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Detection of *Fusarium poae* Infestation in Wheat Grain by Measurement with Two Electronic Noses †

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Abstract: *Fusarium poae* is a pathogen that is widespread in the temperate zone and poses a serious threat to crops due to its wide range of host plants (including cereals). Electronic nose measurements were performed on wheat grains infected with *F. poae* to evaluate the application of early detection of fungal infections. Wheat seeds were artificially inoculated to test the devices. Three same-weight but different infection levels variants of experiments were prepared: 3 g infected seeds with 12 g healthy seeds, 5 g infected seeds with 10 g healthy seeds, and 10 g infected seeds with only 5 g healthy seeds. The seeds were infected with fresh fragments of *F. poae* mycelium. Measurements were carried out for five constructive days, recording the changes in volatile odor compounds released each day. A custom-built, low-cost device based on Figaro Inc. TGS metal-oxide, semiconductor gas sensors, and commercially available PEN3 electronic nose device from Airsense Analytics GmbH was used for the experiment. A non-linear sensor response for measured sample odor was observed with both devices. Spoiled grain in a proportion of 1/15 of the sample could be detected by measuring the volatile components. However, the patterns of the sensor responses were different for various concentrations of spoiled grain in the measured samples.

Keywords: gas sensor; application of e-nose; PEN3; low-cost electronic nose; fungal spoilage detection; odor concentration; wheat infection; seeds infection

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1. Introduction

There are five categories of toxic molds: *Cladosporium*, *Penicillium*, *Fusarium*, *Aspergillus*, and *Stachybotrys*. *Fusarium* species are filamentous fungi, which are commonly found in the environment, particularly in soil, plants, and aquatic systems. *Fusarium* species cause diseases of many crop species. Symptoms include yellowing, dwarfing and death of seedlings and yellowing and dwarfing of older plants. In addition, *Fusarium* species become opportunistic pathogens of humans. They can cause a range of diseases, from superficial, invasive, and disseminated infections through direct inoculation, ingestion, but also by inhalation. Fusariosis [1] is an infection encountered at plants but also at animals, and even at humans, which is caused by various fungi of the genus *Fusarium*. Most *Fusarium* diseases can be transmitted through the soil and, compared to pathogens infecting aboveground plant parts, the processes by which *Fusarium* infects its hosts are not well understood. The

fungus persists in the soil in the form of chlamydospores (small survival structures), which can persist in the soil for years even in the absence of a host organism.

There is no effective fungicide or other treatment for Fusarium wilt. The pathogen almost always kills infected hosts, so prevention and exclusion are the only effective disease control strategies. Hence, there is a need to develop rapid diagnostic methods for detecting the presence of *Fusarium*, e.g., by odor of volatile compounds.

An easy way to detect *Fusarium* is to smell the bulbs, since if they are infested may be distinguished by a sour odor. Infection can be also detected by visual examination by noticing a white mycelium (mold) growing on the surface, usually concentrated at the base of the bulb.

The detection of fungi of the genus *Fusarium* in cereal ears or stored grains, e.g., wheat, is visually difficult, and therefore, the development of an electronic nose for this purpose would be very useful for practical applications.

Fusarium poae is mostly found in temperate regions; therefore, its detection is particularly justified in Europe, the Russian Federation, and the northern part of the USA and Canada. Although the use of e-nose could also be used in South Africa, Australia, and New Zealand [2], where *Fusarium poae* has also been recorded. In Poland, it has been causing damage to plants since 1957, when it was first recorded [3]. It is an important pathogen because it has a wide host range infecting plants in the panicle family (*Poae*), including cereals, sugarcane, and rice. It is also found on carnations, chrysanthemums, many legumes, and on tree seedlings, including conifers [2].

F. poae infects cereals through the seeds, so these too should be carefully monitored. In the USSR, it was frequently found on both autumn and spring-harvested cereals. In large grain storage silos, odor analysis for the detection of this pathogen will be particularly useful because it is not necessary to find infected grain, and it is sufficient to detect volatile secondary metabolites.

Various methods have been developed for the chemical analysis of gases and the detection of odors. The reference and most effective approach is the application of classical chemical analysis methods (e.g. gas chromatography, mass spectrometry), which allows to determine the chemical components and their concentrations. Unfortunately, this method may be applied only under laboratory conditions.

Electronic nose (e-nose) [4–6] applies a series of non-specific gas sensors, which do not allow the analysis of the composition of the gas mixture and identify individual chemical components. It relies on machine learning pattern recognition techniques, allowing differentiation between the studied sample categories. One of the most popular commercially available electronic noses is PEN3 (Airsense Analytics GmbH, Schwerin, Germany). However, many custom-made, low-cost alternatives have also been proposed [7].

