Title: The use of gas phase detection and monitoring of potato soft rot infection in store

Authors

Massimo F. Rutolo [a,*], John P. Clarkson [b], Glyn Harper [c] and James A. Covington [d]

[a] School of Engineering, University of Warwick, Coventry CV4 7AL, UK;
E-Mail: M.Rutolo.1@warwick.ac.uk;

[b] Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne, Warwick CV35 9EF, UK
E-Mail: John.Clarkson@warwick.ac.uk;

[c] AHDB Sutton Bridge Crop Storage Research, Eastbank, Sutton Bridge, Spalding PE12 9YD, UK
E-Mail: Glyn.Harper@ahdb.org.uk

[d] 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 (M.F.R.); Tel.: +44(0) 2476 523523; Fax: +44(0) 2476 418922.

Abstract

Soft rot caused mainly by the bacterium *Pectobacterium carotovorum* is a major cause of potato post-harvest storage losses. This work reports on pre-symptomatic detection and monitoring of soft rot under laboratory and commercial research store conditions by means of an array of gas

sensors (specifically metal oxide, electrochemical, photoionization and non-dispersive infrared).

Two different types of time course experiments were completed. The first set of experiments, under laboratory conditions, evaluated a prototype instrument with various representative sample types for different stages of disease progression in potato tubers. This allowed for optimisation of all parameters for subsequent testing in a potato store. The second set of experiments evaluated an optimised sensor array for soft rot monitoring under realistic potato store conditions. Results showed that a number of gas sensors could detect and monitor early soft rot development with considerable accuracy.

Keywords: gas sensors; potato tuber, soft rot; disease detection

1. Introduction

Post-harvest diseases of potato tubers are a major problem for the industry, with bacterial soft rot being particularly prevalent. In the UK, the bacteria most often associated with soft rot are *Pectobacterium carotovorum* ssp. and *Pectobacterium atrosepticum* (Czajkowski et al., 2011; Czajkowski et al., 2015). The term 'soft rot' is applied to potatoes in store, whilst 'blackleg' is generally employed for the symptoms caused by *Pectobacterium* spp. (and some other bacteria) in the growing crop, where infection causes blackening of the plant stems (AHDB, 2013).

In the UK, following harvest, potato tubers can be stored for up to nine months in purpose-built storage facilities. Potatoes may be stored in boxes or in bulk piles on the floor. Monitoring the disease status throughout the stores is very difficult due to the extremely limited access to the potatoes.

However, to extend the storage life of the potatoes and decrease the incidence of post-harvest diseases, stores are generally environmentally controlled with forced air ventilation around the tubers. This system allows the opportunity for disease monitoring through gas / chemical analysis of the circulating air. The monitoring and early detection of infected potatoes within a commercial storage facility would facilitate prompt action by store managers to remove the diseased material and prevent further spread.

Academic work related to the analysis of gas / volatile organic compounds (VOC) associated with potato soft rot dates back to the 1970s (Varns and Glynn, 1979; Varns and Shaw, 1973) and 1980s (Waterer and Pritchard, 1984a, 1984b) while later work was also carried out in the early part of the century (de Lacy Costello et al., 2001, 1999, 1996; Kushalappa and Zulfiquar, 2001; Kushalappa et al., 2002; Lui et al., 2005; Lyew et al., 2001, 1999; Ratti et al., 1995). These research studies utilised either gas chromatography (GC) or gas chromatograph mass spectrometry (GC-MS), the gold standard for such analysis. In some of these studies, potential bio-markers associated with disease inception and progression were reported, while in others the overall VOC concentration was considered as a possible discriminating factor between healthy and soft rot affected tubers. However, the high purchase / running costs, manual processing of samples, complex data sets, and labour intensive / time consuming processes make GC / GC-MS unsuitable for continuous monitoring in commercial potato stores (Jansen et al., 2011).

