The use of an Electronic Nose to detect early signs of soft-rot infection in potatoes

Authors: Massimo F. Rutolo[a], John P. Clarkson[b] and James A. Covington[c]*

[a]School of Engineering, University of Warwick, Coventry CV4 7AL, UK;

E-Mail: M.Rutolo@warwick.ac.uk;

[b]Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne, CV35 9EF, UK;

E-Mail: John.Clarkson@warwick.ac.uk;

[C]School of Engineering, University of Warwick, Coventry CV4 7AL, UK;

E-Mail: J.A.Covington@warwick.ac.uk;

*Author to whom correspondence should be addressed;

E-Mail: J.A.Covington@warwick.ac.uk; Tel.: +44(0) 2476 574494; Fax: +44(0) 2476 418922.

Abstract

In this paper we report on the detection of soft-rot in potatoes caused by the bacterium *Pectobacterium carotovorum* through the use of an array of low cost gas sensors. This disease results in significant crop losses in store (circa 5%) with associated negative financial impacts. At present, there is no commercial technological solution for soft rot detection in such stores, with store managers having to regularly inspect large volumes of potatoes. As soft-rot is associated with a strong odour and there is forced air movement through potato stores, our aim was to investigate the potential of an array of low-cost gas sensors to detect the disease. In laboratory conditions, 80 potatoes with and without soft rot (evenly split) were analysed by an array of 11 different gas sensors. These were tested at both pre-symptomatic and symptomatic time points. Results indicated that 100% detection accuracy could be achieved at both time points with only 3 sensors. The identified sensors therefore offer promise for an automated in-store monitoring system.

Keywords: soft rot, potato disease detection, electrochemical gas sensors; nondispersive infrared gas sensors;

1. Introduction

Bacterial soft rot disease caused principally by *Pectobacterium carotovorum* (Czajkowski et al., 2015) causes significant losses in UK potato stores, with approx. 5% of of the crop being destroyed each year (AHDB, 2012). At present, there is no technology available for monitoring this disease in commercial stores, but if soft rot could be detected early, the farmer/store manager can make an informed decision of how best to manage the infected crop (usually by selling into the food or animal feed markets, or by changing the storage conditions). Such early identification is not normally possible as potato stores are very large, the tubers are not easily accessible for visual inspection and the characteristic odour associated with soft rot is only detectable by the store manager when the disease is at an advanced stage. However, we believe that automated detection of soft rot could be achieved through modern gas analysis technology.

This concept is not new, with early work being undertaken by Varns and Glynn (1979) followed by the study of Waterer and Pritchard (1984). These and subsequent studies used either GC (Gas Chromatograph) or GCMS (Gas Chromatograph Mass Spectrometer) in an attempt to identify the specific chemicals that were associated with soft rot (Ratti et al., 1995, Lyew et al. 2005, Kushalappa et al., 2001). This resulted in a large number of different potential biomarkers for the disease being reported; however due to a range of experimental differences, there is no consensus over their identity. This is not unexpected as it has been reported that plants produce around 200,000 volatiles before and after harvest (Dixon et al., 2002, Feihn, 2002).

Though these studies are scientifically interesting, they do not provide a solution for practical disease monitoring in potato stores. GC and GCMS are expensive pieces of equipment that require trained staff and significant infra-structure making them unsuitable for a store environment. However, one alternative technology that could be applied is the so called “electronic nose” or “eNose” – an instrument designed to mimic the biological olfactory system. This instrument is already finding favour in precision agriculture, where there is a growing use of sensors and sensor systems to optimise and improve manufacturing in agriculture and forestry (Wilson, 2013). The eNose is relatively cost effective as it can be formed from an array of low-cost chemicals sensors (sub $50), it uses air as carrier gas, can be produced to be portable (even battery powered) and can provide a simple and quick answer to a chemical identification task. This is in stark contrast to higher-end analytical techniques, such as GC-MS. The number of agricultural applications for eNose that have been studied is considerable, from crop protection, floral odours, ecosystem management to wood management and beyond (Wilson, 2013). In relation to potato soft-rot analysis, there have only been a small number of researchers using eNoses (De Lacy Costello et al., 2001; Biondi, 2014; Sinha et al., 2017). We previously demonstrated that early signs of soft rot infection could be detected using ion mobility spectrometry (specifically using an Owlstone Lonestar, UK) and a commercial electronic nose (AlphaMOS Fox 3000, France; Rutolo et al., 2014, 2016). Both of these studies have shown the potential of gas analysis, but have practical issues. The former, though sensitive, uses a technology that is well beyond the financial reach of the potato industry and also requires the use of clean air and a clean environment to operate. The work with the AlphaMOS system showed that it is possible to achieve similar results with an array of gas sensors. However, this system is no longer available (production stopped in 2016) and the exact manufacturers of the sensors are unknown. In addition, these units are constructed from an array of power-hungry, thick-film metal-oxide gas sensors. This severely limits their use in portable/battery powered applications.