Much research focused on the application of electronic noses to studies of odors of fungi infection in cereals [8,9]. Dong et al. [10] described the research on volatile metabolites from malt contaminated with *F. poae* during malting. One can find several reports describing research on the detection of particularly *Fusarium* infestations. Presiecce et al. [9]. Eifler et al. [11] studied the possibility of detection of infection of wheat grains with *F. cerealis*, *F. graminearum*, *F. culmorum*, and *F. redolens*. Also other cereals such as rapeseed wheat and triticale [12–14] and rice [15,16] were investigated. The work of Laddomada et al. [17] described a GC–MS measurements of the volatile profile of durum wheat contaminated with *F. poae*. Mota et al. [18] reviewed the possibilities of identification of fungal species using electronic noses. Nordstorm et al. [19] studied *F. circinatum* and Feng et al. [20] *F. oxysporum* in tomato processing. Labanska et al. [21,22] basal rot infections in onions and shallots. Ji et al. [23] analyzed the volatile metabolites in wheat kernels contaminated with *F. graminearum*.

Only a few authors reported experiments when several electronic noses were applied to the same subject and results compared [24,25].

In the authors' previous reports [26,27] experiments of differentiation of *F. poae* from four other *Fusarium* species were described. Also, differentiation between other *Fusarium* species by electronic nose has been studied [28,29].

Cheli et al. [30] critically reviewed the current status of the e-nose technology and concluded that the performance of detection is limited. Further studies and proposals of devices and data analysis methods are needed to make suitable detection of mycotoxins in the field applications.

2. Materials and Methods

2.1. Electronic Nose Devices

Two electronic nose devices were used in the experiment. First of all, the commercially available PEN3 electronic nose (Airsense Analytics GmbH, Schwerin, Germany). Secondarily, the constructed by the authors, PW8 device, belongs to the low-cost electronic noses category.

2.1.1. PEN3

The PEN3 e-nose applies a sensor array of 10 metal oxide semiconductor (MOS) sensors (Table A1). An important component of the PEN3 electronic nose device is an advanced pneumatic system allowing it to suck the air containing the measured odor from the vicinity of the sample being the source of volatiles. It transports the measured gas to the sensors' chamber. The pneumatic system also allows the device to be cleaned by blowing air purified by the charcoal filter. In the electronic nose, a control and data acquisition module is also included. The PEN3 has various commercial applications but has also been used worldwide for research purposes (the Scopus database reports 180+ research papers using the PEN3 keyword).

2.1.2. PW8

The PW8 devices developed in our laboratory [31] is a simpler device, allowing the application of up to 8 MOX gas sensors of the Figaro TGS series (Figaro, Osaka, Japan). In the current experiment, 7 sensors were used (Table A2). The device is equipped with a much simpler pneumatic system which is used only for cleaning the sensors between consecutive measurements of various samples. A dedicated control electronic system allows to collection of dynamic sensor's response to changes in the chemical composition of the gas in which the sensors are immersed. Operation of the electronic nose has been described in detail in previous reports of the authors [31]. However, unlike the other experiments [27–29], in the current measurements, only the gas adsorption phase of the sensor's response has been exploited.

2.2. Samples Preparation

Fusarium poae isolates from the pathogen bank of the Faculty of Agriculture and Forestry of the University of Warmia and Mazury in Olsztyn (Poland) were used for the analyses. First, the isolates were refreshed by transplanting them onto fresh potato dextrose agar (PDA) medium (20 g dextrose, 15 g agar, 4 g potato starch, and 1 L distilled water). The isolates were incubated until the mycelium covered the entire plate. In the meantime, the wheat seeds were prepared for inoculation. As the measurements were performed with two devices, the preparation process differed depending on the vessel in which the samples were prepared. For PEN 3, sterilized 250 mL jars with prepared holes in the lid were used, through which the odors for the measurements were collected, while for PW8, the seeds were prepared in Petri dishes with a diameter of 9 cm. Three variants were prepared for each device, which differed in the proportion of infected seeds in relation to healthy seeds:

(i) 0/15 = 15 g of healthy seeds,
(ii) 1/15 = 1 g of infected seeds + 14 g of healthy seeds,
(iii) 3/15 = 3 g of infected seeds + 12 g of healthy seeds,
(iv) 5/15 = 5 g of infected seeds + 10 g of healthy seeds.