Previously, we tested a range of alternative gas sensing technologies for detection of potato soft rot caused by *Pectobacterium spp*. These included field asymmetric ion mobility spectrometry (Rutolo et al., 2014), and more recently an electronic nose formed from an array of metal-oxide gas sensors (Rutolo et al., 2016). Similar work has been undertaken with the electronic nose in the agricultural sector (Li et al., 2010; Torri et al., 2010; Wilson, 2013), including some

research on potato soft-rot (de Lacy Costello *et al* 2000*;* Biondi *et al.*, 2014). Though the electronic nose is still not cost effective for this application with retail prices exceeding $10k, it is constructed from an array of relatively low-cost commercial gas sensors.

The purpose of this work was to evaluate the potential of different gas sensors for pre-symptomatic detection and monitoring of potato soft rot. This would enable the production of a low-cost engineering solution formed from a small number of cheap gas sensors, which would then be economic to deploy widely in a commercial potato storage setting. The objectives of the research were; 1) to design, manufacture and test a bespoke instrument based on different gas sensing technologies (metal-oxide, electrochemical, no-dispersive infrared and photoionization detection) based on previous experimental work, 2) to assess which gas sensors could detect and discriminate soft rot development from healthy tubers over time in controlled laboratory conditions and 3) to determine which gas sensors could detect pre-symptomatic soft rot and monitor disease progression over time in an experimental commercial potato store facility.

2. Materials and Methods

2.1 Experimental laboratory work

2.1.1 Sample preparation

In all experiments, tubers from the potato variety 'Maris Piper' were used due to its widespread use and value to the UK industry. To ensure they were all healthy, they were closely visually inspected for any signs of bacterial or fungal infection before use. To initiate soft rot disease, tubers were first soaked in water for approx. 1 h after which they were inoculated with a suspension of *P. carotovorum* (isolate SBEU_08). This was done by growing the pathogen on nutrient broth at 25 °C for 24-48 h to create a bacterial suspension and pipetting 40 µl of this suspension into a stab wound (1 cm deep, made with a 200 µl pipette tip) at the stolon end of each tuber. In a first set of laboratory experiments, inoculated potatoes were suspended on a mesh over water (400 mL) in sealed 4 L plastic boxes to maintain high humidity and incubated at 25 °C to create conditions for rapid disease progression. In a second set of experiments, tubers were suspended on an acrylic mesh over water (400 mL) in 9 L plastic containers for 4 h postinoculation and prior to sampling (Addis Housewares Ltd, 2016), at circa 25 °C (room temperature) with a gas path inlet and outlet added via 1/8" push-fits (SMC Pneumatics Ltd, 2016) as indicated in Fig. 1.

2.1.2 Sampling protocol

For the experimental work a bespoke instrument was developed and then deployed in either the laboratory (Fig. 1) or in the storage facility (Fig. 2, C). The instrument was constructed with the sensors listed in Table 1 and in both cases the instrument was set up for continuous monitoring (without a clean air cycle). For the laboratory work, three types of tuber treatments were set up; 1) an unwounded control (no stab wound, no *P. carotovorum*, 2) a wounded control (stab wound, no *P. carotovorum*) and 3) infected (stab wounded and inoculated with *P.*

carotovorum). Tubers were arranged in four 9 L plastic boxes as follows: box 1, 5 tubers with treatment 1 (unwounded controls); box 2, one tuber with treatment 2 (wounded control) and four with treatment 1; boxes 3 & 4, one tuber with treatment 3 and four with treatment 1. The laboratory background air was also sampled by the instrument before potatoes were added to each box in order to ensure that there were no external contaminants that would affect the results. The use of the different control treatments allowed for the potential effect of wounding on gas / VOC profile to be assessed while the two infected tuber treatments allowed for some variability in soft rot progression to be assessed. Air sampling was carried out for each container with a flow rate of 330 mL/min and the mixture of air and emissions from each sample fed to the purpose-built instrument for data collection.