The challenge of developing a dedicated eNose system that can be deployed within a storage setting for the detection of soft-rot therefore still remains. To achieve this, it is important to understand how and which low-cost gas sensors respond to the disease and if they will map onto store environments.

Furthermore, as most gas sensors are designed to detect inorganic gases (unlike previous work which focussed on organic compounds), new insights may be gained relating to the biomarkers released by the soft rotting bacterium itself or products associated with the enzymatic breakdown of the potato tissue (Smadja et al., 2004). Thus, the main aims of this paper were to identify low cost gas sensors that can detect soft-rot disease and which inorganic gases may play an important role as biomarkers for infection.

2. Materials and Methods

2.1 Electronic Nose system

The majority of electronic nose instruments, in either a commercial or research setting, deploy an array of metal-oxide gas sensors, numbering 6 to 32. The reason for this is that metal-oxide sensors historically have had a higher sensitivity to a target gas than other sensors. However, the latest generation of electrochemical sensors are now achieving similar sensitivities, whilst offering many of the advantages of such sensors. Electrochemical gas sensors have found favour within the industrial safety market and more recently in both indoor and outdoor air quality applications (Mead et al., 2014). Their key advantages include being relatively low-cost (under $50 per sensor), ultra-low power consumption (they generate energy as part of the detection process), room temperature operation and good tolerance to environmental changes (specifically changes in temperature and humidity).

Furthermore, in this specific application, they map extremely well onto a low temperature potato store environment. Temperatures as low as 0^{o}C result in a reduction in electrochemical sensor zero current (the output of the sensor when not being presented with a target gas) and results in a lower limit of detection. In addition, these sensors are tolerant to both wide ranges of humidity and to high humidity due to the way they are constructed.

In this study, we used an in-house electronic nose called the WOLF 4.1 (Warwick OLFaction, with the number referring to the instrument being desktop). The nine sensors selected for testing (Table 1) were all from a special group that are commercially available and specifically designed for outdoor air quality monitoring and thus have very high sensitivity. This array was augmented with additional gas sensors to evaluate if other potential low-molecular weight biomarkers could be identified, specifically carbon dioxide and methane/hydrocarbons which cannot be easily detected using electrochemical means. The sensors were mounted inside a large case, which included fluidic components, valves (ETO-12, Clippard, USA) and flow sensors (Honeywell AWM-3300) and a single PC board. The sensors used commercial interface boards (either an ISB or Digital Transmitter Board, AlphaSense, UK) that produce either a voltage or current output. Any currents are converted to an output voltage and then the output of all the sensors was measured by a National Instrument DAQ card (USB-6009). The unit is controlled by a custom written LabVIEW program (version 2015, National Instrument, USA) that allows the sensor data to be stored to a file for later analysis.

2.2 Sample preparation

The potato variety chosen for all experimental work was ‘Maris Piper’, due to its widespread use in the industry. The *P. carotovorum* isolate (SBEU_08) used was originally isolated by Dr Glyn Harper (AHDB Potatoes, Sutton Bridge Crop Storage Research) from an infected potato tuber (variety Marfona) showing characteristic symptoms of bacterial soft rot. In pure culture, it caused pitting in Crystal Violet Pectate agar at 27 °C and identity confirmed as *P. carotovorum* by PCR (*Pectobacterium* specific primer sets courtesy of Dr J. Elphinstone, FERA, UK). A standard procedure was used for inoculating potato tubers with this *P. carotovorum* isolate in order to initiate disease reliably and reproducibly. Potatoes were first soaked in water for one hour before use and dried with a paper

towel. Each tuber was then stabbed at the stolon end with a sterile 200 µl pipette tip. *P. carotovorum* was grown on nutrient agar at 25°C for 48h, after which 2 ml of sterile water was added and the colonies gently scraped using a sterile plastic loop to create a bacterial suspension. This bacterial suspension (20µl) was then used to inoculate individual potato tubers by pipetting into the stab wounds. A further set of healthy control tubers were stabbed at the stolon but not inoculated. After treatment, the potato tubers were placed in sealed plastic boxes at 25±1 °C in an incubator and suspended on a mesh over 400 ml of water, to create warm and high humidity conditions conducive to soft rot disease development. No determination of latent *Pectobacterium* infection was carried out on the potato tubers used, but controls were checked for infection throughout and at the end of the experiment.