Each variant was prepared in triplicate. First, 1, 3, and 5 g were weighed into dishes or jars and then moistened with distilled water (about 5 mL for each vessel). After 24 h from the time of seed preparation, inoculation was carried out with previously prepared fresh wipes containing *F. poae*. Using a corkscrew with a diameter of 5 mm, fragments of the substrate were cut out together with the mycelium and placed in the containers. For the 3 g variant, 3 agar fragments were added, for 5 g—5 fragments, and for 10 g—10 fragments. The samples prepared in this way were left for a week to allow the mycelium to develop on the seeds. After a week, when the appearance of hyphae was observed, an appropriate amount of fresh wheat seeds was added to each container.

2.3. Measurements with Electronic Noses

The measurements for this experiment were taken using the aforementioned PEN3, shown in Figure 1a, and using our own e-nose, PW8, shown in Figure 1b. The previously prepared samples were measured over 2 consecutive days. Each day the samples were measured in a random order according to the Excel spreadsheet random number generator. The experiment was conducted under controlled conditions at constant temperature and humidity. The samples for PEN3 were sealed so that the emitted volatiles were collected in jars. For this e-nose, the measurement time was set to 120 s. In contrast, the samples for PW8 were sealed in Petri dishes, and the measurement time was 315 s. Before each subsequent measurement, the e-noses cleaned their sensors by blowing clean, filtered air.

Measurements for both devices were performed in pairs. The control sample, without infected seeds, was measured first. Then, samples with infected seeds, at a given ratio, were measured.

In total, we collected 32 observations with the PEN3 and 32 observations with the PW8.

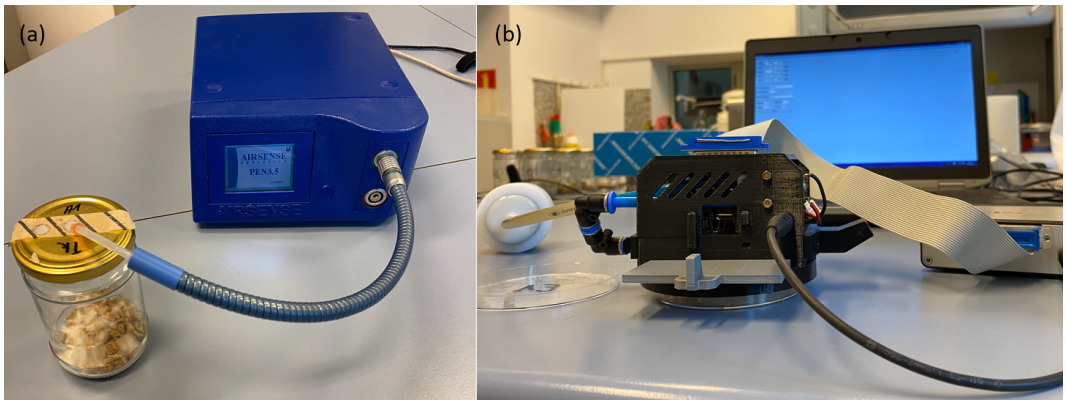

Figure 1. Measurement setup of the used electronic nose PEN3 (**a**) and PW8 (**b**) applied to a sample with *Fusarium poae*-infected wheat grains. The sample measured by the PEN3 device is noticeable in the photography, while in the case of the PW8 device, it is contained in the Petri dish applied under the devices.

3. Results of Measurements

Both electronic noses apply Metal-Oxide Sensors (MOX) working on the same physical principle [32]. The measured sensors response [33] is the electrical conductance (or resistance) of the sensors in the presence of the odor of interest. What is important to notice, the meaningful quantity is the the conductance relative to its magnitude measured in the presence of the reference gas, which in both cases was clean air. That requires that a measurement cycle of one sample starts as a measurement of the sensor conductance in clean air conditions and, after that, in the presence of the measured odor. In the case of the PEN3 device such change of conditions is performed by application of the built-in pneumatic system, while in the case of PW8 device, by opening the sensor chamber shutter. In the cases of both devices, the transient sensor response to the change of conditions (gas adsorption by the sensor) can be measured.

3.1. PEN3 Electronic Nose

In Figure 2, we present a visualization of the trend of the PEN3 electronic nose response collected during the two days of the experiment.