After some initial optimisation of sample interval, two sets of experiments were carried out. In the first, the tubers were placed in sealed plastic boxes at 25 °C for 24 h before being moved into the 9 L containers for sampling. This allowed some disease progression to take place before gas analysis. In the second, the tubers were placed immediately in the sealed 9 L containers at 25 °C following inoculation and sampling started 4 h later. In both cases, gas / VOC sampling was continued for 7 d after which inoculated potatoes had clear soft rot symptoms. Data for each sample type were collected at 10 min intervals. At the end of each experiment, plastic containers were thoroughly cleaned, sterilized and baked in an oven at 50 °C for 3 h in order to remove any potential contaminating odours.

A B

Fig. 1. Laboratory setup. (A) Environmental gas analyser connected to each of the four plastic containers via PTFE tubing. (B) Inside of each container with acrylic mesh on which tubers were suspended over water bath to maintain high humidity.

Gas/VOC detected	Part Number	Manufacturer	Gas sensor type[1]
Alcohols	TGS2620	Figaro Engineering Inc	MOX
Ammonia	SP-53B-00	FIS Inc	MOX
Carbon Monoxide	CO-A4	Alphasense Ltd	EC
Carbon Dioxide	Cirius 2	Clairair Ltd	NDIR
Ethylene Oxide	ETO-A1	Alphasense Ltd	EC
Hydrocarbons	TGS2611-C00	Figaro Engineering Inc	MOX
Methane	TGS2611-E00	Figaro Engineering Inc	MOX
Nitric oxide	NO-A1	Alphasense Ltd	EC
Temperature/RH	SHT15	Sensirion AG	-
VOCs (non-specific)	TGS2602	Figaro Engineering Inc	MOX
VOCs (non-specific)	PID-AH	Alphasense Ltd	PID

Table 1. List of sensors tested in this study. [1]MOX (metal oxide), EC (electrochemical), NDIR (non-dispersive infrared), PID (photoionization detection).

2.2 Experimental storage facility work

2.2.1 Sample preparation

As before, the 'Maris Piper' potato variety was used and inoculated in the same way as described in 2.1.1 except that here 25 µL of *P. carotovorum* suspension was applied to each of three 1 cm deep stab wounds per tuber. Inoculated potato tubers were then incubated in 30 L sealed plastic boxes at 15 °C and 95 %R.H. in a store room for 4 d to allow some disease development.

2.2.2 Sampling protocol

Four 1 tonne wooden boxes of potatoes (Maris Piper) were placed within a 56 m^3 store room located at the AHDB Sutton Bridge Crop Storage Research (SBCSR), Lincolnshire, UK (Fig. 2) and the room slowly brought to 95 %RH and 15 °C. Tubers had previously been treated with an experimental treatment to control sprouting (SmartBlock®, AMVAC Chemical Corporation Inc, 2016). Air sampling was carried out every 10 min under these conditions for 7 d. After this period, tubers (approx. 40 kg) each infected with *P. carotovorum*, as described in 2.2.1, were distributed between four plastic crates which were arranged in two stacks of two crates adjacent to the 1 tonne boxes (Figure 2, C). Air sampling (10 min intervals) was then continued for a further 21 d.

A **B** **C**

Fig. 2. (A) Potato storage rooms at AHDB Sutton Bridge Crop Storage Research; (B), storage room employed for experiments; (C), tubers infected with *P. carotovorum* in crates (left-hand

side) next to four 1 tonne potato boxes (right-hand side) with gas analysis instrument in background.

