Final Report

Early Detection of Potato Storage Diseases by Gas Analysis

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1. SUMMARY

1.1 Aim

The aim of the research was to investigate the efficacy of pre-symptomatic detection and monitoring of potato soft rot by means of gas analysis and understand its application to stores. Early stage detection and precise location of infected potatoes within a commercial storage facility, would help store managers to remove the diseased material and prevent further spread. This would reduce the financial losses to growers and storage facilities and thus levy payers.

1.2 Methodology

The main goal of this research is to identify possible practical solutions for pre-symptomatic and symptomatic soft rot detection in store by means of gas analysis. The detection approach used was based on commercial gas sensing technologies, making future widespread uptake simpler. These technologies are easier to use, deploy and be cost-effective when compared to traditional (analytical) instruments (as used in most previous research). A range of commercial technologies were tested: FAIMS (Field Asymmetric Ion Mobility Spectrometry), PID (Photoionization detection), Electronic Noses based on metal oxide, electrochemical and NDIR (Non Dispersive Infrared) gas sensors. The first part of the research has been carried out in order to evaluate the potential of these types of instruments in a laboratory environment. Potato tubers were inoculated with *P. carotovorum* (the main pathogen causing soft rot) and subsequently sampled and analysed with gas sensing instruments. Two time points (namely 5 and 2 days post-inoculation) were selected for pre-symptomatic and symptomatic detection of the aforementioned disease, with the resultant data analysed to detect potential differences.

1.3 Key Findings

The main finding is that in all laboratory work all gas sensing technologies (though not all sensors) were proven to be effective for both pre-symptomatic and symptomatic detection of soft rot.

1.4 Practical Recommendations

Research for practical implementation of such technologies for store deployment is now being sponsored by the University of Warwick. Levy payers who might be interested in a prototype to be deployed in their stores are encouraged to get in contact with Massimo Rutolo (M.Rutolo@warwick.ac.uk) and James Covington (J.A.Covington@warwick.ac.uk).

2. INTRODUCTION

The United Nations FAO (Food and Agriculture Organization) estimates that between 40 to 50 % of root and tuber crops, fruits and vegetables produce is wasted each year to a range of causes (FAO, 2016). In the United Kingdom one staple crop that is particularly susceptible is potato tubers. Most of this loss is caused by a disease of bacterial origin known as 'soft rot', which causes substantial post-harvest store losses to the industry (AHDB Potatoes, 2012). Therefore, there is a strong need for a simple and cost-effective means for the detection and monitoring of this disease.

The term soft rot refers to the disease when found in store, whilst blackleg is generally employed for the growing crop (Peters et al., 2012). The pathogen found most frequently in the UK associated with soft rot (and blackleg) is *Pectobacterium carotovorum* ssp. *carotovorum*, but *Pectobacterium atrosepticum* is also common (*Bacterial Rots of Potato Tubers*, 2009; Czajkowski et al., 2015). Bacterial soft rot in store and blackleg can also be caused by several strains of *Dickeya spp.* (Czajkowski et al., 2011), more recently identified as *D. Dianthicola, D. dadantii, D. zeae* and *D. Solani* (Czajkowski et al., 2015; Peters et al., 2012; Toth et al., 2011) as well as by *P. carotovorum* subsp. *brasiliensis* (Duarte et al., 2004; Leite et al., 2014) and *P. wasabiae* (Panda et al., 2012; Pitman et al., 2010). Other two infections, namely late blight and dry rot have been reported to provide a means for secondary infection of both subspecies of *Pectobacterium* (Lui et al., 2005).

Due to the nature of consumer requirements, potato tubers are stored for longer periods of time after harvest. They are kept in such storage facilities from late September until June of the following year. On average, each of these store rooms can contain several hundred 1-ton boxes of produce. This is the most widespread practice for storing the crop in the UK, although other approaches are also employed worldwide. Monitoring the disease status of potatoes in stores is difficult, due to poor access and the large volume of product. However, in an attempt to extend the storage life of these potatoes, stores are generally environmentally controlled with air forced through the tubers. Furthermore, soft rot produces a very strong odour that can easily be detected by human olfaction. Therefore, it should be possible to use artificial sensing technologies to detect the odour given off by infected tubers. Previous work on early detection of potato storage diseases has been conducted over a span of decades with results that appear, to date, to have been inconsistent or of no practical implementation. In addition, those studies also proved to be neither cost-effective nor have a practical implementation for commercial facilities.

The purpose of this study is to, in part, validate previous work to show that it is possible to detect soft rot infection by gas analysis and then deploy different sensing technologies (not previously reported in literature) that could be more appropriate to a final, working solution. In particular, attention is given to low-cost and practical implementation of early detection of the aforementioned potato storage disease.

3. MATERIALS AND METHODS

3.1 Overview of technologies employed

For the current research project three main gas sensing technologies were employed, namely FAIMS (Field Asymmetric Ion Mobility Spectrometer), PID (Photoionization detection) and electronic noses.

The basic working structure of a FAIMS (Field Asymmetric Ion Mobility Spectrometer) consists of three core parts, namely an ionization and reaction region (or ionization and reaction chamber), a drift region (or separation chamber) and a detection sensor. Once the sample molecules to be analysed (in gas form) are ionised and pushed through the separation chamber to reach the detector. The peculiar feature of FAIMS is that an asymmetric RF (radio frequency) field is applied to the two electrodes through which the ions flow (the RF field is orthogonal to the motion of the ions flow), thus generating a saw tooth-like trajectory migration of the ions toward the detector plate. However, because of the different mobility K characteristic of each ion type, only a very restricted set of ions are able to reach the sensing plate. Hence, ions with the incorrect mobility are annihilated, via a saw tooth-like trajectory, against the electrodes while those with the right one collide with the sensor plate (with a less pronounced saw-like path), thus generating an electrical signal, as shown in Fig. 3.1. The utility of the instrument comes into play when a direct current field, known as compensation voltage (or CV), is utilized to modify the asymmetric RF waveform in such a way to select different ions with specific mobility, thus, by sweeping the CV, a mobility spectrum is generated for all the chemical compounds under analysis.

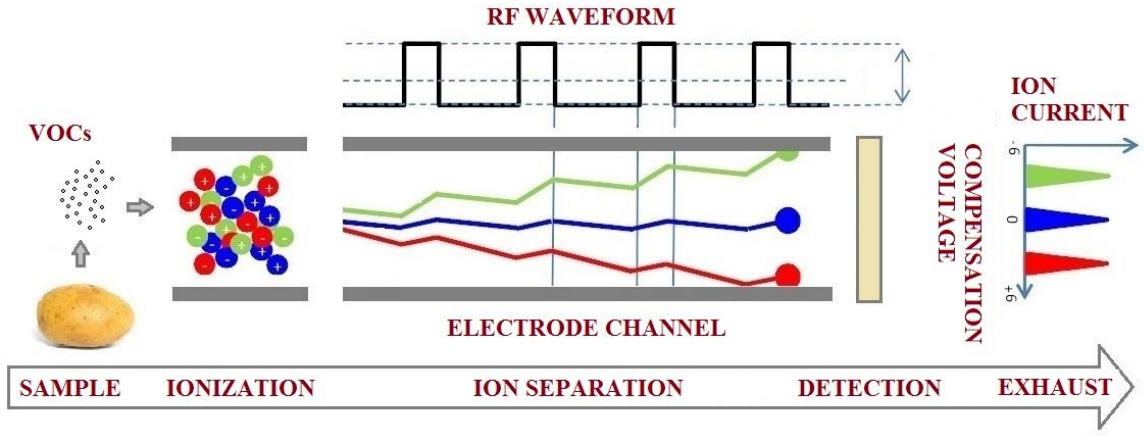


Fig. 3.1. Basic working principle of Field Asymmetric Ion Mobility Spectrometry (FAIMS).

Photoionization detection, or PID, has been employed as a technique for detection of VOCs and is based on a numerical response indicative of cumulative presence of the overall spectrum of volatiles present in the gas sample under analysis. The technology, as indicated in Fig. 3.2, is based on two main components, an ultraviolet (UV) lamp and a sensing unit. The principle of photoionization detector utilizes ultraviolet light to ionize the gas molecules of the chemical under analysis. The gas then becomes electrically charged and the ions produce an electric current by contact with the sensing electrode, thus producing the signal output. UV levels are

usually measured in eV, electron Volts. Each VOC has an ionization potential, or IP also measured in eV. All volatiles with ionization potential below the eV levels of the lamp employed will be ionised. This technology offers a detection range from a wide range of chemical and also for a broad range of concentration, ranging from 1 parts per billion (ppb) to 20,000 parts per million (ppm) with few seconds for both response time and clearing down (Ion Science Ltd, UK.).

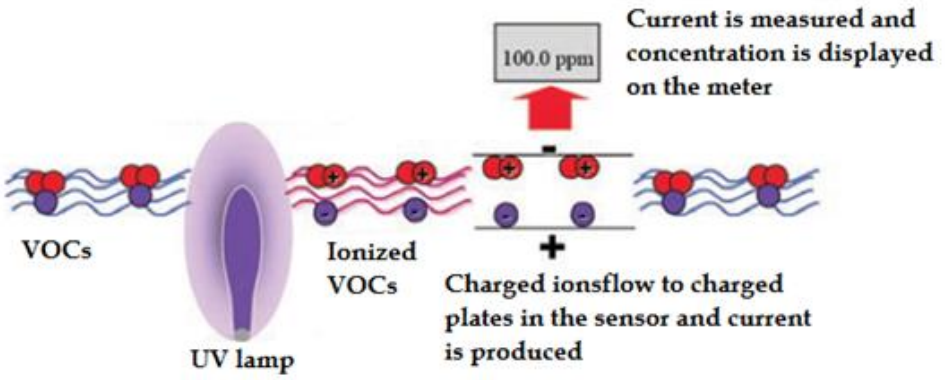


Fig. 3.2. Basic working principle of PID (Photoionization Detector) (RAE Systems - Honewell Inc, 2005).

The electronic nose (Fig. 3.3), unlike FAIMS and PID, does not employ an ionization chamber and the gas molecules of the sample under analysis are transported by a carrier gas until they reach an array of sensors. Each sensor is composed of a material that reacts differently to the sample molecules. The change of the sensor is transduced into an electrical signal and is then processed by a pattern recognition system. The final output is a characteristic 'odour' specific to each sample, odour which can be learned by the instrument by means of artificial intelligence techniques. If a similar chemical pattern is analysed, the electronic nose will then recognise the specific odour under analysis.

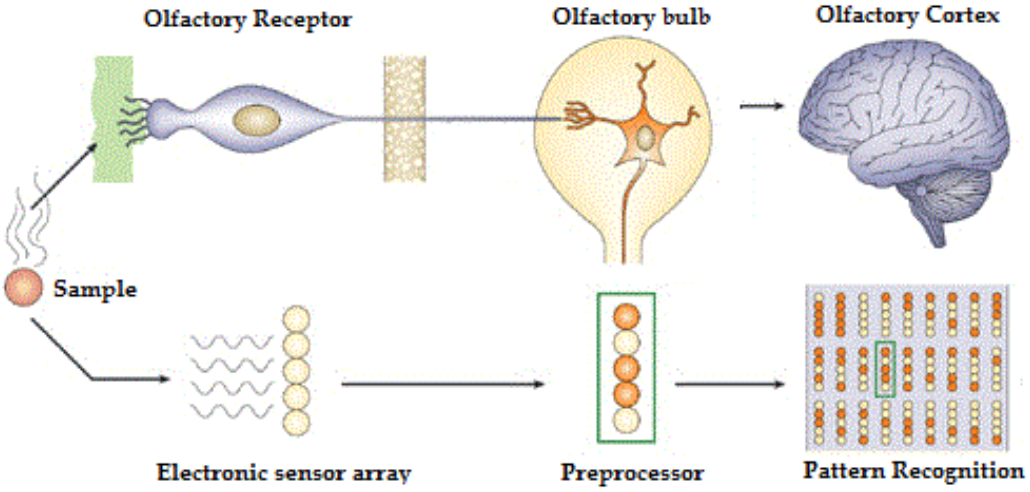


Fig. 3.3. Basic working principle of Electronic Nose (Turner and Magan, 2004).

3.2 Experimental sample preparation

Sample preparation was devised in consultation and under the guidance of researchers from AHDB Potatoes at Sutton Bridge Crop Storage Research for both potatoes variety selection and

sample preparation. The potato tuber chosen for experimental work was ‘Maris Piper’. This variety was preferred over others due to its commercial value to the industry. ‘Maris Piper’ is both the main multipurpose crop for all the requirements of food processing and storage and is also the most widespread one, with an planted area larger to the other three most common varieties (Markies, Maris Peer, Lady Rosetta) combined (Maslowski et al., 2015).