2.3 Experimental setup

Prior to sampling, potatoes were placed each in turn into 1 L polytetrafluoroethylene jars (PTFE ; Fisher Scientific Ltd, UK) with inlet and outlet fittings added (1/8" push-fit, Pneu-store, UK) at both ends. The potatoes (both control and infected) were tested individually. Laboratory zero grade air was then flushed into one end of the container and into the electronic nose. The acquisition time was 120 sec, with a start injection of 20 s, injection time of 10 s, and flow rate of 300 mL/min. The use of different PTFE jars, for control or infected tubers, helped reduce cross-interference and all containers were regularly replaced with cleaned ones. For cleaning, the containers were thoroughly sterilized with 70% ethanol, washed with water, dried out and flushed with zero grade laboratory air for circa 5 min. Potato tubers were inoculated either 2 or 5 days prior to sampling and kept at 25°C in the sealed boxes as described above until sampling in the PTFE containers at laboratory temperature (20 ± 2°C), after which they were returned. After 2 days post-inoculation, tubers showed no visible signs of soft rot and hence this allowed the sensors to be evaluated for early pre-symptomatic disease detection. After 5 days post-inoculation, tubers had begun to exhibit both visual and olfactory signs of infection that could be identified by a store manager; this material therefore tested the ability of the sensors to detect soft rot at an advanced symptomatic stage of disease development. Overall, 80 tubers (40 inoculated, 40 uninoculated) were analysed for both the 2 day and 5 day post-inoculation time points (40 tubers per time point).

2.4 Data Analysis

A number of different feature extraction methods were considered and area of the response was found to be the most suitable pre-processing technique for electrochemical and NDIR gas sensors. This area is represented by the response time (exposure to odorant) above the baseline from the first point to the point of maximum response. This approach was chosen since there is a minimal recovery time for these types of sensors and their high selectivity to target chemicals is accurately represented by the area under the curve. For classification, the data were split 75% for training (using 10-folds for cross validation) and 25% for testing (stratified random split technique was used for selection of train and test sets)). Different analysis models were selected based on their diversity and degree of complexity: LDA, or linear discriminant analysis (Fisher, 1936), MARS, or multivariate adaptive regression spline (Friedman, 1991), Classification and Regression Trees (CART; Breiman, 1984), C5.0 (Quinlan, 1993), Naïve Bayes (Kohavi, Kohavi &Becker, 1997), support vector machine, (SVM; Cortes & Vapnik, 1995; Steinwart & Christmann, 2008), ensemble CART and random forests (Breiman, 2001). In order to test the robustness of the various models, confusion matrices metrics were considered. Of particular interest was the conditional metric known as sensitivity, which is the rate at which the event of interest is correctly predicted for all samples in that event. This metric is particularly useful for potential store deployment since if the event of interest were a healthy control tuber, sensitivity would indicate the ability of the model to accurately predict healthy controls, thus disregarding any

other disease (or variation of disease or other confounding factors) in case other metrics were used (such as specificity).

3. Results

Initially, we simply considered the raw voltage output of the instrument in relation to healthy control and infected tubers (at both time points and without any features extraction). Figure 1 shows a typical instrument response to an infected tuber. Here the output of the sensors is displayed as an output voltage. Figure 2 shows the magnitude of the averaged sensor responses. From visual inspection, significant differences between the infected and control tubers could be identified at both the 5 day symptomatic time point and the earlier (pre-symptomatic) 2 day time point when there were no visual indications of disease.

Features were then extracted from the raw sensor responses and a principal component analysis (PCA) undertaken using these extracted features for all 11 sensors (Figure 3). The plot shows that healthy control samples could be distinguished from infected tubers at both pre-symptomatic and symptomatic time points for the majority of cases. In addition, the analysis suggests that sensor output at the symptomatic time point is greater than at the pre-symptomatic time point.

Following evaluation of the loading values (data not shown) and the raw sensor responses (Figure 2), a small number of sensors could be identified that provided most of the variance in the PCA plot. These sensors were CO (carbon monoxide), ETO (ethylene oxide) and NO (nitric oxide). Further PCA analysis with just these three sensors (Figure 4) showed that there were again clear differences between the infected and healthy control groups, with a similar separation as for the 11 sensor analysis (Figure 3).

Machine learning models were then applied to the data. The data set was partitioned into stratified random splits (75% for training and 25% for testing) and CV (cross validation) was carried out with a k fold of 10. After training, the generated models were evaluated with the test data set. Results indicated a sensitivity of 100 % across many models for all the sensors comprising the original array and also for the selected subset (with the exception in this latter case of lower percentage for the C5 algorithm). In table 2 are reported the values for sensitivity (number of samples with event and predicted to have the event of interest / number of samples with the event of interest) and specificity (number of samples without event and predicted as 'non-events' / number of samples without the event of interest) metrics of the confusion matrices for the selected time points for both all and the shortlisted sensor subset (carbon monoxide, ethylene oxide and nitric oxide). This indicates that all techniques with similar initial conditions and pre-processing can be employed as suitable models for the data set comprising the selected set of sensors. However, a lesser degree of model performance was found for the C5 algorithm, indicating that other sensors may contribute in minor part to disease identification when this technique is employed.