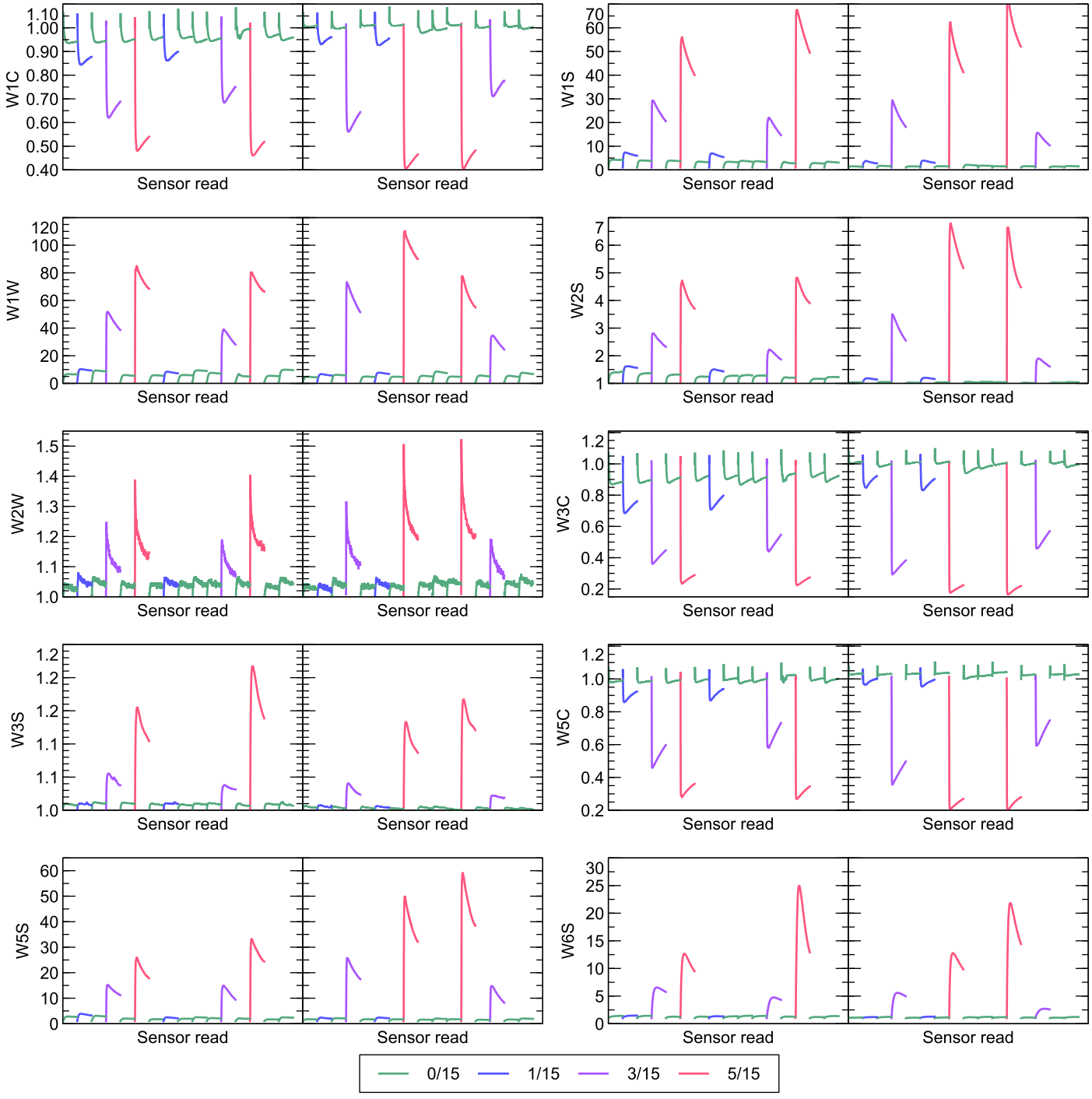

Figure 2. Sensor response collected during two days of the measurements by PEN3 electronic nose. The sub-figures on the left represent the first day of the experiment, and on the right, the second day. The data along the x-axis are squeezed, interruptions in the data collection process, and sensor relaxation are removed. The Y-axis represents the sensor response magnitude normalized by the response in the clean air G/G_0. The sensor type (Table A1) is indicated as the caption of the y-axis.

As one can notice, there is a noticeable difference between the studied categories of samples. The observed magnitude of the sensors response is consistent and very similar when we compare measurements of different samples of the same category.

We can also notice the trend indicating that the sensor response increases with the increase of the proportion of the infected material in the sample. The sensor response is very small in the case of the samples with the smallest studied amount of the infected wheat grain.

A characteristic feature of the sensor response curve, allowing to differentiate between the measured samples may be defined as the extreme magnitude of the sensor response. As one can notice in Figure 2, for some of the sensors it was the minimum of the response, while for some other sensors, it was the maximum of the response, depending on the direction of the response to the presence of the measured gas.