A standard sample preparation procedure was developed for this project and consisted in inoculating ‘Maris Piper’ potato tubers with *P. carotovorum*, in order to cause the pathology known as soft rot. Potatoes were first soaked in water for one hour before use and dried with a paper towel. Each tuber was stabbed at the stolon end with a sterile 200 µl pipette tip. To a 48 h culture of *P. carotovorum* grown on nutrient agar at 25°C, 2 ml of sterile water was added and the colonies gently scraped (using a sterile plastic loop) to create a bacterial suspension. 20µl (high inoculum) of this bacterial suspension was then pipetted into the stab wound in each tuber. A further set of tubers (controls) were stabbed at the stolon but not inoculated. After treatment the tubers were placed in sealed boxes at 25±1 °C in an incubator (to allow rapid disease progression) and suspended on a mesh over water (400 ml), with the expected humidity to be above normal laboratory (neither absolute nor relative humidity levels were measured). No determination for latent *Pectobacterium* prior to infection was carried out, but controls were checked for infection throughout and at the end of the experimental procedure. The *P. carotovorum* isolate used in this study was originally isolated by Dr Glyn Harper (AHDB Potatoes, Sutton Bridge Crop Storage Research) and was isolated from an infected tuber, variety *Marfona*, and showing characteristic symptoms of bacterial soft rot. Isolated and in pure culture it caused pitting in CVP agar at 27 °C, was identified by PCR as *P. carotovorum* (*Pectobacterium* primer sets courtesy of Dr J. Elphinstone, FERA, UK) and could infect potato tubers causing the original symptoms. No strain reference has been used to date for this isolate since it is the first time a paper has been published using it. The strain has been suggested by Dr Glyn Harper to be named SBEU_08.

3.3 Experimental sampling protocol (FAIMS)

Three types of experimental protocols are reported here and all the work was done with 1 L PTFE (Polytetrafluoroethylene) containers (Fisher Scientific Ltd, UK), with a gas path inlet and outlet added via1/8” push-fits (SMC Pneumatics Ltd, 2016), at both ends. PTFE containers were chosen since the polymer is inert to most chemicals and the reason for which it is used in a broad variety of applications. For the first set of experiments with the FAIMS technology (Lonestar, Owlstone Ltd, UK), each tuber was placed into a PTFE container provided and sampling was carried out for each individual tuber by allowing clean air to flow around it, the mixture of gases and VOCs emanating from the potato were then fed for analysis to the FAIMS instrument (Lonestar, Owlstone Nanotech Ltd, Cambridge, UK) at a flow rate of 2 L/min. Other FAIMS parameters included a dispersion field (DF) from 0 to 100% in 51 scanning steps and a compensation voltage (CV) from -6 to 6 V in 512 steps in order to build a 3D data matrix characteristic of the sample under analysis. These settings are typical for normal FAIMS operation. Each potato tuber was scanned in such a manner twice. Prior to entering the sampling container the air was scrubbed clean and dried using moisture and hydrocarbon traps. PTFE containers were employed always separating usage for controls and infected tubers to avoid accidental cross contamination. The containers were also replaced, when appropriate, in order to eliminate potential by-product of potato decomposition that might have affected results. After sampling each tuber was repositioned in the containing box in the incubator. Prior and

after experimentation containers were thoroughly cleaned and sterilized with ethanol at 99% (Fisher Scientific Ltd, UK).

Four sets of experiments were performed where 3 to 4 tubers were inoculated at days 1, 2 and 5 before testing, (with corresponding controls). The sampling procedure was carried out twice (two consecutive days). For the first experiment, on day one of testing, 18 potatoes were analysed and the procedure was repeated on the second day with the same tubers, giving a total of 36 samples. This procedure was carried out for all other experiments with 36 samples for the first three experiments, while the number of samples was increased in the fourth experiment to 48 (24 tubers analysed in the first day and again in the following day for a repeat). The number of potato tubers tested was 78 for a total number of samples of 156 (including repeats). Details of the experiments are shown in Table 3.1. The first part of the protocol had the objective to verify the ability of the Lonestar FAIMS to discriminate between controls and soft rot infected tubers sampled after 5 days of storage in the incubator, when the symptoms of the disease could be identified by olfactive, tactile and visual inspection of the sample. The second part of the experimental procedure had the aim to characterize early detection and consequently to probe the possibility of the instrument to detect the disease and discriminate between control and infection, when no visible, odour or tactile symptoms of the disease where present (one and two days post inoculation). Hence the terms “standard disease detection” or “disease detection” (5 days post inoculation) and “early disease detection” (1 and 2 days post inoculation) were employed. At the end of the sampling procedure all tubers were cut in half and photographed to gather indication of the degree of infection.

Experiment	**Number of Potato Tubers**						**Tests**	
	1 Day post inoculation		2 Days post inoculation		5 Days post inoculation			
	Control	Infected	Control	Infected	Control	Infected	1st	2nd (repeat)
No 1	3	3	3	3	3	3	18	18
No 2	3	3	3	3	3	3	18	18
No 3	3	3	3	3	3	3	18	18
No 4	4	4	4	4	4	4	24	24
Total	13	13	13	13	13	13	78	78

Table 3.1. Experiments carried out and number of samples (total of 156).

For the second set of FAIMS experiments, potato tubers (cultivars Melody, Nectar, Agria, and Annosa) previously inoculated by staff at FERA (Food and Environment Research Agency) with the quarantine pathogens *Ralstonia solanacearum* (brown rot) and *Clavibacter michiganensis* (ring rot) were stored in a controlled environment in cardboard boxes. Samples for FAIMS testing included 30 tubers for controls, 50 for brown rot and 15 for ring rot. After initial experimentation with different laboratory set-ups, tubers for sampling were placed in PTFE containers to optimise air flow and, as before, air was purified by means of moisture and hydrocarbon traps prior to delivery to the FAIMS instrument. All samples potato tubers were washed in a water bath before sampling which was carried out 2 hours after potato tubers had been removed from the storage room.

In the third set of experiments, tubers were inoculated 5 days before sampling, as described in previous section, using 4 tubers per time point (with 4 corresponding controls). Sampling was carried out using FAIMS (as above at 25 ^{o}C), after which the incubator temperature was reduced to 15°C and the next day the sampling procedure was repeated. The total number of samples was 48, with 24 sampled after being stored at 25 ^{o}C and 24 after being stored (for more than 12 hours) at 15 °C. This was repeated but with the temperature on day 2 of sampling further reduced to 10 °C, which is close to commercial store temperature. This latter experiment was carried out as a proof-of-concept in order to show that volatiles coming off from the same infected tuber could be detected at lower temperatures, typical of storage facilities. This also implied that a very similar detected fingerprint for soft rot was present at different temperatures.

3.4 Static headspace for sampling protocol (PID)

The laboratory protocol that was developed for PID technology (Tiger VOC analyser, Ion Science Ltd, UK), differed from other analytical instrument in that static headspace collection was employed rather than a dynamic one (i.e. where clean air is passed over the potato, which is placed in a chamber, and then into the instrument). Tubers were moved into the 1L Fisher Scientific PTFE jar and left in the sealed containers for approximately five minutes, after which sampling was carried out at one end of the container (i.e. an internal pump within the instrument drew a small volume of air to the detector). The output of the instrument was taken in the form a single numerical value per measurement (total VOC level). After having established the specified protocol for collection of volatiles (which was based on 32 samples, although more were tested previously in a tentative manner), a total of 80 samples were measured.

3.5 Dynamic headspace sampling for the FOX and WOLF electronic noses

Sampling was carried out for each individual tuber (placed into the 1 L PTFE container) by passing zero-grade air around it at a flow rate of 200 mL/min and the mixture of air and emissions from the tuber fed to a commercial FOX 3000 electronic nose (AlphaMOS, Toulouse, France) for analysis. Available sensors in the unit as indicated by the manufacturer are listed in Table 3.2. The acquisition time was 120 s, purge of 30 s, start injection of 20 s, injection time of 10 s. For the WOLF 4.1 (sensors list is listed in Table 3.3) acquisition time was of 120 sec, start injection of 20 s, injection time of 10 s, and flow rate of 300 mL/min. Sampling containers were kept separate for controls and infected tubers throughout all experiments to avoid accidental cross contamination. The containers were regularly replaced with clean ones, in order to eliminate the potential of any by-products of tuber decomposition to affect the results. Containers were also thoroughly flushed with zero grade lab air for 5 to 10 minutes before starting any experimental work and after equipment cleaning in order to remove potential residual odorous emissions (originating from either laboratory practice or from the cleaning process) that could affect experimental work. After sampling, each tuber was returned to the sealed plastic boxes in the incubator at 25 °C. Prior to, and after experimentation, containers were thoroughly cleaned and sterilized. Four experiments were carried out where four tubers were inoculated 2 and 5 days before sampling, with method and scope as indicated in the work with FAIMS.

At the end of the sampling procedure, all tubers were cut in half and photographed to assess the degree of infection. In total, 40 potato tubers (20 inoculated, 20 uninoculated) were analysed for each of the two time points.

Sensor Name	**Responsive to:**	**Type of sensor**
P10.1	Hydrocarbons	P Type
P10.2	Hydrocarbons	P Type
P40	Fluorinated and chlorinated compounds, aldehydes	P Type
PA2	Polar compounds, solvents	P Type
SX00	Air quality	SX Type
SY.cG	Amine, amine compounds	SX Type
SY.G	Fluorinated and chlorinated compounds, aldehydes	SY Type
SY.gCT	Hydrocarbons	SY Type
SY.GW	Polar compounds, solvents	SY Type
SY.W	Hydrocarbons	SY Type
T30.1	Polar compounds, solvents	T Type
T70.2	Alcohol and aromatic compounds	T Type

Table 3.2. Description of FOX3000 sensors as indicated in the FOX2000-4000 Manual (Release 4.01) by Alpha MOS Ltd.

Sensor Name	Responsive to:	Type of sensor
Cirius CH_4 NDIR	Methane	NDIR (Nondispersive infrared)
Cirius CO_2 NDIR	Carbon Dioxide	NDIR (Nondispersive infrared)
Carbon Monoxide (CO)	Carbon Monoxide	Electrochemical
Ethylene Oxide (ETO)	Ethylene Oxide	Electrochemical
Hydrogen (H_2)	Hydrogen	Electrochemical
Hydrogen Sulphide (H_2S)	Hydrogen Sulphide	Electrochemical
Nitric Oxide (NO)	Nitric Oxide	Electrochemical
Nitrogen Dioxide (NO_2)	Nitrogen Dioxide	Electrochemical
Oxygen (O_2)	Oxygen	Electrochemical
Ozone (O_3)	Ozone	Electrochemical
Sulphur Dioxide (SO_2)	Sulphur Dioxide	Electrochemical

Table 3.3. Description of WOLF 4.1 sensors employed for experimental work as indicated by Alphasense Ltd and Clair Air Ltd.

4. RESULTS

4.1 FAIMS laboratory responses for all time points

Fig. 4.1 shows photographs ((A), (B), representative positive ion matrices ((C), (D)), cross section for each ion matrix at 45% dispersion field ((E), (F)), positive ion matrices (logarithmic base 10 for ion current axis - (G), (H)), for a control tuber and an infected one as representative of the two groups. Results for each of the four sets of experiments are shown in Fig. 4.2 to Fig. 4.4 for all three time points (5, 2, 1 days post inoculation). In all graphs PCA and k-means clustering have been used for data representation. PCA scree plots for all experiments showed that most of the variance was explained by the first two principal components. Variance explained in the first two principal components of the time point 'detection' is 41.1% and 35.2%, for the time point at two days post inoculation 69.6% and 14.5% while 35.3% and 25.5% for the last. Analysis of data was carried out by assuming no prior knowledge of the data. Results are presented in only two categories, "control" and "infected", regardless of degree of infection of inoculated potato tubers. However, some controls showed clear signs of infection while a number of inoculated tubers manifested varying degrees of mild infection. Following a first analysis with PCA and k-means for the whole data set of the experiments, an interpretation of the principal components has been attempted by employing the Lonestar DF matrices and photographic analysis of the internal part of samples cut in half after the sampling procedure. By looking at the data sets it has been noticed that the height of the Ion Current increases without any major shape difference going along the ordinate (i.e. 2nd principal component) while, instead of an intensity change, a shape change along the abscissa. Hence, the first principal component appears to be correlated with a change in total volatile metabolites of the same type while the second principal component seems to be related to a change in the type of volatiles emitted. All samples outside the two confidences ellipses have shown intermediate characteristics between the two.