4. Discussion

The United Nations Food and Agriculture Organization estimates that between 40 to 50 % of root and tuber crops, fruits and vegetables produce is wasted each year (FAO, 2013). In the UK, one of the major losses of potato tubers in store is due to bacterial soft rot caused by *Pectobacterium* spp. In this paper, we used an in-house electronic nose instrument (WOLF 4.1) to test an array of commercially available low-cost sensors based on electrochemical and optical detection methods and identify those that have good potential for early detection of soft rot in potato stores.

Results indicated that soft rot could easily be detected at both pre-symptomatic and symptomatic stages of infection. In addition, almost the same selectivity could be achieved with just three sensors, specifically carbon monoxide, ethylene oxide and nitric oxide. Interestingly, the separation of disease and control samples was slightly better with all the sensors over the subset. This suggests that the rest of the sensor array provides a small amount of additional information that can aid separation of the diseased and healthy sample datasets.

One potential limitation of this study is that experiments were carried out at a higher temperature, at lower humidity and higher chemical concentrations than are routinely found in potato stores where conditions of 3-10°C and >90% RH are maintained to extend storage times and prevent the tubers drying out (Cunnington & Pringle, 2012). However, the electrochemical sensors used in this study map well onto this store environment. Lower temperatures will result in higher sensitivities (through lower zero current) and these sensors are either tolerant to very high humidity levels (as they are constructed with a humidity barrier) or can have their electrolyte concentration altered to make them tolerant. In terms of the sensitivity required, potato tubers are stored in large volumes, either loose or in 1 tonne boxes, with a store containing many hundreds of tons of tubers. This will therefore result in a substantial dilution of the chemical biomarker components. However, as these sensors were developed for environmental monitoring applications, we believe they will have the required sensitivity as their detection limits are as low as single figure parts per billion for most molecules. These questions related to practical application are currently being addressed in the next phase of this work which is focussed on in-store experimentation.

None of the chemicals detected above (carbon monoxide, ethylene oxide and nitric oxide) have been identified previously as being associated with soft rot disease in previous studies with GC-MS. Of specific interest is the substantial sensor response to carbon monoxide uniquely associated with diseased tubers. This raises an additional issue of health and safety in food storage. It is also interesting to note the lack of responses to the two NDIR sensors, namely carbon dioxide and methane. Carbon dioxide is known in the industry as being associated with tuber respiration and is monitored in store. The lack of response for CO_2 could be caused by the experimental technique adopted here (dynamic headspace sampling) and further experiments in store will help answer this question. At this stage of the research, it is still not clear what metabolic processes might be involved with the chemical compounds detected by the sensors.

5. Conclusions

In this paper, we report on the use of gas analysis equipment to detect and investigate odours associated with bacterial soft rot of potatoes. Past research on the early detection of potato storage diseases by gas analysis has been conducted over many years, dating back to the 1970s. However, there is currently still no cost effective, non-destructive, reliable and practical approach for soft rot detection in commercial storage facilities. In previous work, the authors presented the case for the use of a commercial electronic nose, using metal-oxide sensors for soft rot detection. Here we have identified other gas sensing technologies that could be employed as a viable solution for deployment in store. Electrochemical and infrared commercial technologies offer the advantage of a high degree of selectivity and fast response time to the chemical of interest. The results show that a subset of these sensors, namely carbon monoxide, ethylene oxide and nitric oxide can be employed for both the symptomatic and pre-symptomatic detection of soft rot under laboratory conditions.

252 Acknowledgments

253 The authors wish to acknowledge and thank the AHDB Potatoes for supporting this work (AHDB
254 Potatoes project reference R461). We also thank Claire Handy and Alison Jackson at Warwick Crop
255 Centre for technical support.

256
257

References:

AHDB Potato Council. *War on waste in the potato supply chain*. http://www.potato.org.uk/news/war-waste-potato-supply-chain, June 2012.

Biondi, E., Blasioli, S., Galeone, A., Spinelli, F., Cellini, A., Lucchese, C., & Braschi, I. (2014). Detection of potato brown rot and ring rot by electronic nose: From laboratory to real scale. *Talanta, 129*, 422-430.

Breiman, L., Friedman, J. H., Olshen, R. A., & Stone, C. J. (1993). *Classification and regression trees*. Chapman Hall, New York.

Breiman, L. (2001). Random forests. *Machine learning*, *45(1),* 5-32.

Cortes, C., & Vapnik, V. (1995). Support-vector networks. *Machine learning, 20(3),* 273-297.

Cunnington A. & Pringle R. (2012). *AHDB Potato Council Store Managers Guide*. AHDB.

Czajkowski, R., Pérombelon, M. C. M., Jafra, S., Lojkowska, E., Potrykus, M., Van Der Wolf, J. M., & Sledz, W. (2015). Detection, identification and differentiation of Pectobacterium and Dickeya species causing potato blackleg and tuber soft rot: a review. *Annals of Applied Biology, 166(1)*, 18-38.

