root segments. Root sample types include: Controls, *H. annosum*-inoculated, and *A. mellea*-inoculated roots.

4.2. Principal component analysis

Principal component analysis (PCA) test results are presented graphically in Figure 4. In addition, pairwise comparisons and statistical differences between samples types are indicated by discrimination powers (DPs) for corresponding root sample types in Table 3. The PEN3 instrument did not discriminate between healthy controls (uninoculated) and inoculated roots of *P.* × *acerifolia*, indicated by low PCA discrimination powers (DP < 0.5). By contrast, the PCA discrimination power (DP), determined by comparing healthy and inoculated roots of *P.* × *acerifolia* incubated in urban soil, was significantly higher (DP = 0.594), probably due to chemical or physical characteristics of the professional soil substrate that inhibited the growth and development of root-decaying fungi.

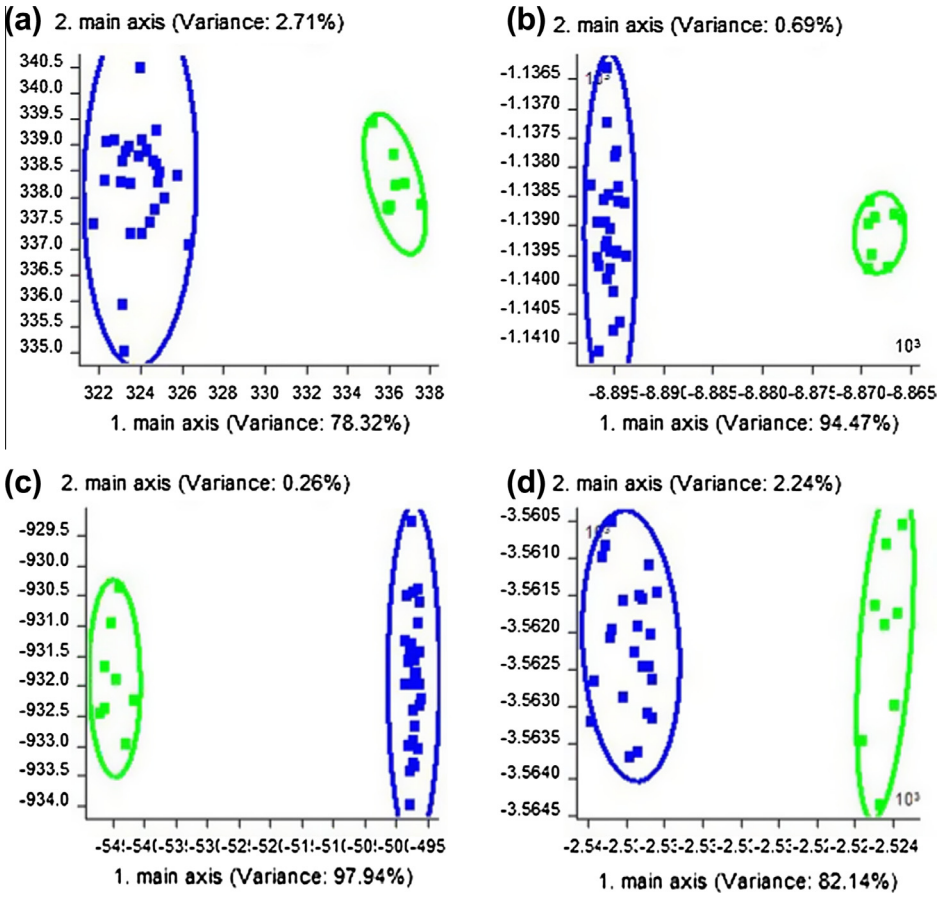

Fig. 3. Linear discriminant analysis (LDA) of PEN3 data derived from sensor responses to headspace VOCs of healthy control root segments (green data points) and inoculated or decayed root segments (blue data points) of the tree species: (a) *A. hippocastanum*, (b) *C. deodara*, (c) *L. styraciflua* and (d) *Q. robur*, 12 months after fungal inoculation (data points include all fungal species tested). The percentages of total variance, accounting for the variability explained by each linear component (LC), are indicated by main axes 1 and 2, representing the *x*-, and *y*-axis, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Linear discriminant analysis (LDA) results for comparisons between healthy (uninoculated controls) and decayed (inoculated) root segments of each tree species: *A. hippocastanum*, *C. deodara*, *L. styraciflua*, *P. × acerifolia*, and *Q. robur*. The percentage variance in the data attributed to linear discrimination components LD-1 (*x*-axis) and LD-2 (*y*-axis) are indicated with total variance (LD-1 + LD-2). Analyte 1 consisted of all e-nose data pooled and analyzed for wood decayed by fungi *Armillaria mellea*, *Ganoderma lucidum*, and *Heterobasidion annosum* collectively (all together as a group) or individually (3 separate fungi) compared against corresponding healthy wood controls (Analyte 2).