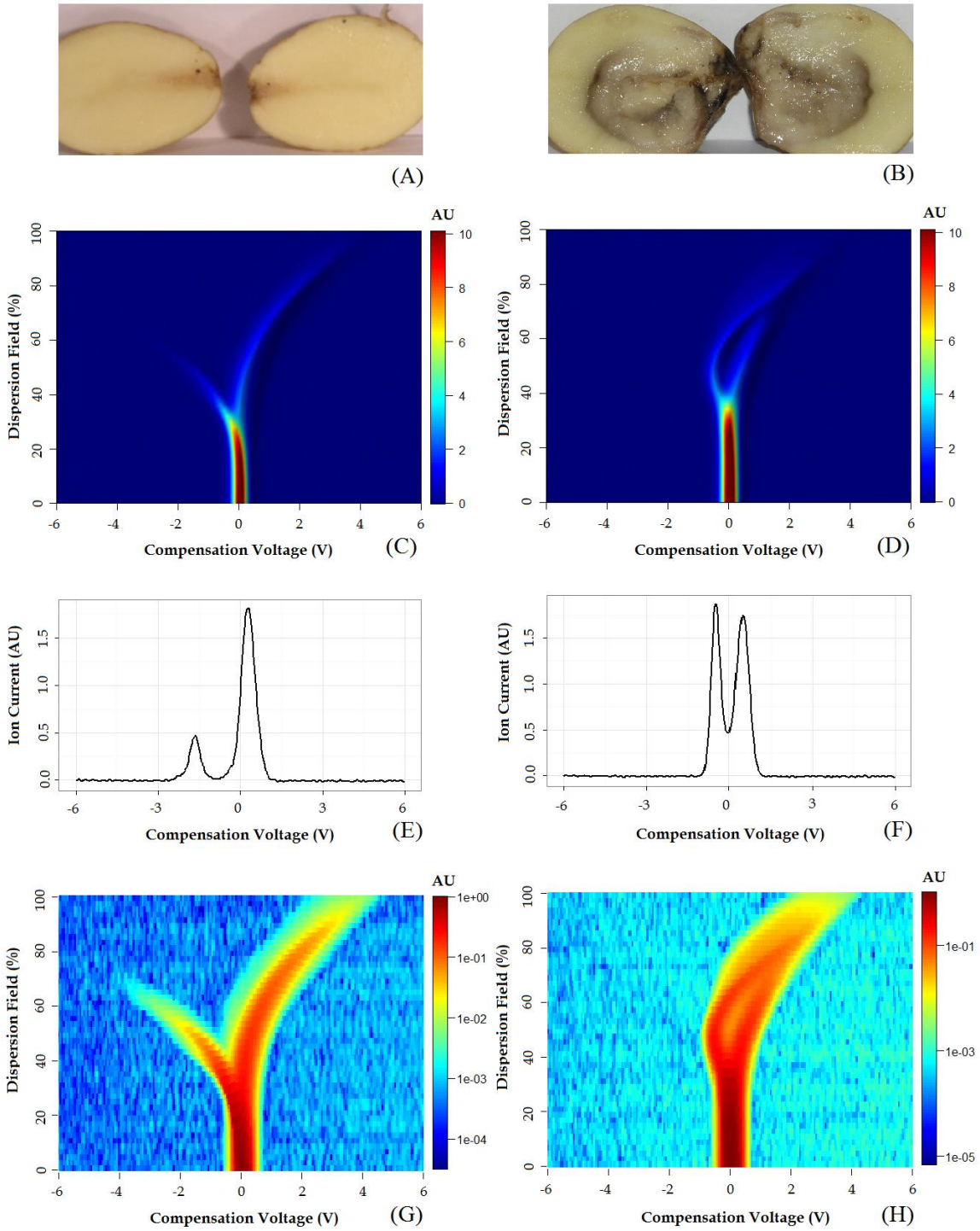


Fig. 4.1. Control (A, C, E, and G) and tuber infected with soft rot (B, D, F, H). Photographic analysis for control (A) and infected potato (B). (C) and (D) are positive ion matrices in A.U. (arbitrary units) while (E) and (F) show ion currents at 45% DF. (G) is the logarithmic representation on the ion current axis of (B), for control and (H) for the infected tuber in (D).

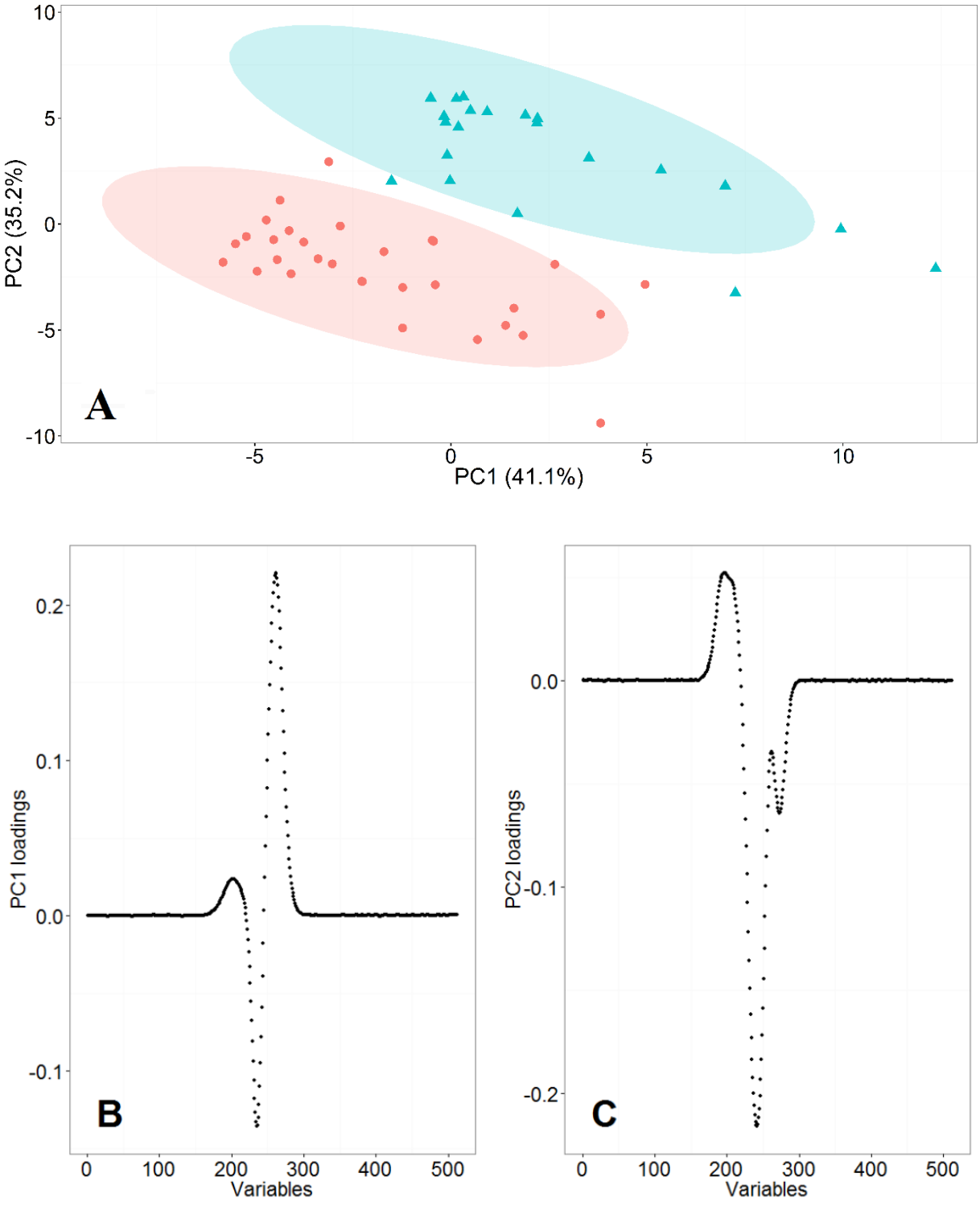


Fig. 4.2. PCA score and k-means clustering (A) for two groups of potato tubers with controls (red circles) and infected (cyan triangles) that have been grouped with 95% confidence ellipses around the centroid identified by the k-means algorithm) for 5DPI (5 days post inoculation). Loadings for the two main principal components in (B) and (C).

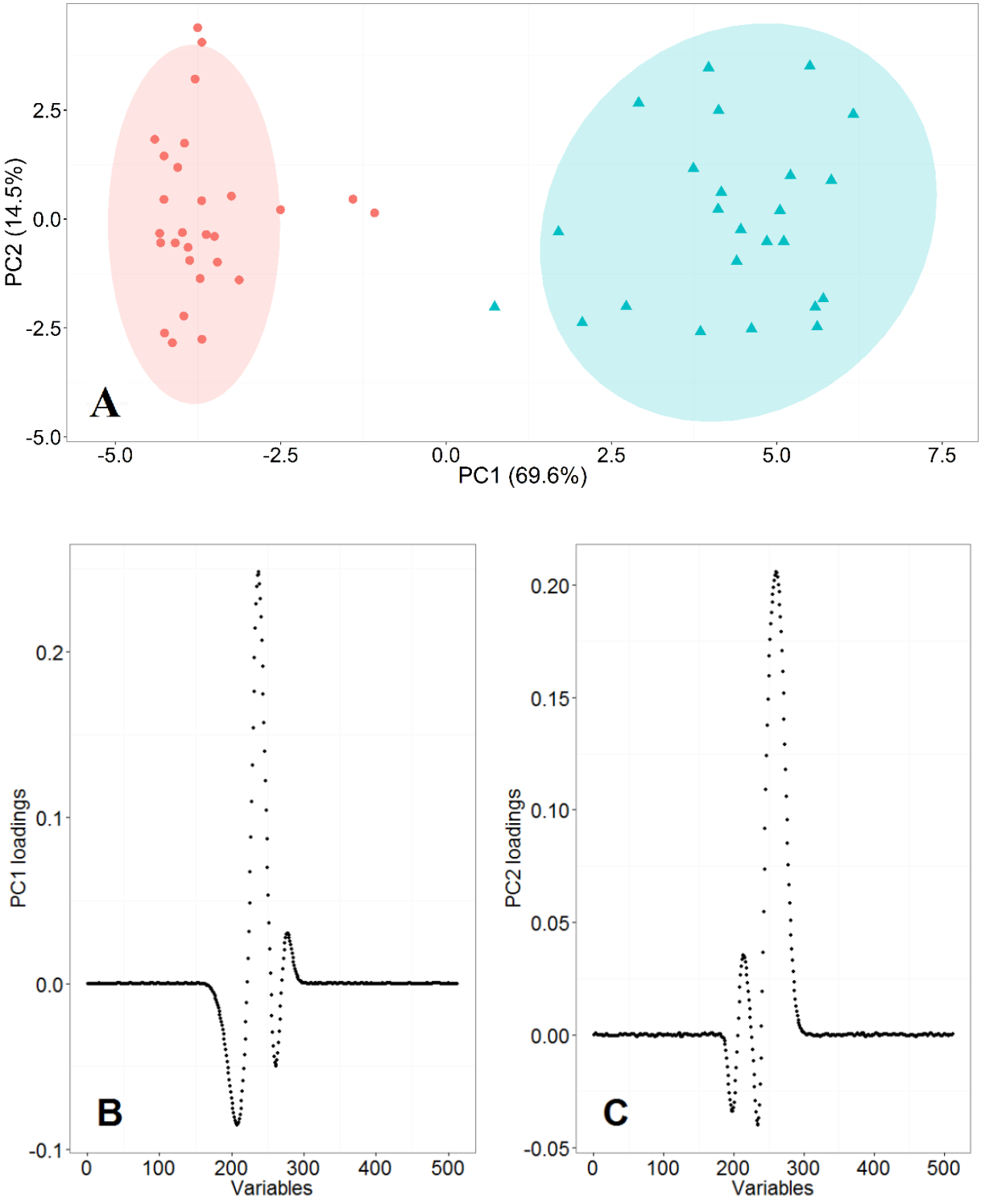


Fig. 4.3. PCA score and k-means clustering (A) for two groups of potato tubers with controls (red circles) and infected (cyan triangles) that have been grouped with 95% confidence ellipses around the centroid identified by the k-means algorithm) for 2DPI (2 days post inoculation). Loadings for the two main principal components in (B) and (C).

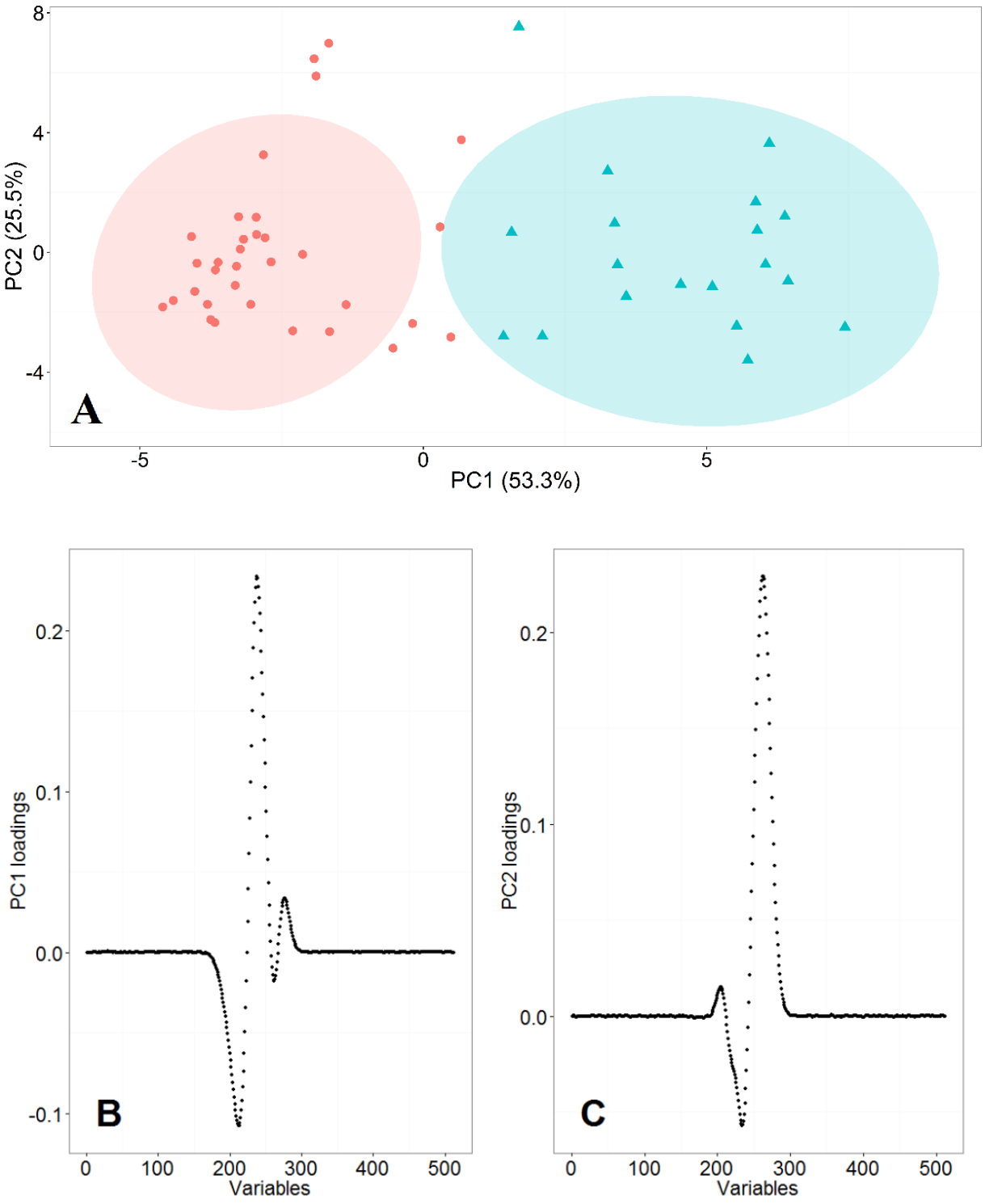


Fig. 4.4. PCA score and k-means clustering (A) for two groups of potato tubers with controls (red circles) and infected (cyan triangles) that have been grouped with 95% confidence ellipses around the centroid identified by the k-means algorithm) for 1DPI (1 day post inoculation). Loadings for the two main principal components in (B) and (C).