In Figure 3, we present box-plot visualization of the extreme sensor response for various studied categories of samples.

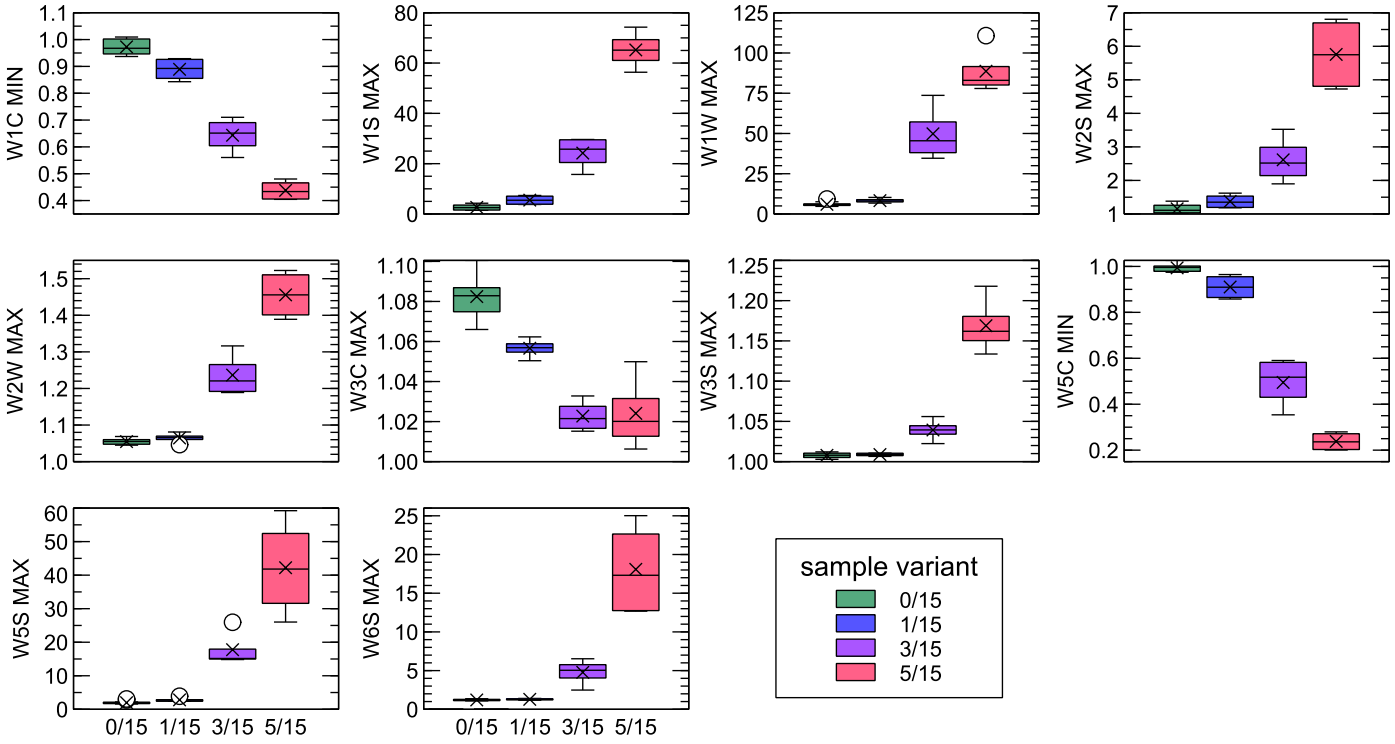

Figure 3. Comparison of the distribution of PEN3 electronic nose sensors response versus studied sample variant. The response was measured at the characteristic moment when the extreme magnitude (minimum or maximum) was reached. The sensor type (Table A1) is indicated at the y-axis caption.

One can observe that there is a reaction of all sensors to the odors emitted by the infected grain, and the reaction depends on the level of the spoilage. It should be noted here, that the sensor response is expressed here as a proportion of the reaction to the measured odor and the response to the clean air conditions (G/G_0). As one can notice, for most of the sensors, the response to the control sample (healthy grain) is close to 1, which signifies that the emitted volatile organic components do not cause a reaction of the PEN3 sensors. The difference is in the case of the W3C sensor, for which the response to the control sample is stronger than to the infected samples. That may signify that the spoilage process probably reduced the amount of some chemical components present in the "wheat aroma" [34].