Host species	Analyte 1	Analyte 2	Variance (%)		
			LD-1	LD-2	Total
A. hippocastanum	Inoculated + all fungi	Healthy	78.3	2.7	81.0
C. deodara	Inoculated + all fungi	Healthy	94.5	0.7	95.2
L. styraciflua	Inoculated + all fungi	Healthy	97.9	0.3	98.2
Q. robur	Inoculated + all fungi	Healthy	82.1	2.2	84.3
A. hippocastanum	Inoculated + 3 fungi	Healthy	50.5	15.0	65.5
C. deodara	Inoculated + 3 fungi	Healthy	49.0	33.7	82.7
L. styraciflua	Inoculated + 3 fungi	Healthy	33.6	29.4	63.0
P. × acerifolia	Inoculated + 3 fungi	Healthy	79.2	7.2	86.4
Q. robur	Inoculated + 3 fungi	Healthy	51.8	17.9	69.7

The capabilities of the PEN3 e-nose to discriminate between healthy and inoculated (decayed) root segments as well as between the different etiologic agents of different plant species, independent of soil substrate, are indicated in Fig. 4. Graphic outputs of linear discriminant analysis demonstrated good segregation between different root sample types, while the discrimination powers of PCA did not often show a valid statistical discrimination. Roots of *C. deodara* (Fig. 4b) show higher significant statistical differences between healthy and *G. lucidum*-inoculated roots (DP = 0.557), but no significant differences were found between healthy and *A. mellea*-inoculated roots (DP = 0.236) and *H. annosum*-inoculated roots (DP = 0.343). Again, uninoculated control roots of *P. × acerifolia* (Fig. 4d) showed significant statistical differences only for *A. mellea*-inoculated roots (DP = 0.772), and no significant differences for *G. lucidum*-inoculated (DP = 1.179) and *H. annosum*-inoculated roots (DP = 0.159).

The capabilities to discriminate between healthy and diseased roots of different plant species were not dependent on the substrate used to incubate the samples (Fig. 3), but other data presented in Table 2 suggest that substrate did have an effect on discrimination. This might be explained by differences in root colonization and decay by individual rot fungi in dissimilar substrates causing the concentration or mixture of VOCs emitted by the fungus to be different in one soil substrate compared with the other. Alternatively, the concentration of VOCs released by the substrates themselves could have been high enough to affect the output of sensor responses. This explanation was supported by PCA results comparing diseased (inoculated) and healthy roots segments of all tree species (Table 2). Different soil substrates did influence the results. Nevertheless, the PEN3 e-nose was capable of discriminating between root samples with different decay fungi regardless of the contribution of substrate VOCs to the headspace aroma mixture of the root-fungus sample.

Table 3
Discrimination power (DP) values of PCA pairwise comparisons between uninoculated (healthy controls) and inoculated (decayed) root segments of each tree species: (a) *A. hippocastanum*, (b) *C. deodara*, (c) *L. styraciflua*, (d) *P. × acerifolia*, and (e) *Q. robur*. The title of every row and column are labeled according to the following 2-tiered (decay fungus, soil substrate) abbreviation scheme: Decay fungi, *Armillaria mellea* (am); *Ganoderma lucidum* (gl); *Heterobasidion annosum* (ha); healthy controls (ctr); and Soil substrata, urban soil substrate (us); professional soil substrate (ps). Data values indicate the decimal fraction overlap of PCA elements that paired sample-analytes share in common. By definition, a sample analyte shares all principal components when compared against itself and the theoretical decimal fraction overlap is 1.000 (for self-comparisons). DP values of 0.80–0.90 are significantly different only at approximately the $P = 0.10$ level of significance; DP values ranging from 0.55 0.75 are significantly different at approximately the $P < 0.05$ level; whereas DP values in bold (DP < 0.50) are significantly different at the $P \leqslant 0.01$ level.