4.2 Other FAIMS results

4.2.1 Potato quarantine pathogens at FERA

Laboratory work at FERA (Food and Environment Research Agency, York, UK) was carried out with the Lonestar FAIMS (after the most appropriate set-up was devised at the University of Warwick for their environment) for two quarantine diseases, namely brown rot and ring rot. Brown rot is caused by the bacterium *Ralstonia solanacearum* while ring rot is caused by the *bacterium Clavibacter michiganensis* subsp. *sepedonicus* (DEFRA, 2005a, 2005b). Brown rot is characterized by browning and necrosis of the vascular ring and surrounding tissue that may cause secondary rotting. Symptoms of ring rot are cheese-like degradation around the vascular tissue, as it can be seen from Fig. 4.5.

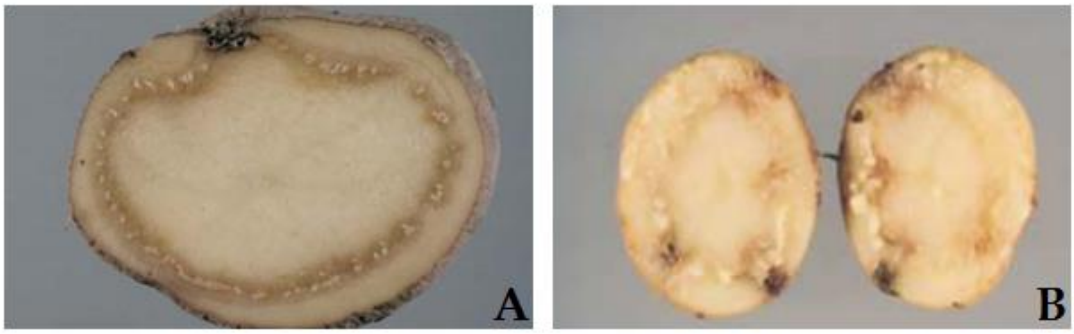


Fig. 4.5. Characteristics early symptoms of brown rot (A) and ring rot (B) (DEFRA, 2005a, 2005b).

Unlike other potato tubers diseases, brown rot cannot be easily detected because of the lack of distinctive “odour” identifiable by human olfaction. The core idea was to evaluate the potential of “artificial olfaction” with FAIMS for the detection of the selected infection, at different stages of disease development. Some results for the experiments with brown rot and ring rot, another quarantine disease selected for study, are shown in Fig. 4.6.

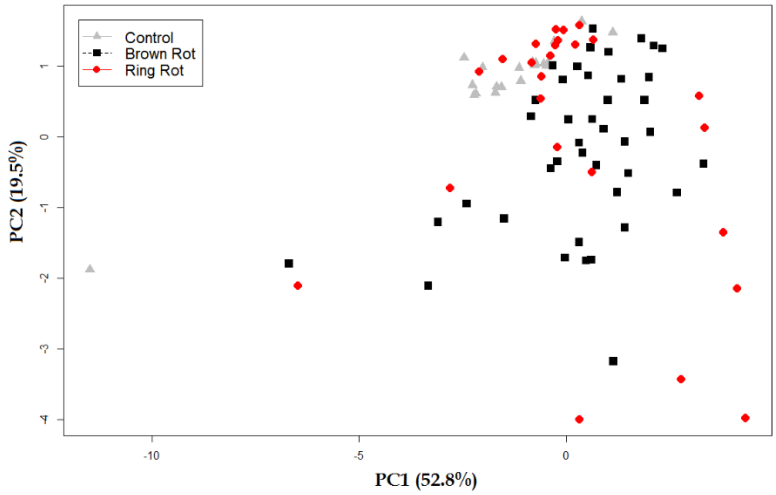


Fig. 4.6. Lonestar FAIMS PCA results obtained at FERA (Food and Environment Research Agency) for control (20 samples), brown rot (50 samples) and ring rot (15 samples).

Partial discrimination between control and brown rot was achieved while the data for ring rot yielded no results, as shown in Fig. 4.6. Because of the initial experimental conditions, the results raised the possibility that temperature could affect volatile emissions in such a manner to make gas analysis by FAIMS or any other technology unfeasible (which has led to further study on effect of temperature on volatile emissions as described later). The graph in Fig. 4.7 shows part of the DF matrix of data, the Ion Current (ordinate) versus Dispersion Field at 50% (abscissa), in short the first being the quantity of ions reaching the detector of the device while the latter the range of (direct current) voltage, which were applied to the ions, acts as a filtering system. Apart of few samples the control and ring rot features a similar profile (hence not distinguishable) while the brown rot showed a more complex profile.

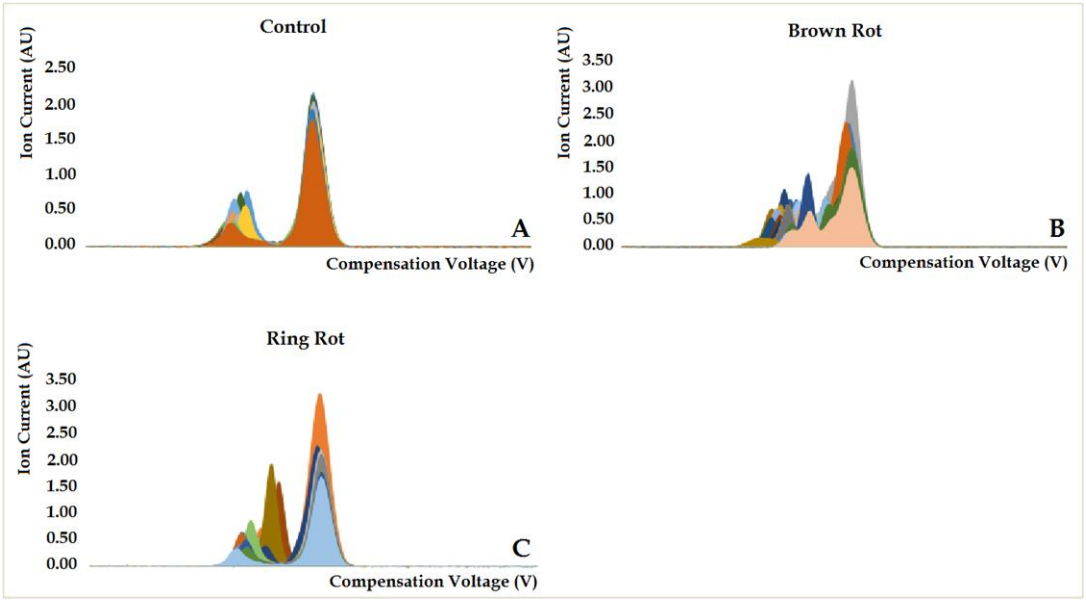


Fig. 4.7. FAIMS data for all potato tuber samples: Ion Current (ordinate, units in AU, arbitrary units) versus Compensation Voltage (abscissa, units in V) at Dispersion Field of 50% for control tubers (A), tubers infected with brown rot (B) and ring rot (C).

4.2.2 Effect of temperature variation on soft rot disease detection

This next set of experiments had the objective to ascertain if low temperatures could affect the FAIMS profile for gas/vapour/volatiles, and if so to what extent. Experiments were devised with the objective to have a first estimate for the potential of the instrument to detect signs of disease at conditions in which the tubers could be commonly stored. Fig. 4.8 shows what can be considered the characteristic outline of a 'double plume' for each infected tuber when sampled (after being kept at 25ºC) that appeared to be very similar in shape to the one after the same tuber was stored at 10ºC for 24h, as shown in Fig. 4.8. Results when temperature for storage was lowered from 25ºC to 15ºC (as in the first set of experiments) yielded similar outcome when conditions for storage of tubers were changed from 25ºC to 10ºC (second set of experiments). An important point to consider is that each tuber was taken from the incubator and immediately sampled. Data are shown PCA and k-means in Fig. 4.9 for tubers at 25-15ºC and Fig. 4.10 for the ones at 25-10ºC. Data collected in each Figure represents all controls for each temperature pair (25-10ºC or vice versa 25-15ºC) against all diseased for the same temperature pair. Apart of the expected number of outliers and variation of experimental work, the data points are closely related together in a similar fashion to what was obtained in the previous section. Hence

it can be concluded that gas analysis can be performed at low temperatures, which is relevant to potato storage.

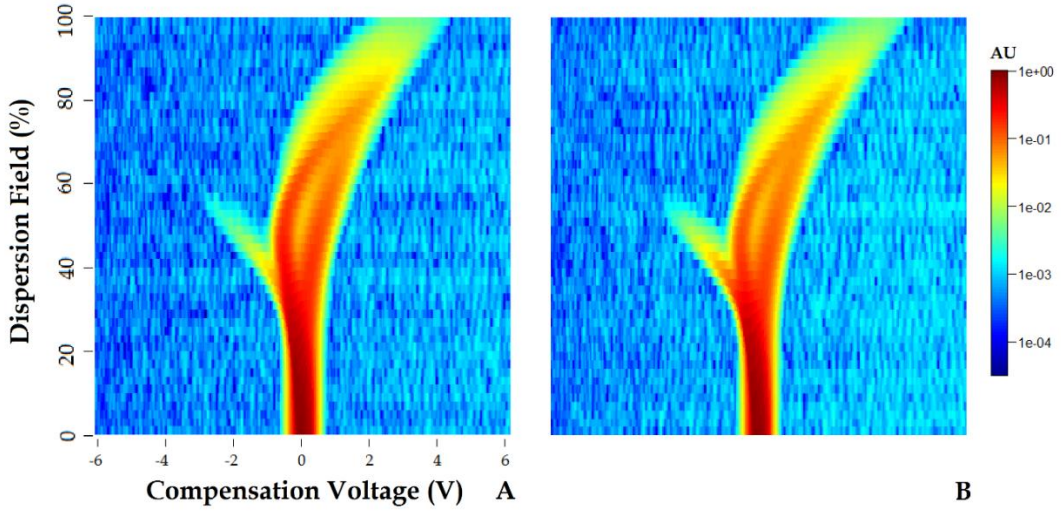


Fig. 4.8. Logarithmic representation of the DF matrix for the same tuber infected with soft rot stored at 25 ^{o}C (A) for four days and 10 ^{o}C (B) for 24 hours after inoculation and prior to sampling.

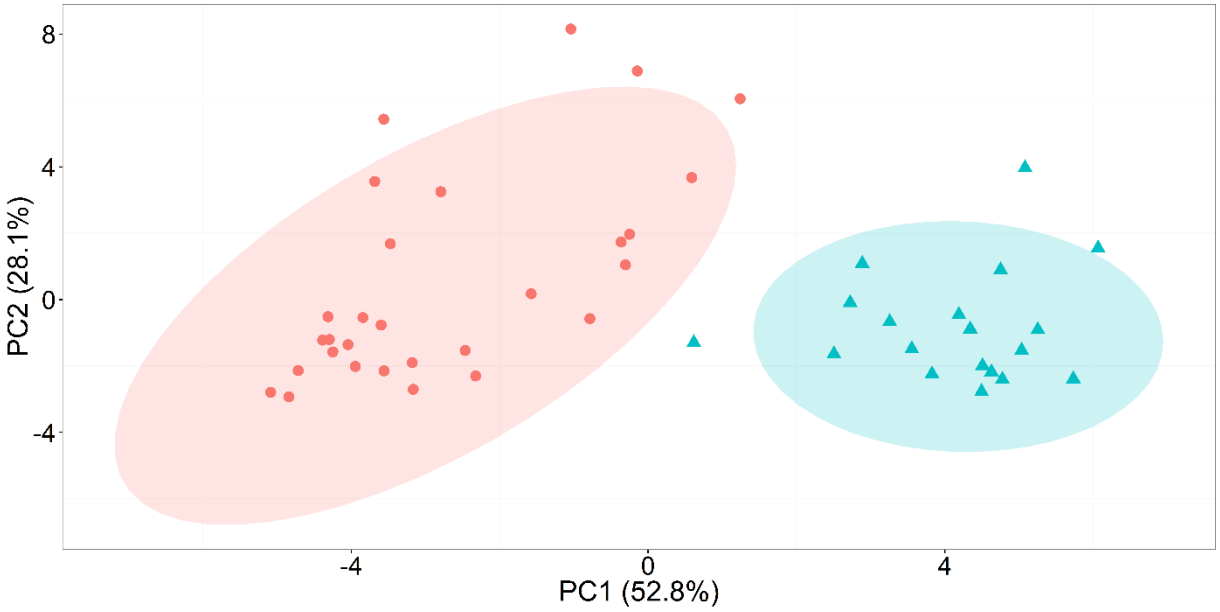


Fig. 4.9. PCA score and k-means clustering for two groups of potato tubers with controls (red circles) and infected (cyan triangles) that have been grouped with 95% confidence ellipses around the centroid identified by the k-means algorithm). Each of the tubers was first stored at 25 ^{o}C for 4 days post inoculation and then for 24h at 15 ^{o}C.