We can also observe a clear trend that an increase in the amount of spoiled grain in the samples led to an increase in the observed sensors' response. The sensor reaction

is not linear with the increase in the amount of spoiled grain. However, that could be expected as it is a known property of MOX sensors. We can also observe, that in most of the cases, the variability of the responses observed for the case of the most spoiled samples category (5/15) is the greatest. That also seems reasonable. The samples were prepared by measuring the amount of the infected and healthy grain. The amount of infected grain is correlated with the amount of produced volatiles, but no one should expect that that is a constant process.

3.2. PW8 Electronic Nose

In Figure 4, we present a trend of the PW8 electronic nose sensors' response collected during the two days of the experiment. In this figure, we skipped the part of the response that could be collected during the sensor relaxation after closing the sensor chamber and blowing purified air used for sensor cleaning.

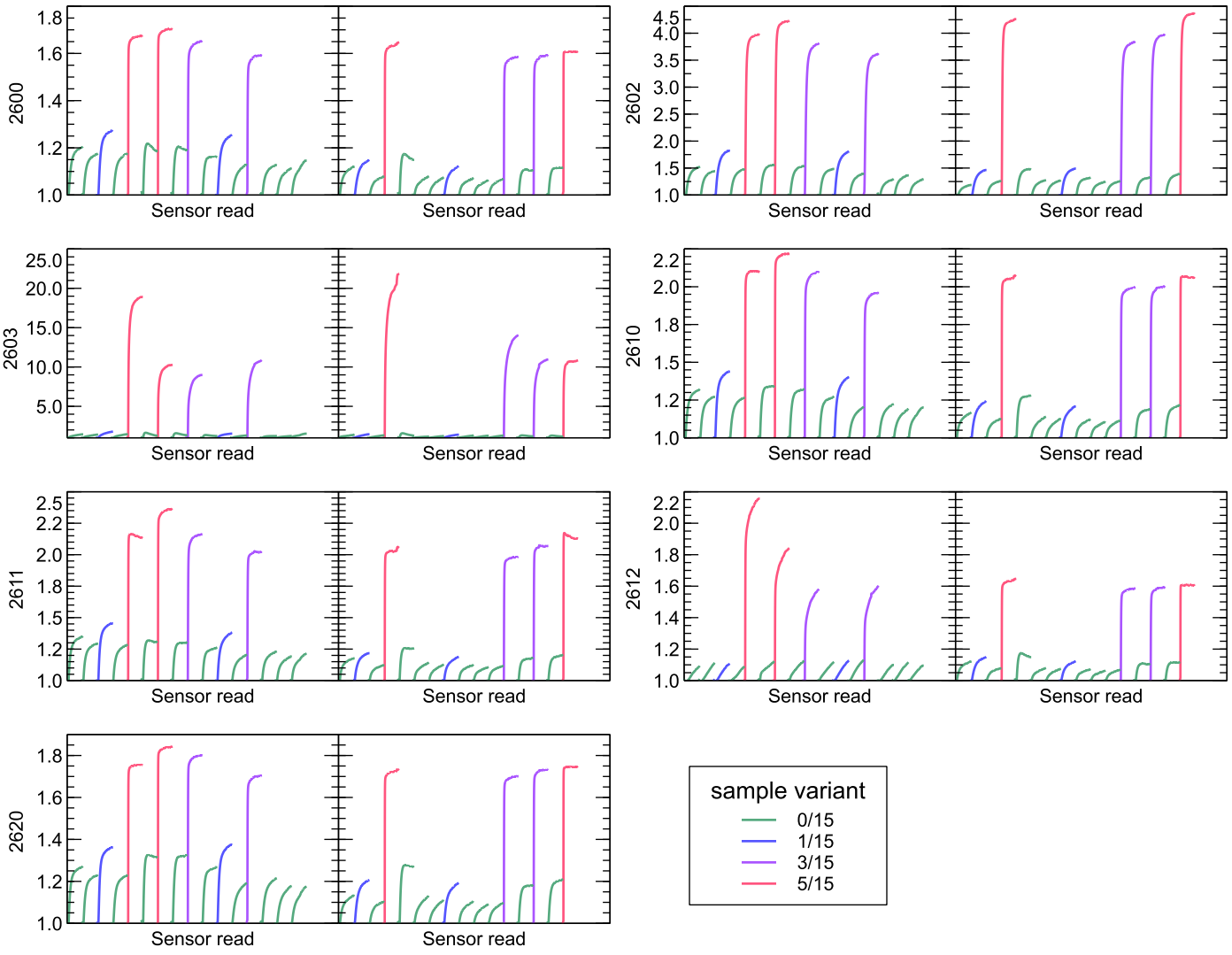

Figure 4. Sensor response collected during two days of the measurements by PW8 electronic nose. The sub-figures on the left represent the first day of the experiment, and on the right, the second day. The data along the x-axis are squeezed, interruptions in the data collection process and the sensor relaxation time are removed. The Y-axis represents the sensor response magnitude normalized by the response in the clean air U/U_0. The sensor type (Table A2) is indicated as the caption of the y-axis.