De Lacy Costello, B. P. J., Evans, P., Ewen, R. J., Gunson, H. E., Jones, P. R., Ratcliffe, N. M., & Spencer-Phillips, P. T. (2001). Gas chromatography–mass spectrometry analyses of volatile organic compounds from potato tubers inoculated with Phytophthora infestans or Fusarium coeruleum. *Plant Pathology, 50(4),* 489-496.

Dixon, R. A., Achnine, L., Kota, P., Liu, C.-J., Reddy, M. S. S. & Wang, L. (2002). The phenylpropanoid pathway and plant defence-a genomics perspective. *Mol. Plant Pathol., 3,* 371–90.

Fiehn, O. (2002). Metabolomics – the link between genotypes and phenotypes. *Plant Mol. Biol., 48,* 155–171.

Fisher, R. A. (1936). The use of multiple measurements in taxonomic problems. *Annals of eugenics, 7(2)*, 179-188.

FAO (2013). Global initiative on food losses and waste reduction. http://www.fao.org/docrep/018/i3300e/i3300e00.htm

Friedman, J. H. (1991). Multivariate adaptive regression splines. *The annals of statistics,* 1-67.

Kohavi, R., Becker, B., & Sommerfield, D. (1997). Improving simple bayes.

Kushalappa, A. C. & Zulfiquar, M. (2001). Effect of wet incubation time and temperature on infection, and of storage time and temperature on soft rot lesion expansion in potatoes inoculated with Erwinia carotovora ssp.carotovora*. Potato Res., 44*, 233–242.

Lyew, D., Gariépy, Y., Raghavan, G. S., & Kushalappa, A. (2001). Changes in volatile production during an infection of potatoes by Erwinia carotovora. *Food Res. Int*., *34*, 807–813.

Mead, M.I., Popoola, O.A.M., Stewart, G.B., Landshoff, P., Calleja, M., Hayes, M., Baldovi, J.J., McLeod, M.W., Hodgson, T.F., Dicks, J. & Lewis, A. (2013). The use of electrochemical sensors for monitoring urban air quality in low-cost, high-density networks. *Atmospheric Environment, 70*, 186-203.

Patel, H. K. (2014). *The electronic nose: artificial olfaction technology*. Springer India.

Persaud, K., & Dodd, G. (1982). Analysis of discrimination mechanisms in the mammalian olfactory system using a model nose. *Nature*, *299*, 352-355.

Quinlan, J. R. (2014). *C4. 5: programs for machine learning*. Elsevier.

Ratti, C, Y Gariépy, G. R. (1995). Collection and analysis of headspace volatiles for disease detection in stored potatoes. *Proceedings of the International Conference on Harvest and Postharvest Technologies*. 255–261.

Röck, F., Barsan, N., & Weimar, U. (2008). Electronic nose: current status and future trends. *Chemical reviews*, *108(2),* 705-725.

Rutolo, M., Covington, J. A., Clarkson, J., & Iliescu, D. (2014). Detection of potato storage disease via gas analysis: a pilot study using field asymmetric ion mobility spectrometry. *Sensors, 14(9),* 15939-15952.

Rutolo, M. F., Iliescu, D., Clarkson, J. P., & Covington, J. A. (2016). Early identification of potato storage disease using an array of metal-oxide based gas sensors. *Postharvest Biology and Technology, 116*, 50-58

Sinha, R., Khot, L. R., Schroeder, B. K., & Si, Y. (2017). Rapid and non–destructive detection of Pectobacterium carotovorum causing soft rot in stored potatoes through volatile biomarkers sensing. *Crop Protection, 93,* 122-131.

Smadja, B., Latour, X., Trigui, S., Burini, J. F., Chevalier, S., & Orange, N. (2004). Thermodependence of growth and enzymatic activities implicated in pathogenicity of two Erwinia carotovora subspecies (Pectobacterium spp.). *Canadian Journal of Microbiology, 50(1),* 19-27.

Steinwart, I., & Christmann, A. (2008). *Support vector machines*. Springer Science & Business Media.

Varns, J. L., & Glynn, M. T. (1979). Detection of disease in stored potatoes by volatile monitoring. *American Journal of Potato Research*, *56(4),* 185-197.

Waterer, D. R., & Pritchard, M. K. (1984a). Monitoring of volatiles: A technique for detection of soft rot (Erwinia carotovora) in potato tubers. *Canadian Journal of Plant Pathology, 6(2),* 165-171.

Waterer, D. R., & Pritchard, M. K. (1984b). Volatile monitoring as a technique for differentiating between E. carotovora and C. sepedonicum infections in stored potatoes. *American Journal of Potato Research, 61(6)*, 345-353.