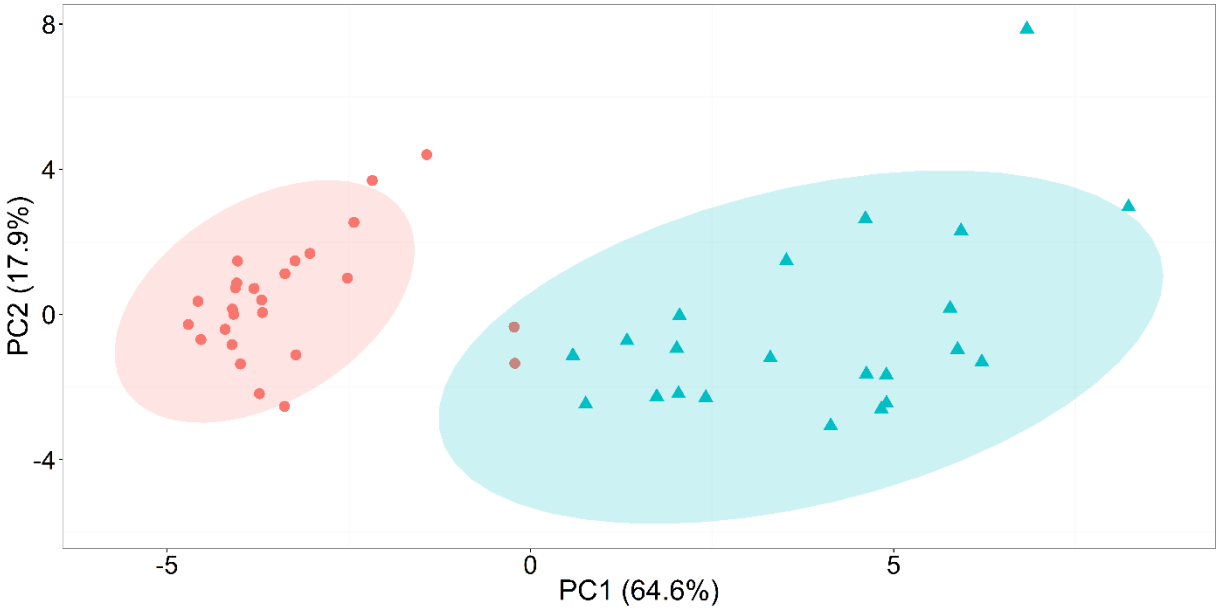


Fig. 4.10. PCA score and k-means clustering for two groups of potato tubers with controls (red circles) and infected (cyan triangles) that have been grouped with 95% confidence ellipses around the centroid identified by the k-means algorithm). Each of the tubers was first stored at 25 ^{o}C for 4 days post inoculation and then for 24h at 10 ^{o}C.

4.3 PID response for ‘detection’ and ‘early detection’ time points

Fig. 4.11 shows results for the first experiments with the Tiger PID analyser (Ion Science Ltd, UK). The aim of this experimental work was to determine the optimal amount of storage time for volatile collection with regard to the 5 DPI time point. 60, 30, 5 and 1 m time periods were used for storing each potato tuber prior to sampling.

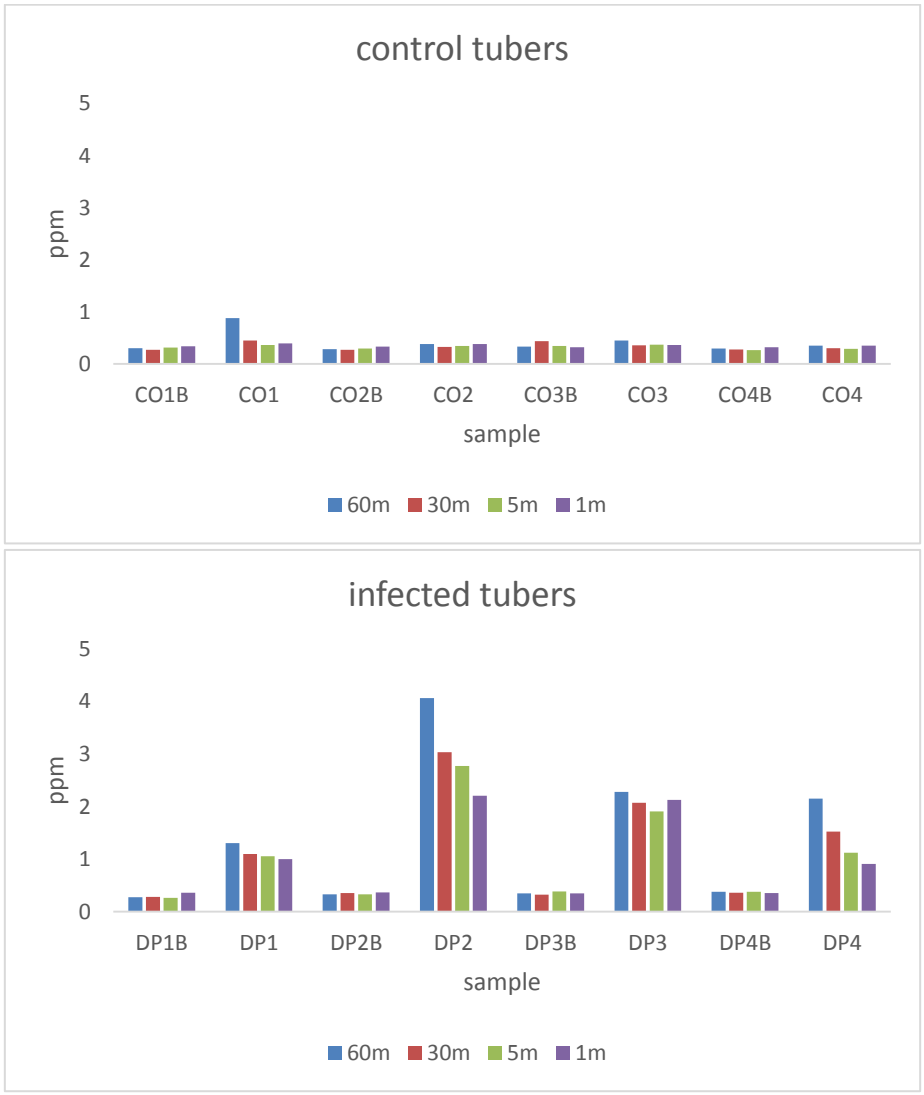
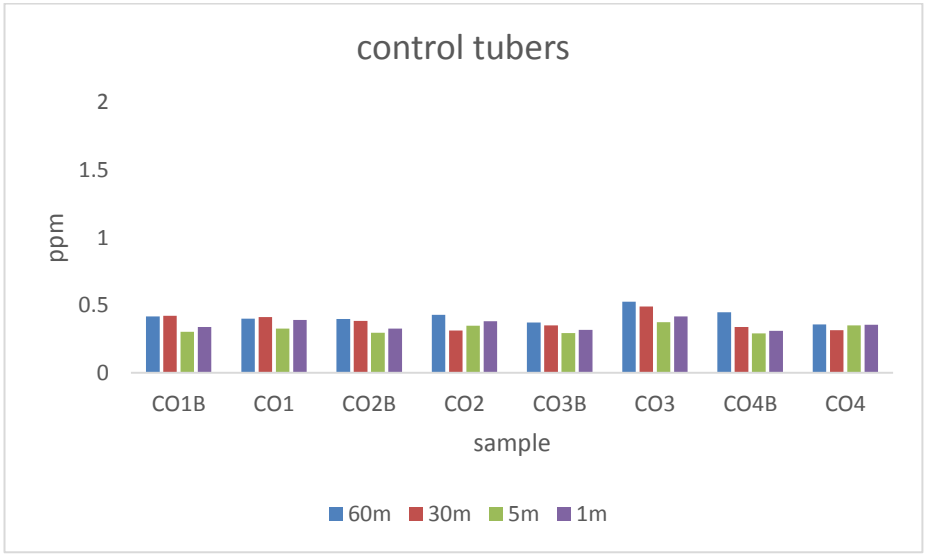


Fig. 4.11. Data results for the Tiger VOC analyser for ‘detection’ time point, i.e. 5 DPI (days post inoculation). “CO” refers to control, “DP” to infected tuber and “B” to background reading before sampling. The legend for 60, 30, 5 and 1m indicates the time period of storage of tuber in the PTFE jar prior to sampling. Units are in ppm (parts per million).

The background value was also taken prior to tuber datum collection and no significant difference was found between background and sample reading for control tubers. However, substantial difference was identified when background and infected tubers were compared. Similar results were obtained for the other time point ‘early detection’ (Fig. 4.12), the only difference being the smaller amount of headspace volatiles for infected tubers. Based on this early work, a time collection period of 5 minutes was chosen in order to corroborate previous data as it best accounted for both the biological course of disease spread and practical experimental considerations. The results for these experiments are shown in Fig. 4.13 for the ‘detection’ time point for both controls and infected potatoes. The data further substantiates the

fact that storage time did not affect the increase in VOCs for uninfected potatoes. In the same Figure only two tubers deviated from this conclusion, but once cut open for inspection they were found to be severely and mildly infected. The outcome for infected tubers thus showed that total VOCs from a tuber increases with the presence of disease (and this can quantified). Furthermore, this implies that a lower cost technological approach can achieve the similar results as FAIMS. Fig. 4.14 indicates that for the second time point 'early detection' and, for both sample types, results still holds albeit with a substantial change in VOC emission between the two time points, which could be associated with severity of disease progression. There is then a substantial difference with FAIMS outcome, since in that former case the Lonestar instrument was able to detect the same amount of an unknown chemical compound(s) (probably due to the high sensitivity of the technology) whilst in the latter case, the PID could also offer a quantitative evaluation of overall VOCs trend associated with disease increase over time, as would be expected and achieved in a real storage facility.



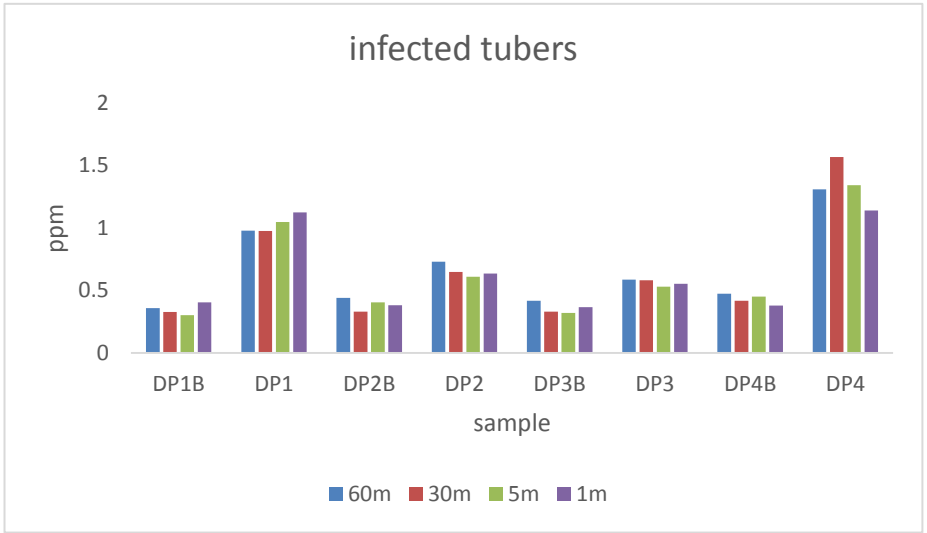


Fig. 4.12. Data results for the Tiger VOC analyser for 'early detection' time point, i.e. 2 DPI (days post inoculation). "CO", "DP" and "B" are as indicated in Fig 4.11. The legend for 60, 30, 5 and 1m indicates the time period of storage of tuber in the PTFE jar prior to sampling. Units are in ppm (parts per million).