3. Results

3.1 Experimental laboratory work

In the first set of experiments, where tubers were pre-incubated at 25 °C for 24 h before sampling, all of the sensors (Table 1) could distinguish tubers inoculated with *P. carotovorum* from the background laboratory environment, unwounded healthy and wounded healthy potato controls with the exception of the NO and ETO sensors, potentially due to a lack of sensitivity. This was observed as an increasing sensor response over time, corresponding to an increase in gas / VOC concentration as illustrated for the carbon monoxide sensor in Fig. 3. The change in output signal for all sensors was plotted as a function of fractional response (defined as the change in sensor output voltage before and after exposure divided by the initial sensor output voltage) to allow all the sensors with different baseline values to be visualised on the same graph (Fig. 4). This indicated that many of the sensors followed the same trend of increasing response over time. Results also showed that the response of the methane sensor was very similar to that of the hydrocarbons sensor (data not shown) which could indicate that a common chemical is being detected by both sensors and is likely to be methane, or a similar lightweight hydrocarbon. This could include ethane which has previously been associated with soft rot in potato (Lui et al., 2005).

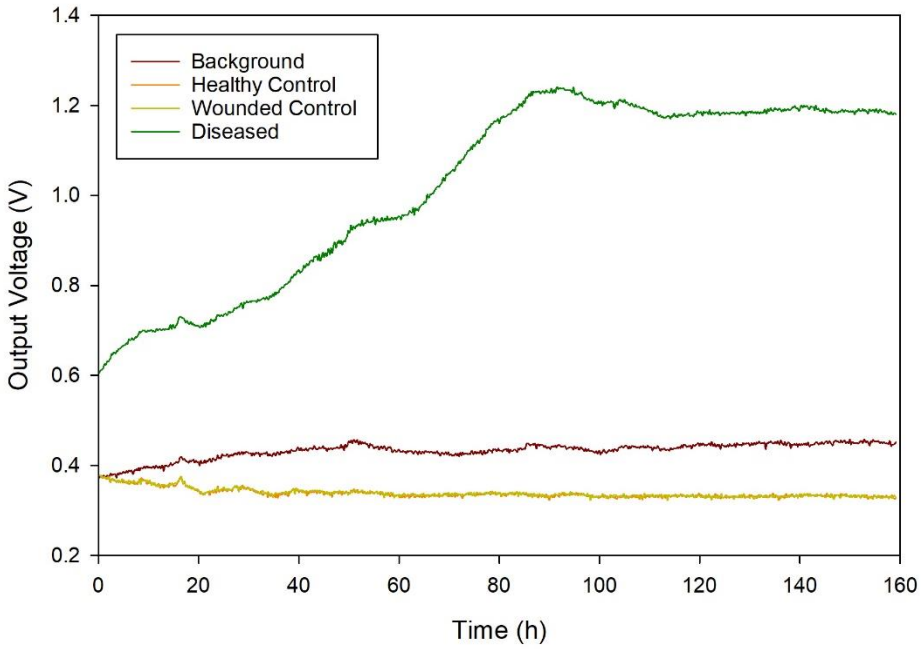


Fig. 3. Carbon monoxide electrochemical gas sensor response over time for potatoes inoculated with *P. carotovorum* (diseased) and healthy control treatments pre-incubated at 25 °C for 24 h before sampling.