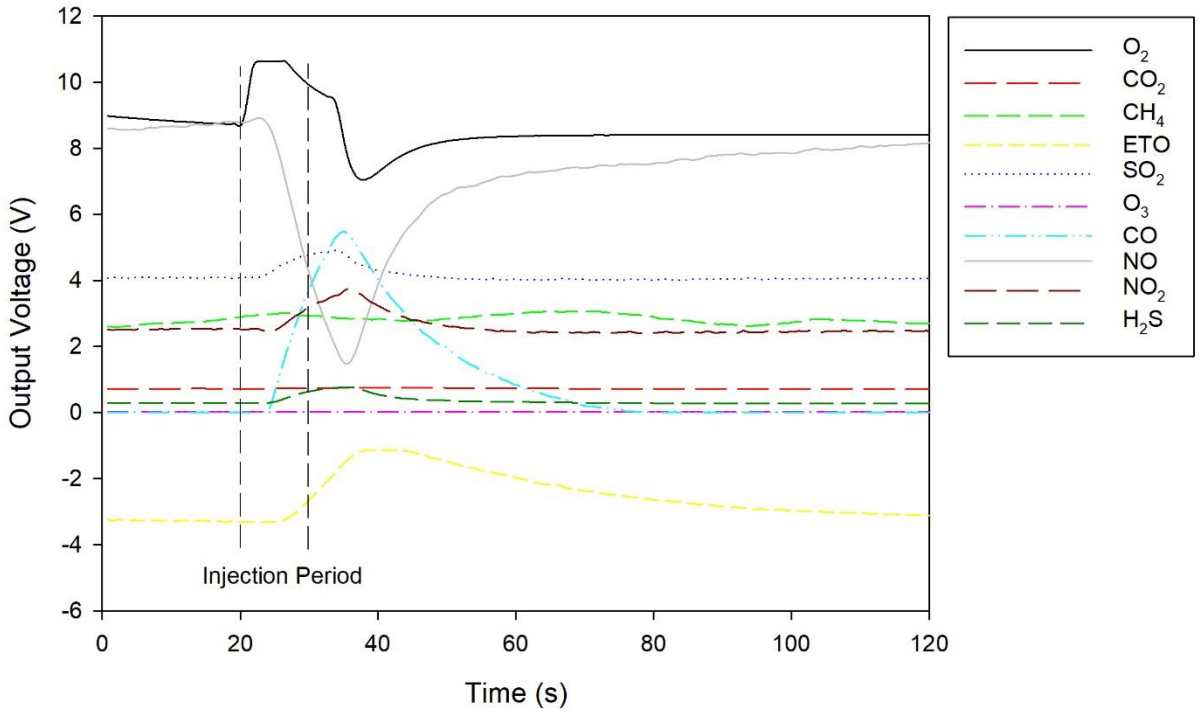
Wilson, A. D. (2013). Diverse applications of electronic-nose technologies in agriculture and forestry. *Sensors*, *13(2),* 2295-2348.

Sensor Name	Manufacturer	Responsive to:	Type of sensor
Cirius CH4 NDIR	Clairair Ltd	Methane	NDIR (Nondispersive infrared)
Cirius CO2 NDIR[1]	Clairair Ltd	Carbon Dioxide	NDIR (Nondispersive infrared)
CO-B4	AlphaSense Ltd	Carbon Monoxide	Electrochemical
ETO-A1	AlphaSense Ltd	Ethylene Oxide	Electrochemical
H2-AF	AlphaSense Ltd	Hydrogen	Electrochemical
H2S-B4	AlphaSense Ltd	Hydrogen Sulphide	Electrochemical
NO-B4	AlphaSense Ltd	Nitric Oxide	Electrochemical
NO2-B4	AlphaSense Ltd	Nitrogen Dioxide	Electrochemical
O2-A2	AlphaSense Ltd	Oxygen	Electrochemical
OX-B431	AlphaSense Ltd	Ozone	Electrochemical
SO2-B4	AlphaSense Ltd	Sulphur Dioxide	Electrochemical

Table 1: Chemical Sensors used inside the WOLF 4.1 instrument for detection of potato soft rot.

Model									Sensor Array
Accuracy Measures (95 % Confidence Interval)	LDA	C5	CART	MARS	Naive Bayes	SVM (Radial Basis)	RF - Ensemble	CART - Ensemble	
Symptomatic									
Sensitivity	100%	80%	100%	100%	80%	100%	100%	100%	All
Specificity	100%	100%	100%	100%	100%	100%	100%	100%	
Sensitivity	100%	60%	60%	100%	80%	100%	100%	80%	Selected
Specificity	80%	100%	100%	100%	100%	100%	100%	100%	
Pre-Symptomatic									
Sensitivity	100%	60%	80%	80%	80%	80%	100%	80%	All
Specificity	100%	100%	100%	100%	100%	100%	100%	100%	
Sensitivity	100%	80%	80%	80%	60%	80%	80%	80%	Selected
Specificity	100%	100%	100%	100%	100%	100%	100%	100%	

Table 2: Confusion matrices metrics for symptomatic and pre-symptomatic detection time points for all sensors and the selected sensor subset (CO, ETO, NO).