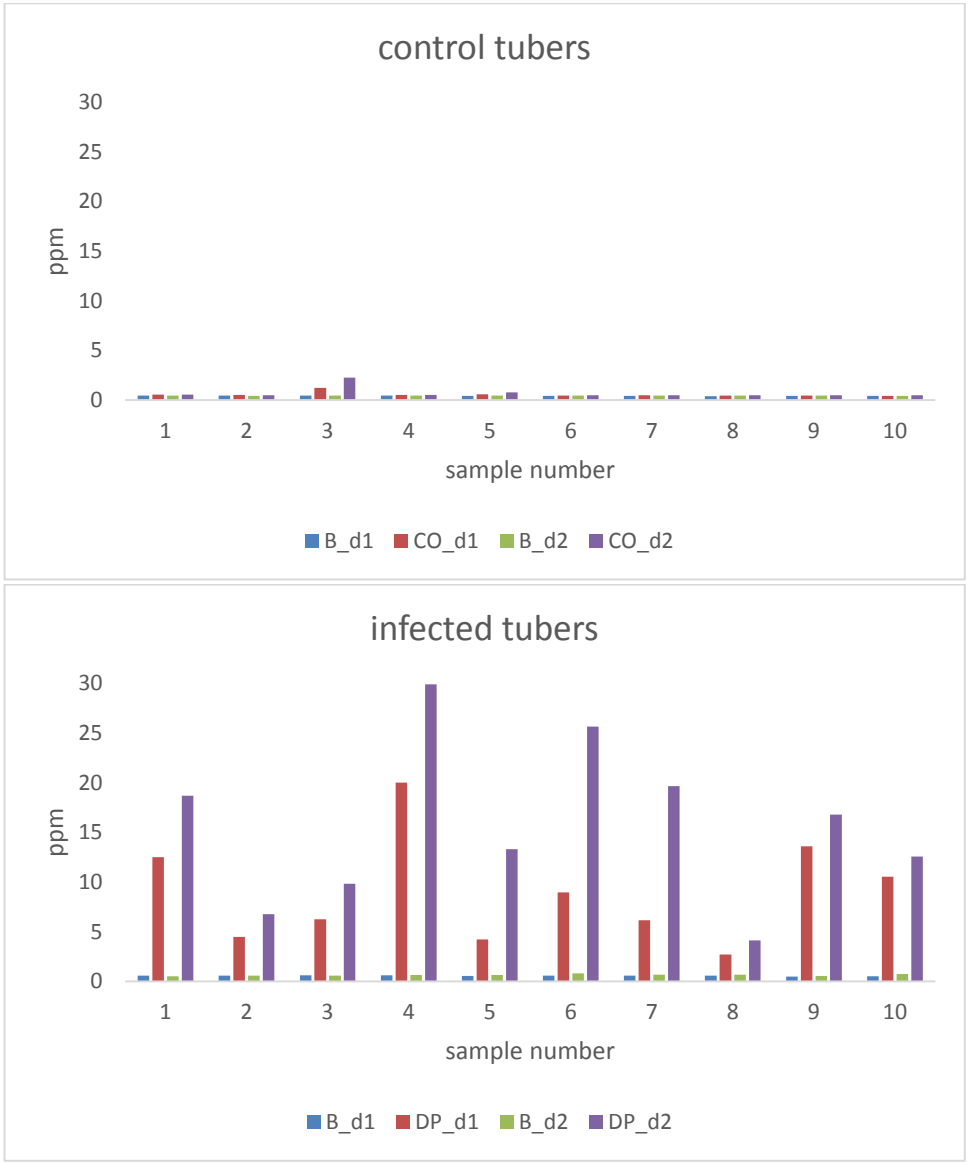


Fig. 4.13. Data results for the Tiger VOC analyser for 'detection' time point, i.e. 5 DPI (days post inoculation. "CO" refers to control, "DP" to infected tuber, "B_" to background reading before sampling, "_d1" and "_d2" to the first and second day of sampling. 5m was the period of storage of tuber in the PTFE jar before sampling. The abscissa indicate potato tuber sample number while the ordinate instrument values in ppm (parts per million).

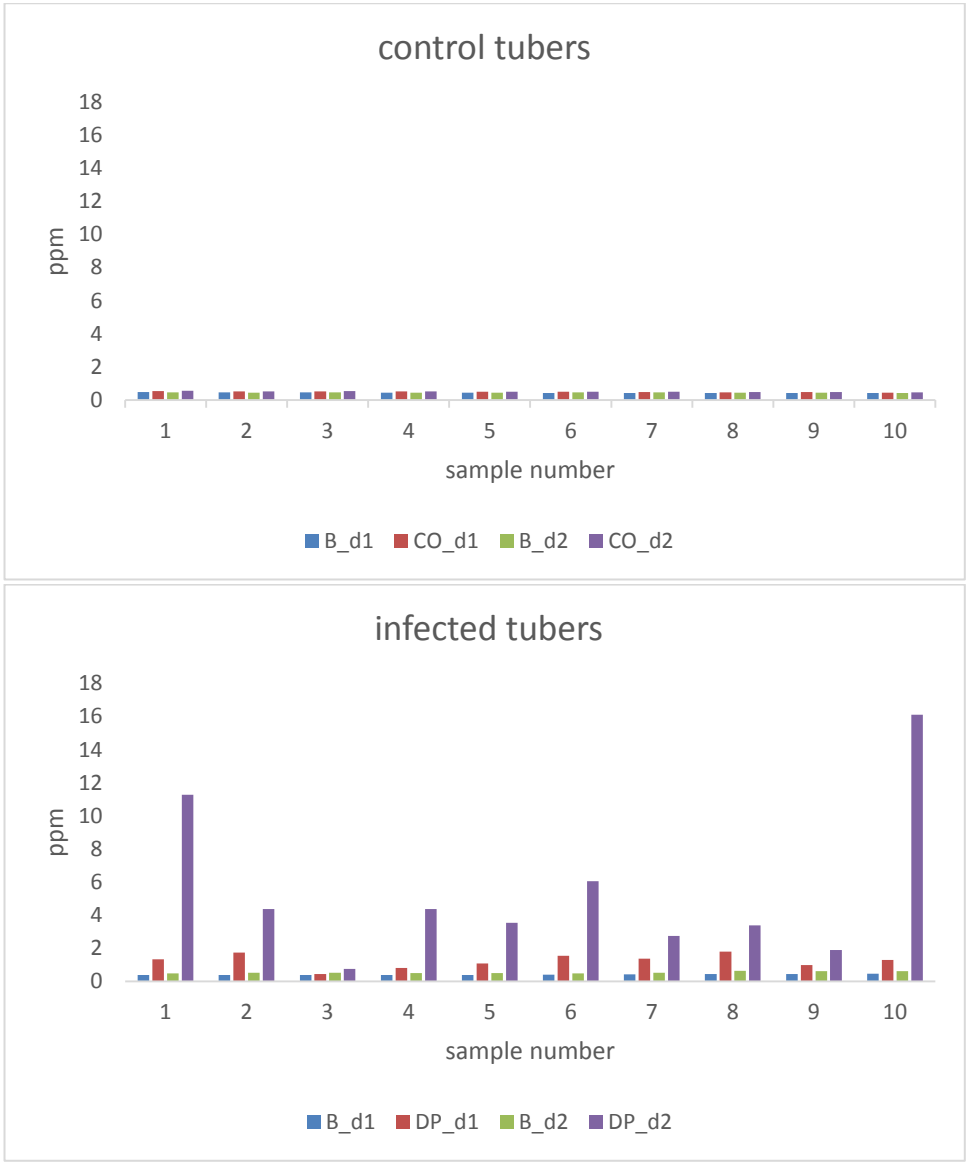


Fig. 4.14**.** Data results for the Tiger VOC analyser for 'early detection' time point, i.e. 2 DPI (days post inoculation. "CO" refers to control, "DP" to infected tuber, "B_" to background reading before sampling, "_d1" and "_d2" to the first and second day of sampling. 5m was the period of storage of tuber in the PTFE jar before sampling. The abscissa indicate potato tuber sample number while the ordinate instrument values in ppm (parts per million).

4.4 Metal oxide gas sensors response to 'detection' and 'early detection' time points

Fig. 4.15 shows a bar plot with cumulative values for the extracted features from all sensors employed for sampling (with standard error over the sample class), for both time points, 'detection' and 'early detection'.

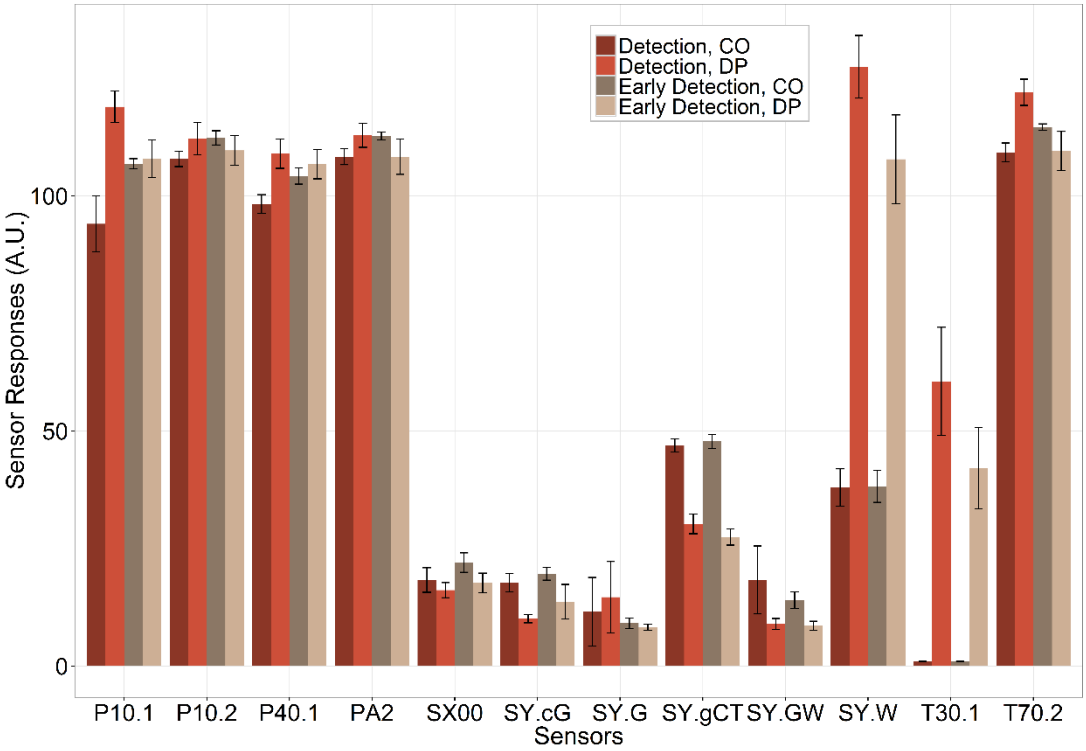


Fig. 4.15. Bar plot of raw data indicating differences in responses for all sensors at two time points ('tp'). 'CO' indicates healthy controls and 'DP' to diseased potato tubers. The error bars represent standard errors of the mean values. The sensors nomenclature refers to Alphamos FOX 200-4000 Manual Release 4.0.1.

Fig. 4.16 shows PCA scores and k-means clustering for the time point referred as 'detection' and indicates the features extracted for all the raw sensors data. Fig. 4.17 is the equivalent biplot for the first two principal components, which accounts for most of the predictor's variance (79.8 %) in the data set. The biplot also indicates that most of the variance characteristic for the two groups can be attributed to a few MOX sensors among those comprising the original array of the FOX3000 electronic nose, namely SY.W, T30.1 and SY.gcT. SY.W is reported to be responsive to hydrocarbons, while T30.1 to solvent, alcohol and polar compounds. The first sensor, SY.W, might be indicative of response to hydrocarbons pertaining to presence of disease, such as ethene, as indicated by Lui (Lui et al. 2005). Acetone vapour or ethanol, that the second sensor would be detecting, appear to be the only common polar solvents that were identified in past studies with GCMS (Kushalappa, Lui, Chen, & Lee, 2002; Lui et al., 2005; Varns & Glynn, 1979). The other sensor of interest, labelled as SY.gcT, is reported to be responsive to hydrocarbons in a similar fashion to SY.W, but is produced by a different manufacturer. In this latter case, it may be very possible that this response may be related to general organic decomposition, in a similar manner to landfill gas emissions. The final results for the subset of sensors, which have been selected as representative of a potential detection system for soft rot, are shown Fig. 4.18.

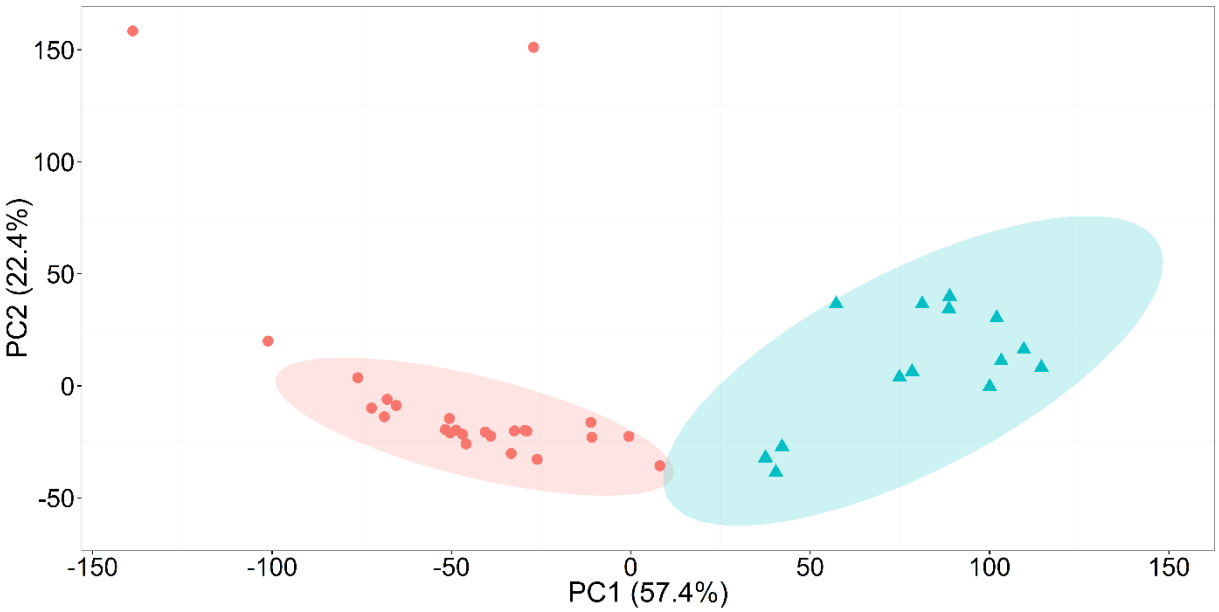


Fig. 4.16. PCA score and k-means plot with 95% confidence intervals based on CMOS technology measurements for all sensors at time point 'detection'. Data points indicate healthy controls (red, circles) and diseased potato tubers (cyan, triangles).