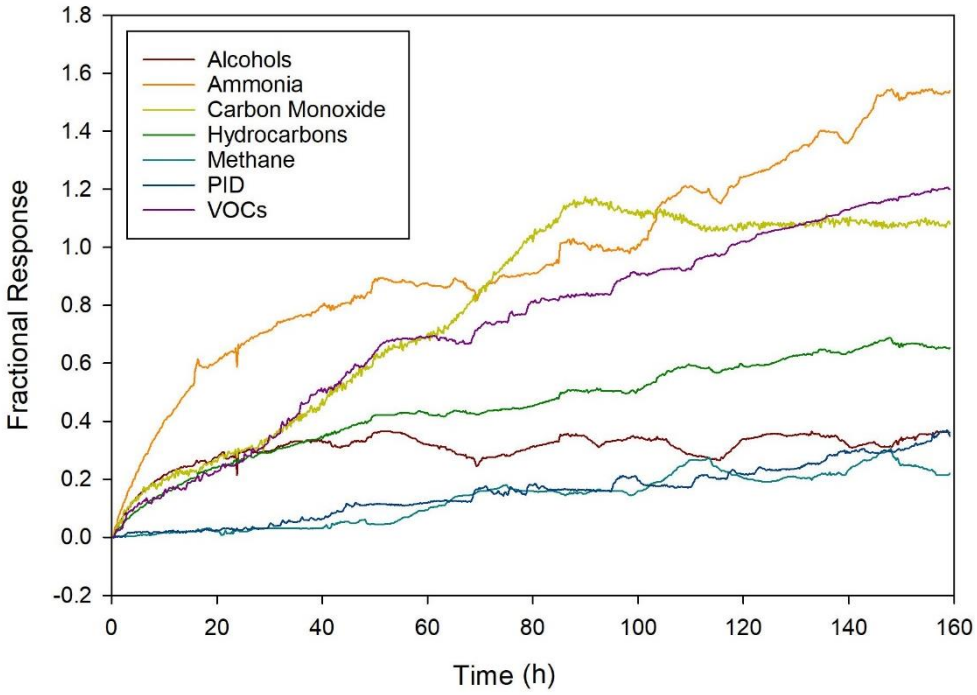


Fig. 4. Gas / VOC sensor outputs (by target molecule) over time for potatoes inoculated with *P. carotovorum* (diseased) and healthy control treatments pre-incubated at 25 °C for 24 h before sampling.

In the second set of experiments, where tubers were sampled from just 4 h post-inoculation with *P. carotovorum* before sampling, a similar increase in sensor output was observed as illustrated for the CO electrochemical sensor in Fig. 5. As before, when the change in output signal for all sensors was plotted as a function of fraction response, sensors to detect alcohols, ammonia, hydrocarbons and methane gave a similar increasing response. Interestingly, methane would not be detected by the PID sensor (due to the specific ionization potential of the PID lamp employed here, i.e. 10.6 eV), but ethane, ethanol and ammonia would be. This could indicate that either of the three chemicals compounds may be associated with disease development.