Fungus, soil type	ctr, ps	ctr, us	am, ps	am, us	gl, ps	gl, us	ha, ps	ha, us
(a) Aesculus hippocastanum								
ctr, ps		0.903	**0.209**	0.783	0.539	0.719	0.678	0.957
ctr, us	0.903		**0.488**	0.782	0.665	0.604	0.672	0.938
am, ps	**0.209**	**0.488**		**0.471**	**0.241**	0.648	**0.437**	0.654
am, us	0.783	0.782	**0.471**		0.726	0.562	0.743	0.743
gl, ps	0.539	0.665	**0.241**	0.726		0.764	**0.243**	0.820
gl, us	0.719	0.604	0.648	0.562	0.764		0.762	**0.276**
ha, ps	0.678	0.672	**0.437**	0.743	**0.243**	0.762		0.783
ha, us	0.957	0.938	0.654	0.743	0.820	**0.276**	0.783	
(b) Cedrus deodara								
ctr, ps		0.745	0.525	0.926	0.881	0.995	0.859	0.943
ctr, us	0.745		0.770	**0.362**	0.877	0.985	0.895	**0.475**
am, ps	0.525	0.770		0.633	0.813	0.966	0.511	0.606
am, us	0.926	**0.362**	0.633		0.870	0.985	0.863	0.555
gl, ps	0.881	0.877	0.813	0.870		0.836	0.727	0.866
gl, us	0.995	0.985	0.966	0.985	0.836		0.971	0.978
ha, ps	0.859	0.895	0.511	0.863	0.727	0.971		0.854
ha, us	0.943	**0.475**	0.606	0.555	0.866	0.978	0.854	
(c) Liquidambar styraciflua								
ctr, ps		**0.321**	**0.297**	**0.212**	**0.167**	**0.411**	**0.168**	**0.263**
ctr, us	**0.321**		0.992	0.999	0.953	0.995	0.750	0.987
am, ps	**0.297**	0.992		0.561	**0.174**	0.881	**0.285**	0.905
am, us	**0.212**	0.999	0.561		**0.419**	0.844	**0.346**	0.557
gl, ps	**0.167**	0.953	**0.174**	**0.419**		0.623	**0.109**	0.504
gl, us	**0.411**	0.995	0.881	0.844	0.623		0.605	**0.458**
ha, ps	**0.168**	0.750	**0.285**	**0.346**	**0.109**	0.605		**0.451**
ha, us	**0.263**	0.987	0.905	0.557	0.504	**0.458**	**0.451**	
(d) Platanus × acerifolia								
ctr, ps		0.907	0.837	0.859	0.730	0.847	0.822	0.774
ctr, us	0.907		0.888	0.976	0.878	**0.491**	0.890	0.883
am, ps	0.837	0.888		0.872	0.765	0.839	0.851	0.819
am, us	0.859	0.976	0.872		0.889	**0.313**	0.905	**0.424**
gl, ps	0.730	0.878	0.765	0.889		0.936	0.827	0.858
gl, us	0.847	**0.491**	0.839	**0.313**	0.936		0.814	**0.348**
ha, ps	0.822	0.890	0.851	0.905	0.827	0.814		0.903
ha, us	0.774	0.883	0.819	**0.424**	0.858	**0.348**	0.903	
(e) Quercus robur								
ctr, ps		0.832	0.881	0.918	0.812	0.961	0.799	0.934
ctr, us	0.832		0.606	0.711	0.717	0.641	0.796	**0.474**
am, ps	0.881	0.606		0.911	0.618	0.933	0.776	0.947
am, us	0.918	0.711	0.911		0.908	**0.384**	0.852	0.641
gl, ps	0.812	0.717	0.618	0.908		0.895	0.703	0.902
gl, us	0.961	0.641	0.933	**0.384**	0.895		0.836	**0.375**
ha, ps	0.799	0.796	0.776	0.852	0.703	0.836		0.829
ha, us	0.934	**0.474**	0.947	0.641	0.902	**0.375**	0.829	

Uninoculated healthy control samples of *A. hippocastanum* root chips (ctr,ps and ctr,us) showed good discrimination powers (DP > 0.5) with *G. lucidum* and *H. annosum*-inoculated samples regardless of the soil substrate type (Table 2a). The e-nose could not discriminate between healthy and *A. mellea*-inoculated root fragments in professional soil substrate or urban soil substrate.