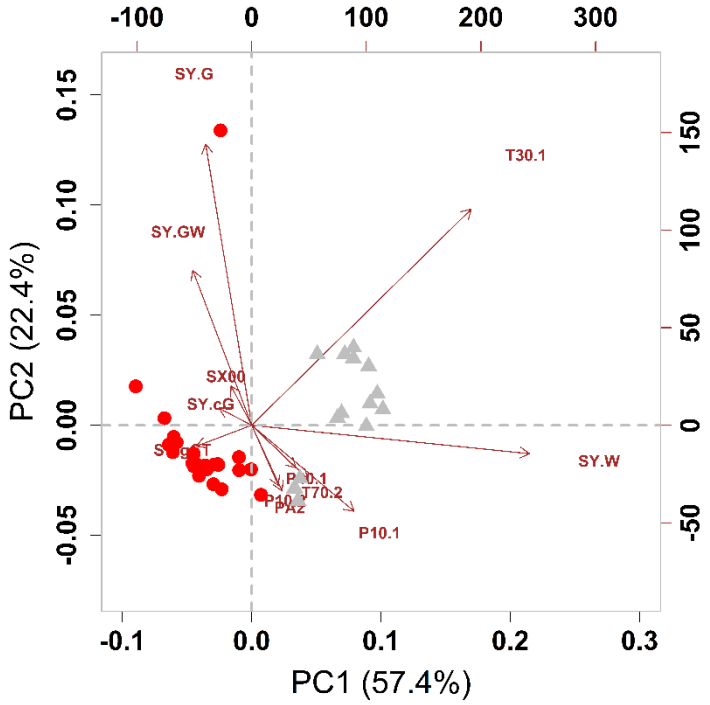


Fig. 4.17. Biplot for all sensors at time point 'detection'. Data points indicate healthy controls (red, circles) and diseased potato tubers (cyan, triangles).

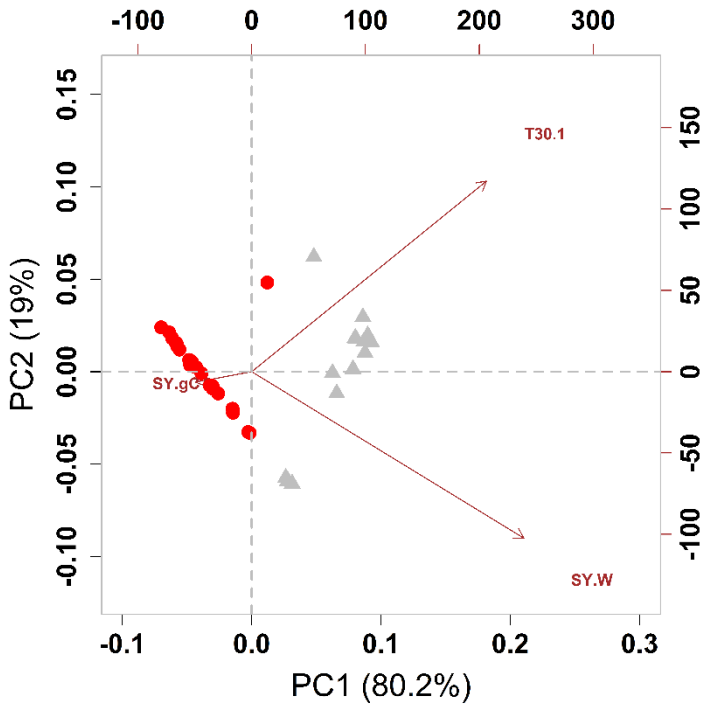


Fig. 4.18. Biplot for selected sensors (SY.W, T30.1, SY.gcT) at time point 'detection'. Data points are as indicated in Fig. 4.17.

A similar approach to the one described above was carried out for the second time point, 'early detection'. The experimental outcome is shown in Fig. 4.19 and Fig. 4.20 for all data points. As found previously, a smaller set of sensors can be selected for this detection time point (Fig. 4.21). For 'early detection', discrimination between healthy controls and infected tubers was achieved with variance related to the chemical substances identified previously with the later 'detection' time point. Further data analysis was also done by means of the features extracted using fewer sensors (SY.W, T30.1 and SY.gcT). For both time points, again various models were selected and comparison carried out across different techniques using the same resampling approach (k-fold cross validation). All of the modelling techniques showed very high accuracy, sensitivity and selectivity, with the positive class being the control tubers.

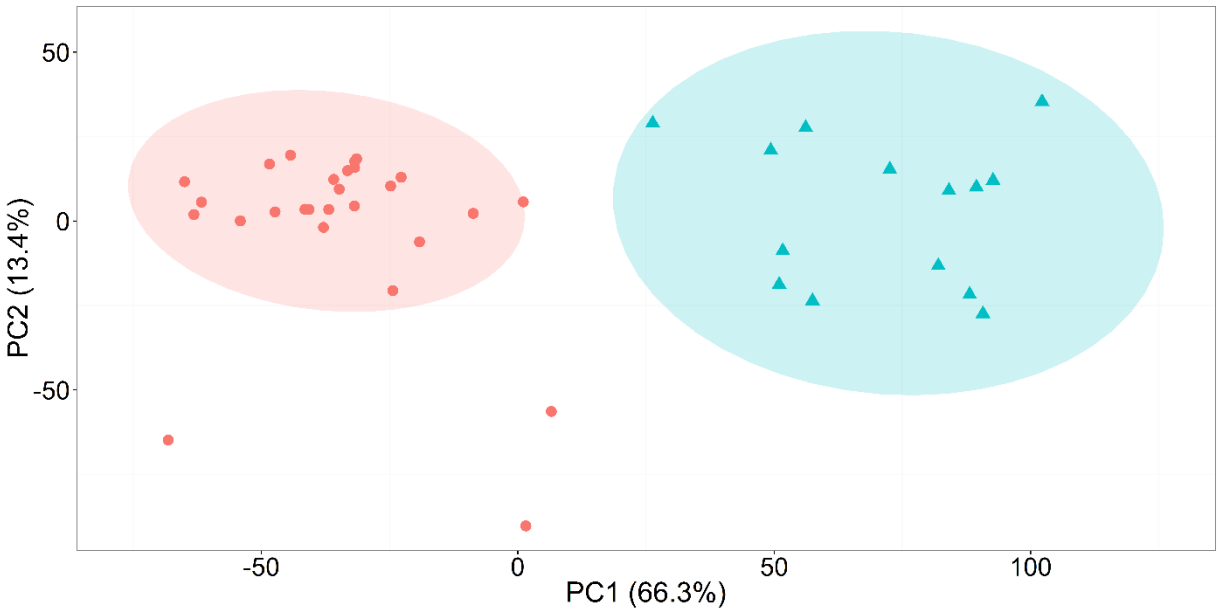


Fig. 4.19. PCA score and k-means plot with 95% confidence intervals based on CMOS technology measurements for all sensors at time point ‘early detection’. Data points indicate healthy controls (red, circles) and diseased potato tubers (cyan, triangles).

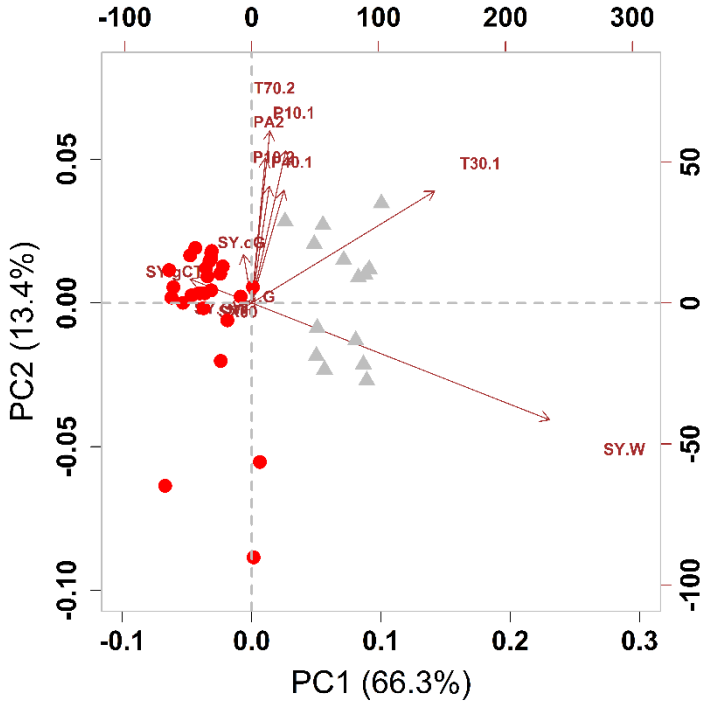


Fig. 4.20. Biplot for all sensors at time point ‘early detection’. Data points indicate healthy controls (red, circles) and diseased potato tubers (cyan, triangles).

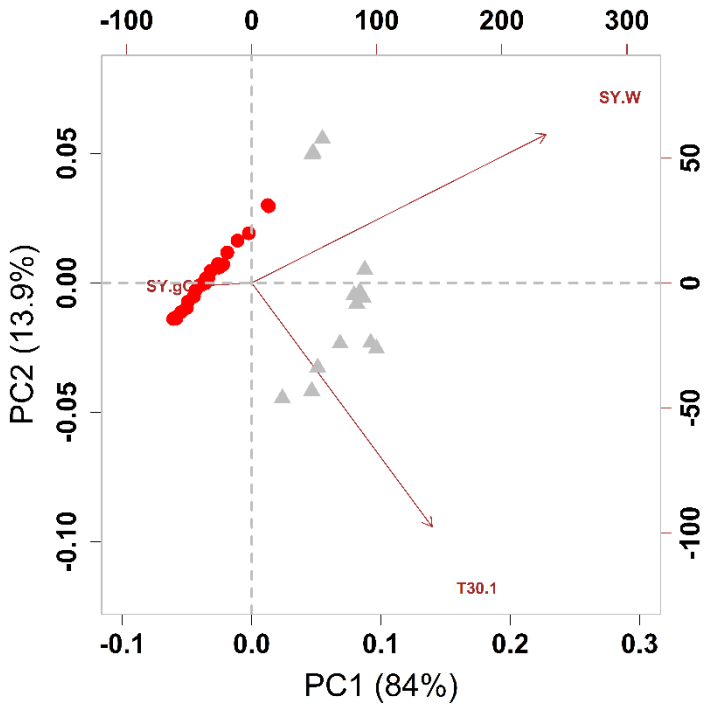


Fig. 4.21. Biplot for selected sensors (SY.W, T30.1, SY.gcT) at time point 'early detection'. Data points are as indicated in Fig. 4.20.

4.5 Electrochemical/NDIR gas sensors response to 'detection' and early detection time points

As in previous section for metal oxide sensors, Fig. 4.22 shows a bar plot with cumulative values for the extracted features from all sensors and for both the time points 'detection' and 'early detection'.

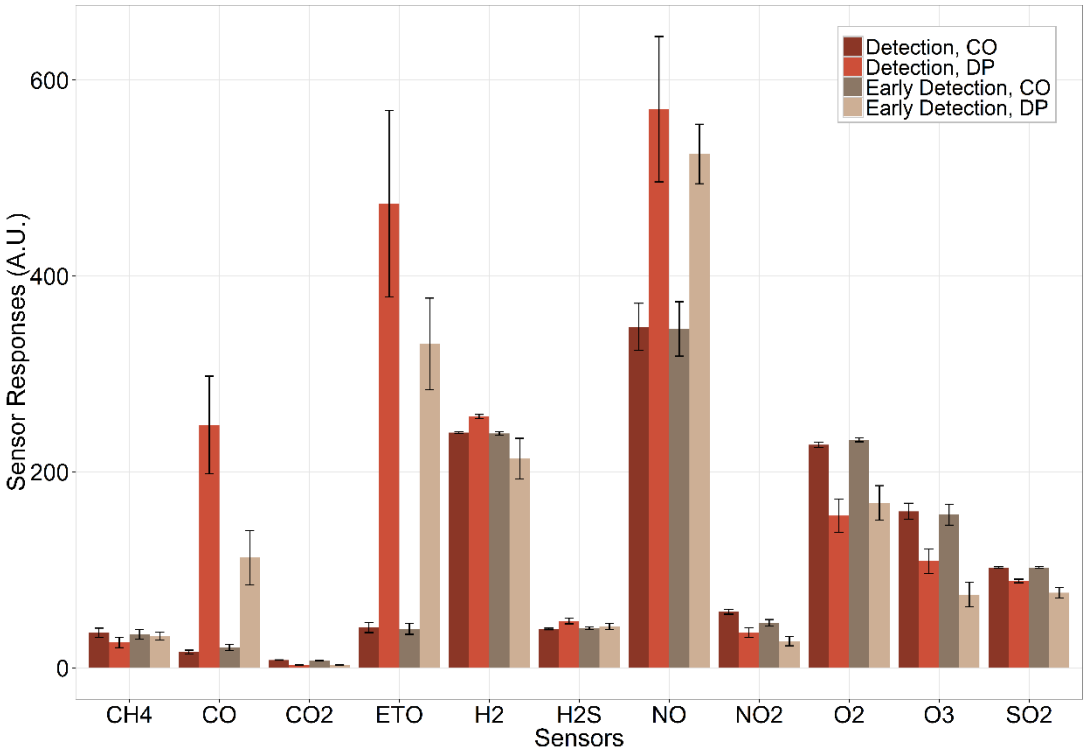


Fig. 4.22. Bar plot of raw data indicating differences in responses for all sensors at two time points ('tp'). 'CO' indicates healthy controls and 'DP' to diseased potato tubers. The error bars represent standard errors of the mean values. The sensors nomenclature refers to the chemical compounds to which sensors are responsive.