343

344 Figure 1: Raw sensor response of sensors within the WOLF 4.1 instrument to a potato tuber infected
345 with *P. carotovorum*. Sample injection occurred after 20 seconds and the injection period was 10
346 seconds. The legend refers to the target gas as specified by the supplier.

347

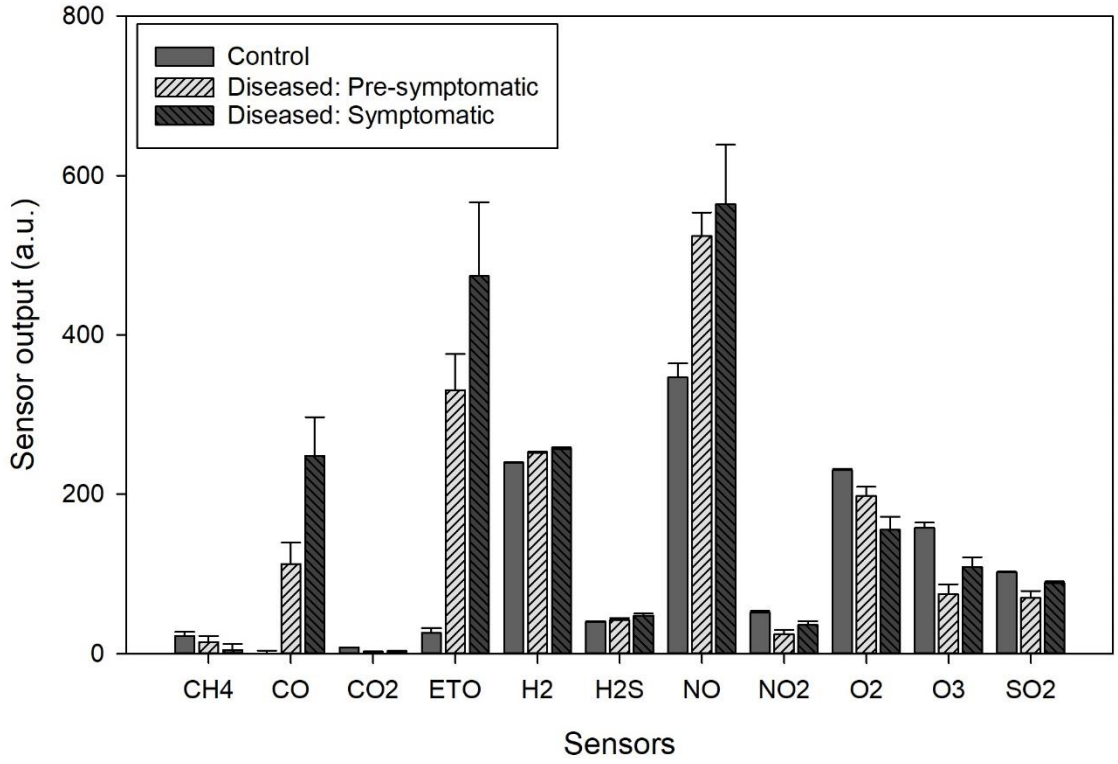


Figure 2: Bar graph showing the averages of the raw sensor responses for healthy control tubers (sample size 40) and tubers infected with *P. carotovorum* at 2 days (pre-symptomatic – sample size 20) and 5 days (symptomatic – sample size 20) time points.