One can observe in this figure that in both days of the experiment, the characteristics of the sensor response were similar. We have not observed noticeable sensor drift or poisoning, changing the characteristics of the response of the electronic nose.

As a feature allowing to differentiate between the studied samples categories, using the electronic nose response, we used the response at the end of the observation time. As one can notice in the Figure 4 this is the stationary state of the sensor response reached at the end of the gas adsorption phase. Distribution of this characteristics of the sensor's response collected in the experiment are presented in Figure 5.

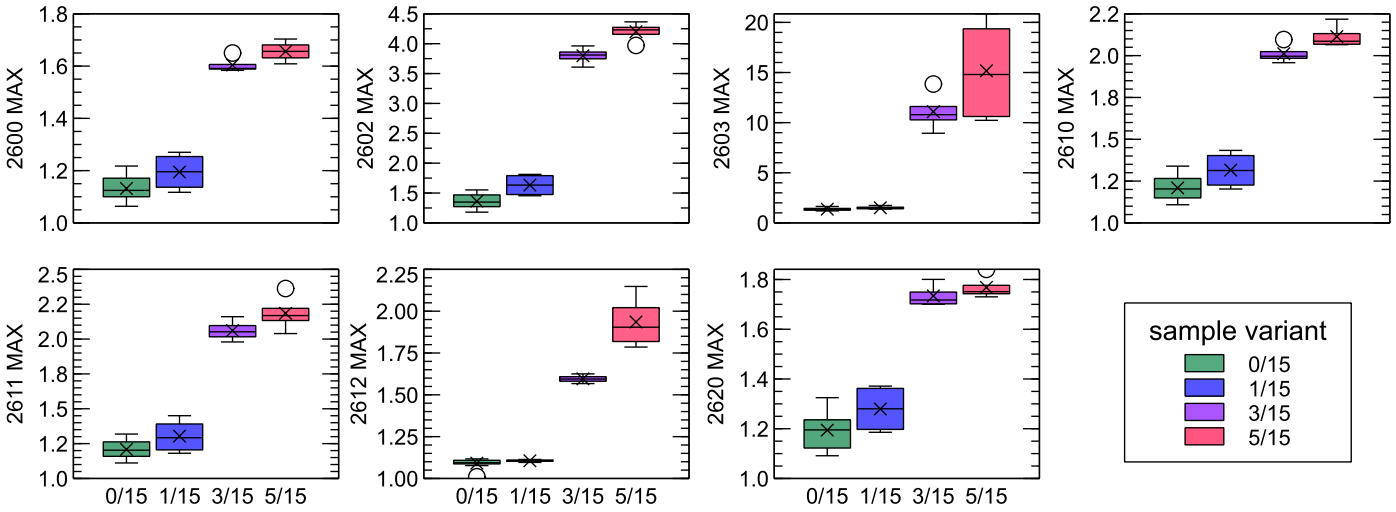

Figure 5. Comparison of the distribution of PW8 electronic nose sensors response versus studied sample variant. The response was measured at the characteristic moment when the maximum magnitude was reached. The sensor type (Table A2) is indicated at the y-axis caption.

In these results, we can observe that all sensors react to the volatile components emitted by the spoiled grain. The reaction to the healthy grain was much smaller and the magnitude of the response expressed as the proportion to the response in clean air conditions was much closer to one. One can also notice that the sensor response to the smallest considered amount of the infected grain (proportion 1/15), which means the smallest amount of produced volatiles and thus the lowest concentration, the response of the sensors was very similar to that observed for the control category. Another pattern in the distribution of data, that could be observed in this figure is very close to the sensor response for the two highest studied categories of samples (3/15 and 5/15), for most of the sensors in the PW8 electronic nose.

These two observed patterns could indicate that the detection of low concentrations of spoiled grain may be difficult by the constructed electronic nose. Also, it may be difficult to estimate the concentration of the spoilage odor since the saturation of the sensor response was observed. However, the former effect may help build a classification model for the detection of spoilage since, above a certain level, the observed pattern of the sensor response is very similar regardless of the concentration.

4. Summary and Conclusions

Both the PEN3 and PW8 e-nose allowed for the differentiation of the tested samples of *Fusarium poae* infected wheat seeds from healthy samples in various proportions.