The PEN3 e-nose was capable of discriminating between all root sample types of *C. deodara* at very high levels of discrimination (DP > 0.90) as indicated in Table 2b. The results are similar in performance to the human olfactory sense that easily recognizes and distinguishes the rich aromatic resinous woods of coniferous tree species. Moreover, there was strong differences between the same decay fungus and different soil substrates (DP > 0.75) for most comparisons.

PEN3 sensor-output data in response to VOCs in headspace volatiles derived from *L. styraciflua* root segments did not indicate good segregation between different root sample types (Table 2c). For example, healthy control samples in professional substrate soil were not discriminated from any other root sample types. However, healthy root samples of all tree species in the urban substrate showed very high discrimination power values (DP > 0.98) in most cases, indicating that healthy roots could be easily distinguished in the urban soil. Also, root segments inoculated with *A. mellea*, *G. lucidum* and *H. annosum* in the professional soil substrate were discriminated by only a few data sets of each type. This could be explained by a low level of decay development on *L. styraciflua* root segments in that soil substrate type.

The PEN3 e-nose could not only discriminate between healthy control and diseased (inoculated) root fragments of *P. × acerifolia* and *Q. robur* tree species from substrate with very high levels of confidence (DP > 0.5), but the PEN3 e-nose could discriminate between the different etiologic agent with only two exceptions: (1) healthy root samples of *P. × acerifolia* in urban substrate (sample ctr,us of Table 2d) where not statistically different from