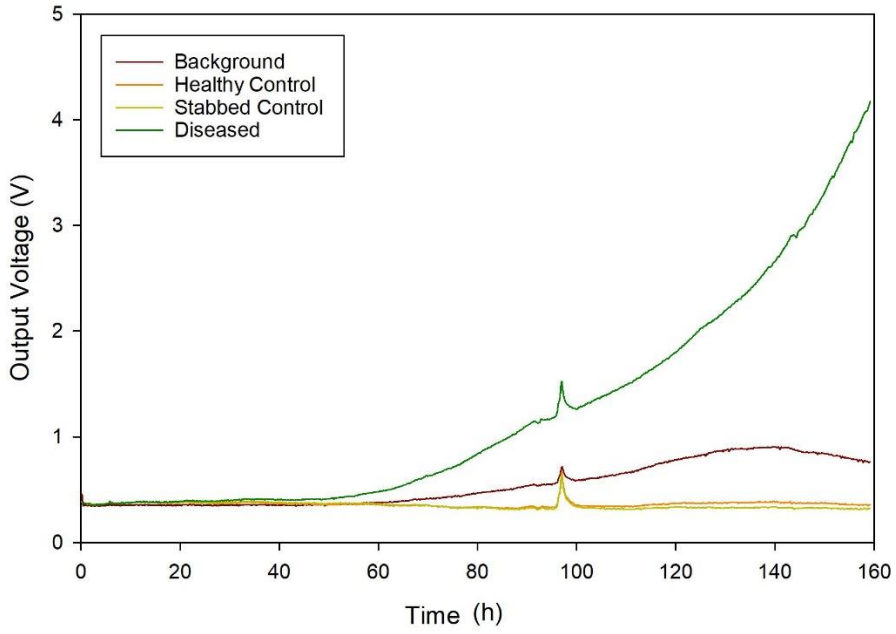
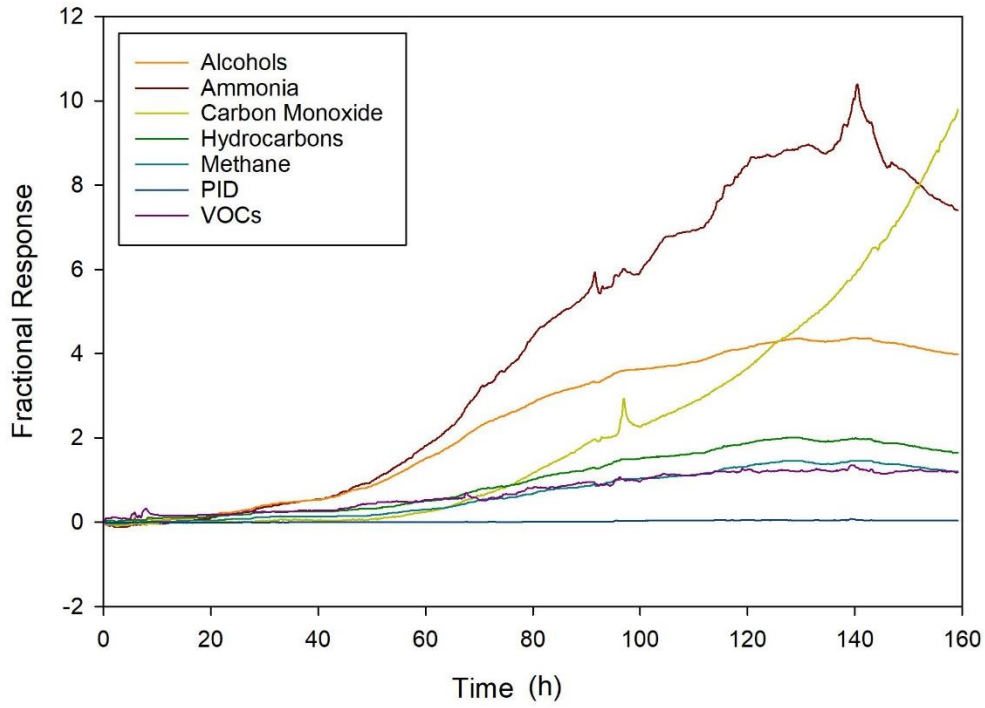


Fig. 5. Carbon monoxide electrochemical gas sensor response over time for potatoes inoculated with *P. carotovorum* (diseased) and healthy control treatments sampled from 4 h post inoculation.



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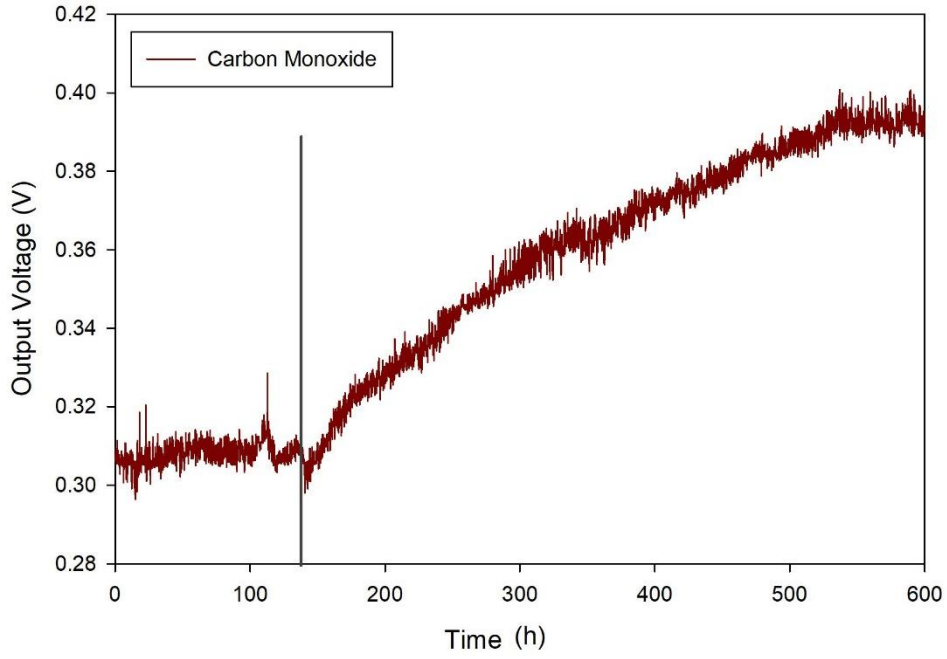
204