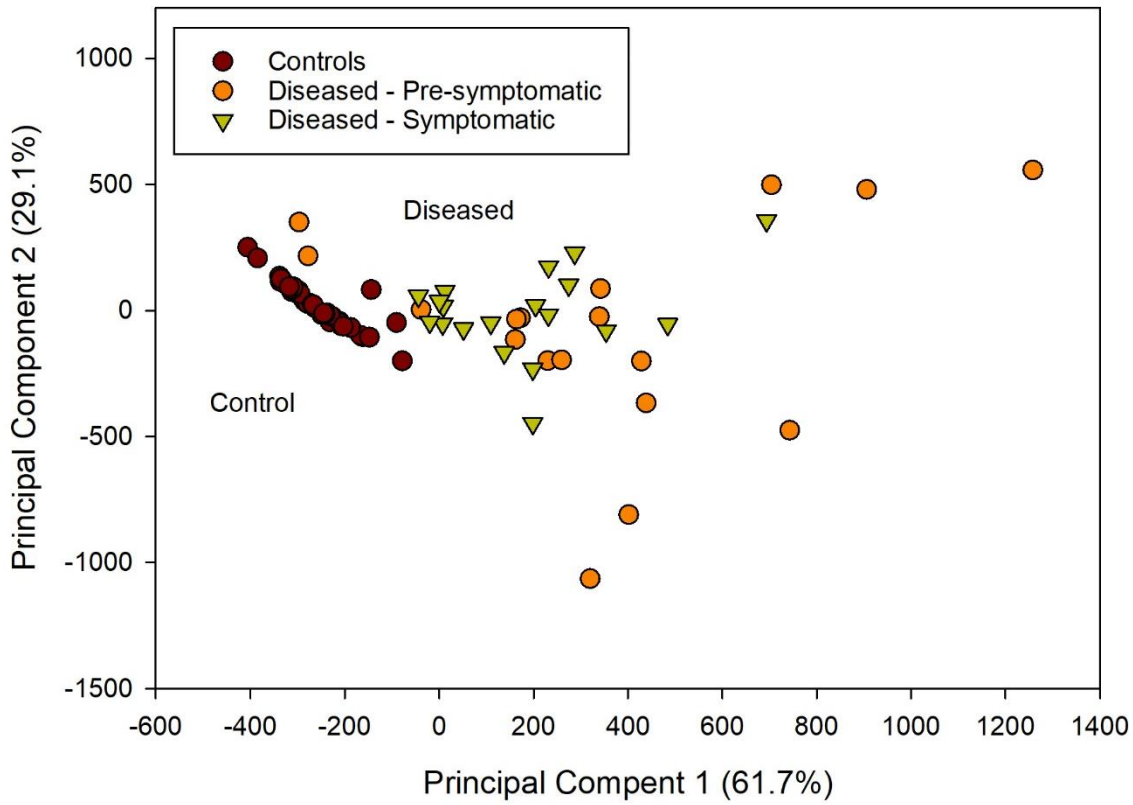


Figure 3: PCA of the sensor responses from the WOLF 4.1 for healthy control tubers and tubers infected with *P. carotovorum* at 2 days (pre-symptomatic) and 5 days (symptomatic) time points.

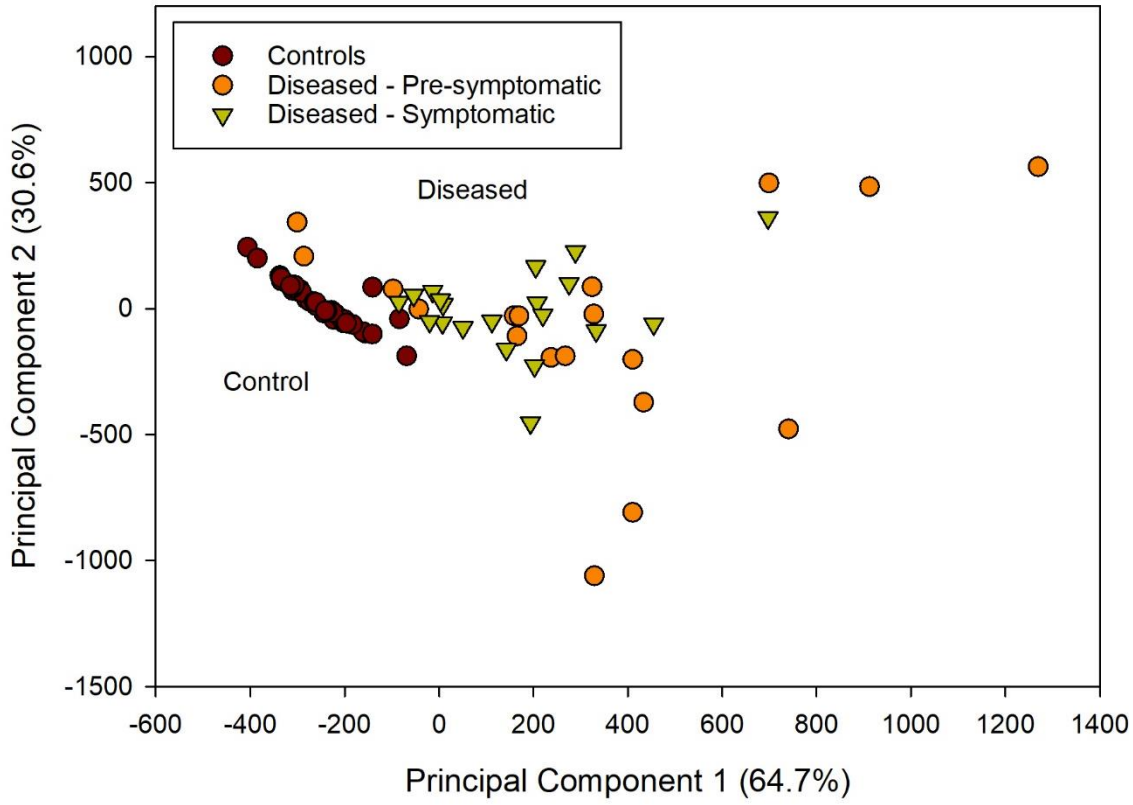


Figure 4: PCA of the sensor responses for CO, ETO and NO sensors for healthy control tubers and tubers infected with *P. carotovorum* at 2 days (pre-symptomatic) and 5 days (symptomatic) time points.