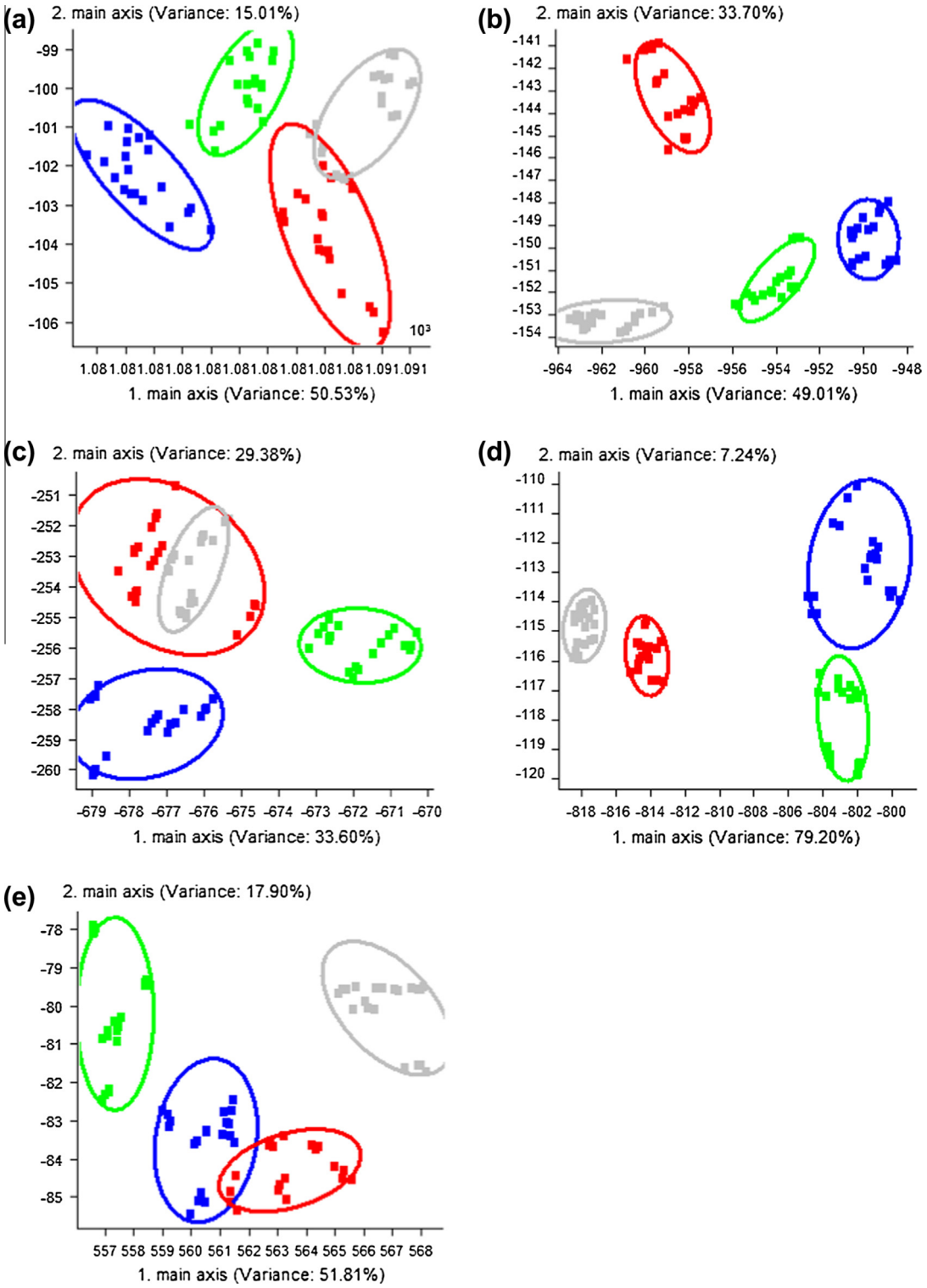

Fig. 4. Linear discriminant analysis (LDA) of healthy control root segments (green data points) of (a) *A. hippocastanum*, (b) *C. deodara*, (c) *L. styraciflua*, (d) *P.* × *acerifolia*, (e) *Q. robur* and decayed (diseased) root segments, 12 months after fungal inoculation. The wood decay fungi responsible for decay in inoculated root segments are indicated by the following color scheme: *A. mellea* (blue data points), *G. lucidum* (red data points), and *H. annosum* (gray data points). The percentages of total variance, accounting for the variability explained by each linear component (LC), are indicated by main axes 1 and 2, representing the *x*-, and *y*-axis, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

G. lucidum-inoculated samples incubated in the same substrate (gl,us); and (2) healthy root samples of *Q. robur* in urban substrate (sample ctr,us of Table 2e) could not be discriminated from *H. annosum*-inoculated samples in the same substrate (ha,us).

The PEN3 e-nose, like other portable e-nose devices, has several advantages over conventional methods for wood decay detection. Conventional methods require destructive sampling, are time consuming and very difficult to use to detect root decays. By contrast, e-nose instruments are capable of root decay detection with

nondestructive sampling and they produce rapid accurate determinations. In addition, conventional methods only indicate whether or not decay is present, but do not tell what type of decay is present (i.e., the specific species of wood decay fungi causing the decay) whereas e-nose methods are capable of identifying the type of decay present when aroma signature reference libraries are used. The PEN3 e-nose does not currently utilize aroma reference libraries, but this capability is available with other e-nose types.

5. Conclusions

Fungal sapwood decays are among the most damaging diseases of trees in the urban environment because structural failures, resulting in limb or bole breaks, can cause serious damage to people and their personal property when these tree parts fall to the ground. In the case of wood decay fungi attacking root tissues, the dangers are even greater due to the inability to detect the decay below ground until it is too late. Our data and critical evaluation of the PEN3 e-nose indicate that this gas-detection device is capable of discriminating between healthy and decayed tree root samples of all tree species tested. We have also demonstrated that this electronic nose can discriminate between most fungal etiologic agents of decay tested in roots of five tree species, regardless of the contribution of soil-substrates VOCs to the complex root aroma mixture. The cases where the e-nose did not detect differences between healthy and decayed wood was most likely attributed to the host wood not being susceptible to decay which prevented the development of a significant "decay" aroma signature. In this case, the absence of significant decay precluded the effective e-nose discrimination between healthy and decayed wood for these certain host wood-decay fungus combinations. This situation would not be a significant problem in field conditions because the absence of decay for a particular host-fungus combination (where the tree species is essential a non-host) would result in a correct healthy determination by the e-nose. We obtained very similar results previously indicating the capability of the PEN3 e-nose and two other types of e-nose devices to detect most fungal decays, caused by 24 decay fungi, in bole (trunk) samples from nine tree species (Baietto et al., 2010). These positive results suggest the need to further test the potential application of the PEN3 e-nose in live field situations (during urban tree surveys) to determine whether this instrument is capable of early detections and diagnoses of root rots in living urban trees.

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