205 Fig.6. Gas / VOC sensor outputs (by target molecule) over time for potatoes inoculated with *P.*
206 *carotovorum* (diseased) and healthy control treatments sampled from 4 h post inoculation.

207

208 *3.2 Experimental work at the SBCSR potato store facility*

209 Results from the store experiment again found that the electrochemical gas sensors NO and
210 ETO did not respond to the presence of diseased tubers. More interestingly, and in contrast to
211 the laboratory tests, the PID and the metal oxide VOC sensors also did not respond. However,
212 the electrochemical (CO) and three metal-oxide (alcohols, ammonia, hydrocarbons) sensors
213 showed an increased response to the diseased potatoes (Fig. 7 and 8). The metal-oxide sensors
214 performed the best in store conditions, with the ammonia sensor showing the most significant
215 response (Fig. 7).



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216 Fig. 7: Response of electrochemical carbon monoxide gas sensor over time to the presence
217 *P. carotovorum* infected tubers. The grey line indicates when diseased tubers were placed in
218 the potato store.

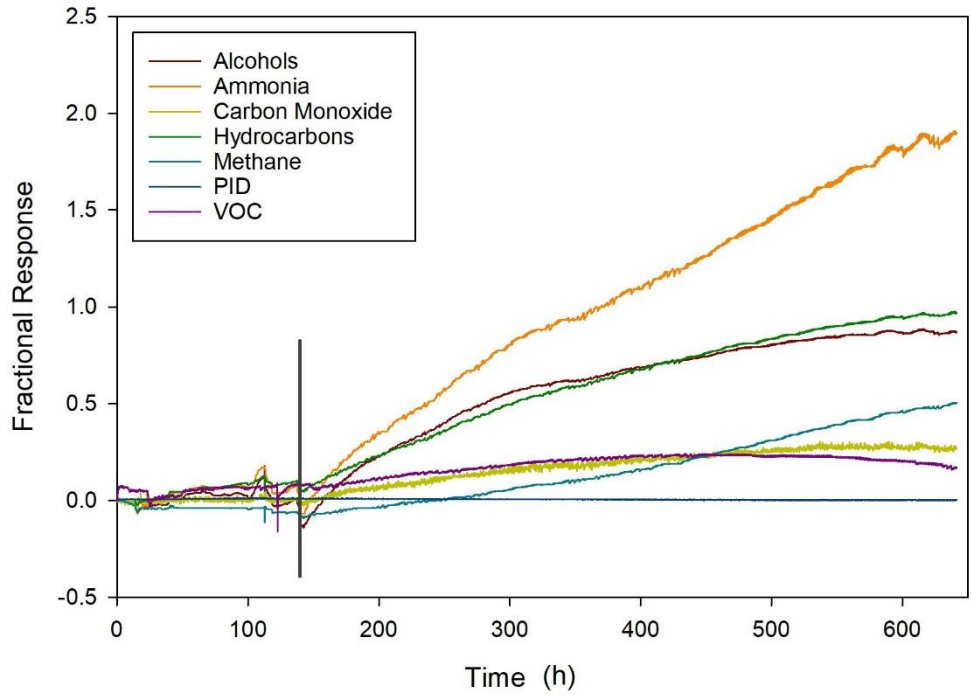

Fig. 8. Response of gas / VOC sensor outputs (by target molecule) over time to the presence *P. carotovorum* infected tubers. The grey line indicates when diseased tubers were placed in the potato store.