Measurements taken using the PEN3 e-nose allowed for the differentiation of infected samples from the control sample containing only healthy seeds. A larger proportion of infected seeds in the sample enables a more certain determination of the presence of *Fusarium poae*. In samples with a higher proportion of infected seeds to healthy seeds (3/15 and 5/15), most sensors registered significantly higher levels of response compared to the control sample (0/15) and the sample with the smallest mixing ratio (1/15). All tested

infected samples were distinguishable from healthy samples by all sensors. Therefore, it can be concluded that healthy wheat grains do not emit volatile compounds that cause reactions in the PEN3 sensors (except for the W3C sensor), allowing the identification of *Fusarium poae* infected samples. The extreme sensor response magnitude is a characteristic feature allowing the differentiation of the measured samples. Depending on the direction of the response to the presence of the measured gas, these can be either minimum or maximum values. Recorded values were also generally higher on the second day of measurement.

The results of the measurements with PW8 also made it possible to distinguish between the samples tested. In this e-nose, all sensors reacted to the presence of *Fusarium poae* infected seeds. However, as with PEN3, the smaller the ratio of healthy to spoiled seeds, the stronger the sensor response. In measurements with this e-nose, the smallest seed mixing ratio (1/15) was very similar to the control sample (0/15). This indicates a problem with detecting low concentrations of infected grains. However, with higher proportions of infected seeds relative to healthy grains (3/15 and 5/15), there is no difficulty in detecting the presence of *Fusarium poae*. Recorded values with this e-nose remained rather consistent over the two days of measurements.

The PEN3 proved to be more effective in detecting low concentrations of infected seeds compared to the PW8. Although both e-noses are effective and allow for the detection of *Fusarium poae* infected wheat seeds.

It can also be noted that in the case of both electronic noses, the patterns of the response strongly depend on the concentration of the infected material in the sample. That can be viewed as various odor concentrations. Such a nonlinear response may be a problem for differentiation between the studied samples by the methods of machine learning algorithms of pattern recognition as the required size of the training sample may grow. One can also notice that for stronger concentrations of the odor, the patterns of response registered by the PW8 device are more similar, so the recognition may be more effective.

Overall research demonstrates possible difficulties in early detection of low concentration of grain spoilage by *F. poae* fungi. Also, changes in response patterns with odor concentration, in our opinion, require further studies.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A Electronic Noses Sensors

Table A1. The list of PEN3 electronic nose sensors.

Sensor	Main Gas Targets
W1C	Aromatic organic compounds.
W5S	Very sensitive, broad range sensitivity, reacts to nitrogen oxides, very sensitive with negative signals.
W3C	Ammonia, often used as sensor for aromatic compounds.
W6S	Detects mainly hydrogen gas, selective (breath gases).
W5C	Alkanes, aromatic compounds, and less polar organic compounds.
W1S	Sensitive to methane (environmental). A broad range of organic compounds detected.
W1W	Detects inorganic sulfur compounds, e.g., H_2S. Also sensitive to many terpenes and sulfur-containing organic compounds.
W2S	Detects alcohol, partially sensitive to aromatic compounds, broad range.
W2W	Aromatic compounds, sulfur organic compounds.
W3S	Reacts to high concentrations of methane (very selective).

Table A2. List of sensors of the electronic nose and their target gases and odors [32].

Sensor	Target Detection
TGS 2600	Highly sensitive to low concentrations of gaseous air pollutants such as hydrogen and carbon monoxide, for example in cigarette smoke. Can detect hydrogen in a concentration of several ppm.
TGS 2602	Highly sensitive to low concentrations of odor-intensive gases such as ammonia and H_2S originating from waste materials in office and residential environments. Very sensitive to low concentrations of VOCs such as toluene from wood surfaces and building products
TGS 2603	Very sensitive to low concentrations of odorous gases such as odors from the amine range and sulfurous odors from waste materials or spoiled food such as fish.
TGS 2610	Uses filter material that eliminates the influence of interfering gases such as alcohol and is highly selective for LP gas.
TGS 2611	Uses filter material that eliminates the influence of interfering gases such as alcohol and is highly selective for methane gas.
TGS 2612	Highly sensitive to methane, propane, and butane, targeted for LNG and LPG monitoring. With low sensitivity to alcohol vapors (a typical interference gas in the residential environment), the sensor is often used in consumer market gas alarms.
TGS 2620	Has a high sensitivity for organic solvents and other volatile vapors and is, therefore, suitable for organic vapor detectors/alarms.

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