4. Discussion

The ultimate goal of our work is to develop a simple means for store managers to detect and monitor potato soft rot infection predominantly caused by *P. carotovorum* in UK stores. Our work is the first to report a successful approach using a gas sensor array, formed of commercially identified sensors, utilising a wide range of different sensor families (electrochemical, metal-oxide, optical and photoionization), in a store environment. This research identified the most effective sensors (metal-oxide sensors detecting alcohols, ammonia and hydrocarbons and one electrochemical detecting carbon monoxide) and hence also provided insight into the type of chemical components detected.

In laboratory testing, seven of the sensors showed a strong response to a soft-rot affected tuber, with two that showed no response (ETO and NO). This is likely to be due to a lack of sensitivity or that these molecules being released were not being detected. Of the remaining group, the ammonia, VOCs and the carbon monoxide sensors showed the strongest response. The ammonia response is interesting in that it has not previously been reported as a potential biomarker for potato infection by *P. carotovorum* and could be associated with the potato breakdown products and therefore be a component of the overall smell of an infected tuber. The VOC sensor detected all VOCs present and therefore, would also in part detect ammonia; hence this may be why its response is of a similar magnitude. The carbon monoxide sensor response may also be detecting chemical breakdown products through cross-sensitivity or CO was simply being produced by the tuber. In addition, many of the sensors showed an initial response that then flat-lined or increased at a reduced rate (specifically the alcohol, PID, methane and hydrocarbon sensors). This may indicate that chemical components initially associated with infection change over time as disease progresses.

Once the efficacy of our system had been established in laboratory conditions, we moved to a research store setting, where after a baseline period, a loading of less than 1% infected tubers was detected. Our results closely mimicked those found in the laboratory setting with the ammonia sensor showing the highest sensitivity, followed by the VOC sensor and hydrocarbon. Furthermore, the output of these sensors continued to increase as the infection developed and it is notable that the hydrocarbon sensor showed better results in the store than in the lab environment. The remaining sensors either did not respond or had a much lower change in output. This is likely be due to the detection limit not being sufficiently high or are being affected by the environmental conditions. This would require further investigation, though the results indicate that a few sensors (ammonia, VOCs and hydrocarbons) within a store could be used to monitor bacterial soft rot.

There are however some limitations to this study; for example it did not take into account other possible external factors that could affect the sensors in a commercial store (specifically external air pollution from farm equipment) and any final solution may still require additional sensors to compensate for this. However, the results did indicate that it was possible to monitor the progression of potato soft rot disease over time. Using a longitudinal approach to identify possible disease development has potential, as it will remove some of the issues surrounding short incidence that may affect the sensor response, such as air pollution or movement of people/crop in and out of the store. Finally, the quantity of tubers used in this study (4 tonnes), was relatively small compared to a commercial store and the incidence of infected samples relatively high for a store environment. In the future, we aim to evaluate this and other potential interferents that may affect such a detection system in practice.

4. Conclusions

In this paper, we report on the use of an array of low-cost commercially available gas sensors deployed within a laboratory and store setting to detect and monitor soft-rot of potato caused by *P. carotovorum*. We have developed a custom gas/volatile analysis system formed from metal-oxide, electrochemical, optical and photo ionising sensors. Results in the lab showed that the instrument was easily able to detect differences between infected and control tubers with high accuracy. This instrument was then deployed in the store, where 1 % of infected potatoes was added to 4 tonnes of healthy controls. Results indicated that the system was easily capable detecting the progression of disease within the store environment, with the ammonia metal oxide sensor showing the best performance. This is the first report if the use of such an array in a store setting. Further work will be need to investigate issues of cross-interference chemicals and sensors responses present in a storage facility. In conclusion, the approach and gas sensing

technologies employed here appear to hold promise for early detection and monitoring of potato storage diseases. The sensors chosen offer the opportunity to develop a store monitoring system at a price point acceptable to the agricultural community.

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