Fig. 4.23 (PCA scores and k-means for time point 'detection') indicates the features extracted for all the raw sensors data, while Fig. 4.24 is the equivalent biplot for the first two principal components, which accounts for most of the predictor's variance in the data set. The biplot indicates that most of the variance in the data set can be attributed to a three sensors, namely Carbon Monoxide (CO) Ethylene Oxide (ETO) Nitric Oxide (NO). None of these chemicals were identified by other researchers in past studies with the use of GCMS (Kushalappa, Lui, Chen, & Lee, 2002; Lui et al., 2005; Varns & Glynn, 1979). Of particular interest is the relative abundance of carbon monoxide in the presence of tubers infected with soft rot. The final results for the subset of sensors, which have been selected as representative of a potential detection system for soft rot, are shown in Fig. 4.25.

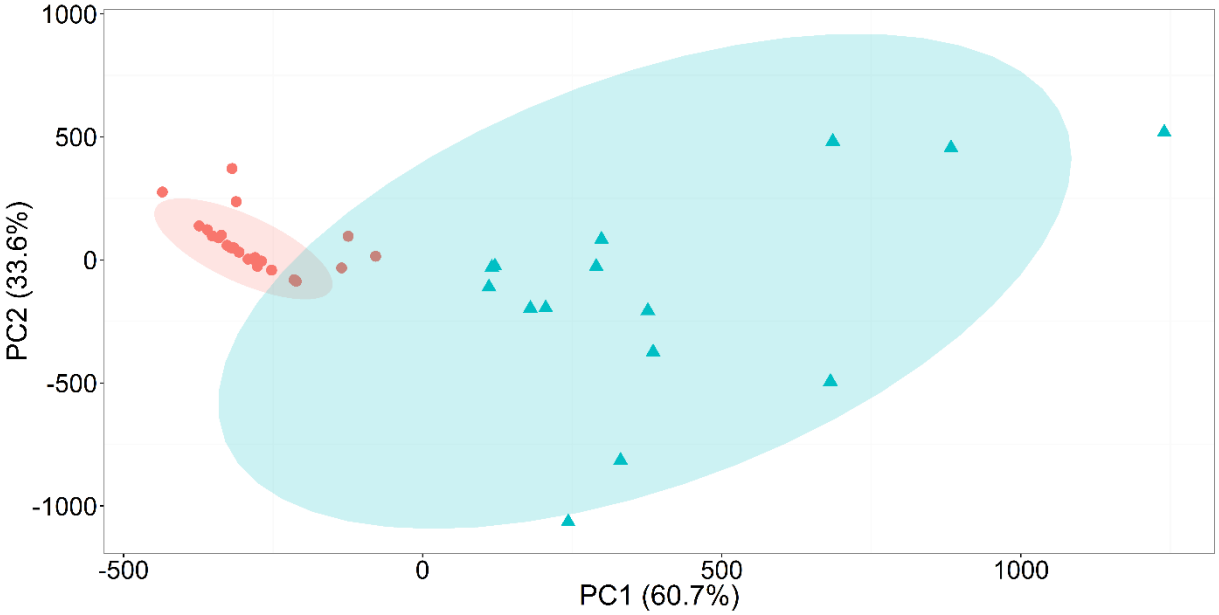


Fig. 4.23. PCA score and k-means plot with 95% confidence intervals based on electrochemical gas sensors technology measurements for all sensors at time point 'detection'. Data points indicate healthy controls (red, circles) and diseased potato tubers (cyan, triangles).

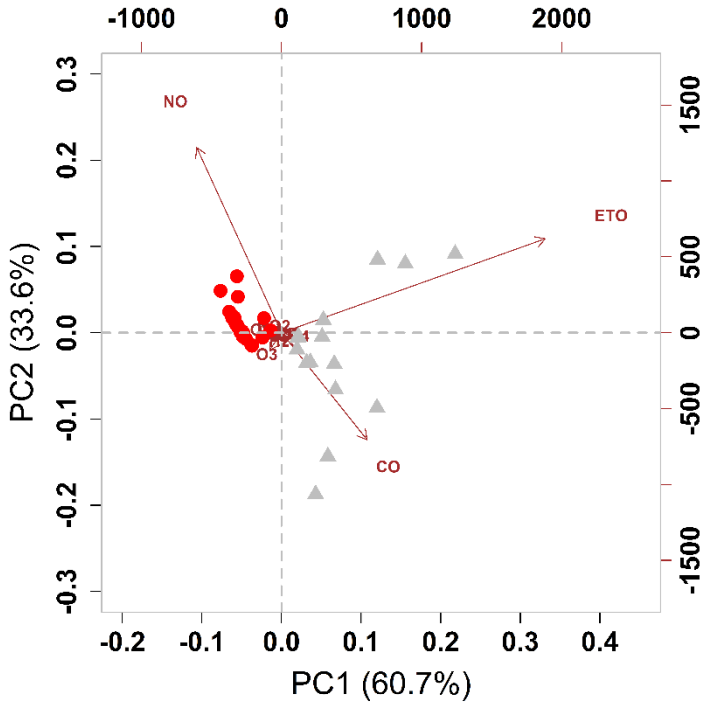


Fig. 4.24. Biplot for all sensors at time point ‘detection’. Data points indicate healthy controls (red, circles) and diseased potato tubers (cyan, triangles).

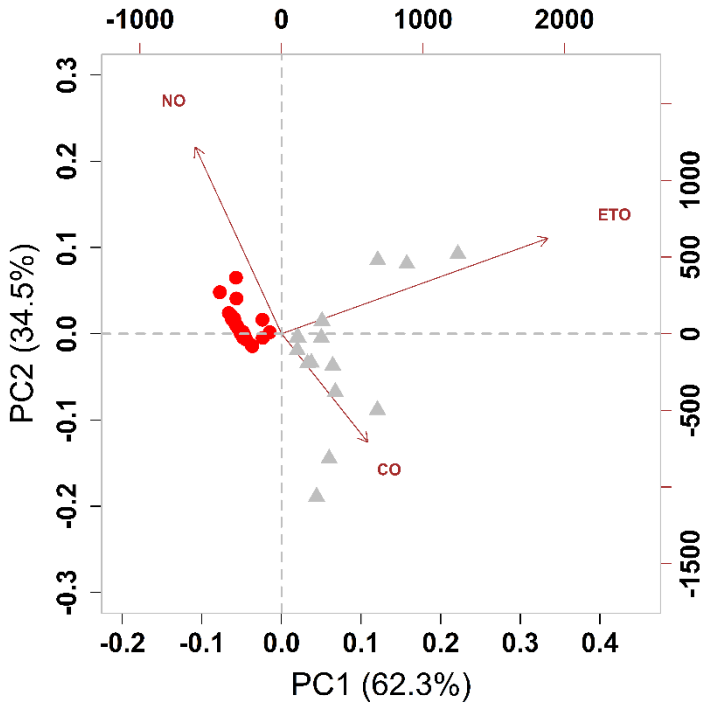


Fig. 4.25. Biplot for selected sensors (CO, ETO, NO) at time point ‘detection’. Data points are as indicated in Fig. 4.24.

The experimental outcome for the second time point, ‘early detection’ is shown Fig. 4.26 and Fig. 4.27 for the whole data set. As in the previous case, sensors were shortlisted and they are

reported in Fig. 4.28. These sensors are Carbon Monoxide (CO), Ethylene Oxide (ETO) and Nitric Oxide (NO). For both 'detection' and 'early detection' these three sensors yield the same discrimination between healthy controls and infected tubers, albeit with a varying degree of variance related to the accumulation of chemical substances related to disease progression. For both time points, various models were also selected and comparison carried out across various models and by employing the same resampling approach (k-fold cross validation). All of the models showed very high accuracy, sensitivity and selectivity, with the control tubers being the positive class.

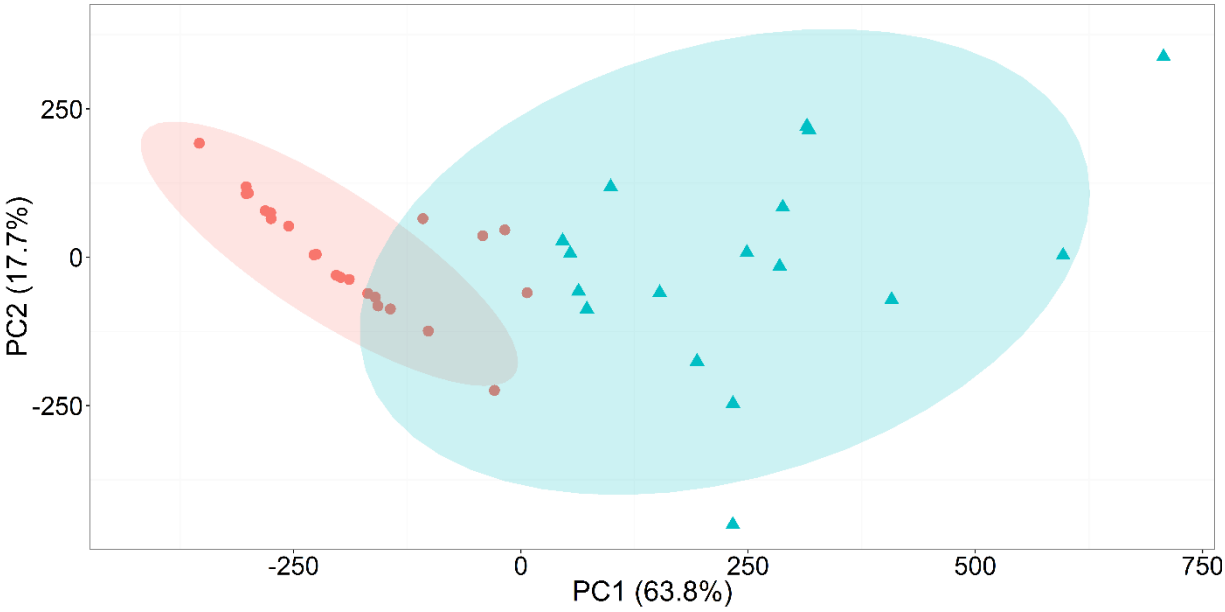


Fig. 4.26. PCA score and k-means plot with 95% confidence intervals based on electrochemical gas sensors technology measurements for all sensors at time point 'early detection' Data points indicate healthy controls (red, circles) and diseased potato tubers (cyan, triangles).

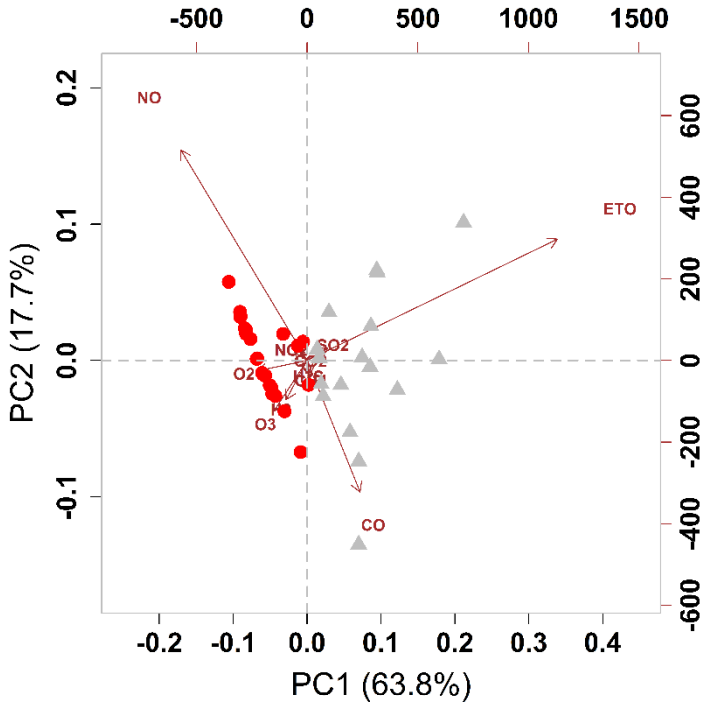


Fig. 4.27. Biplot for all sensors at time point ‘early detection’. Data points indicate healthy controls (red, circles) and diseased potato tubers (cyan, triangles).

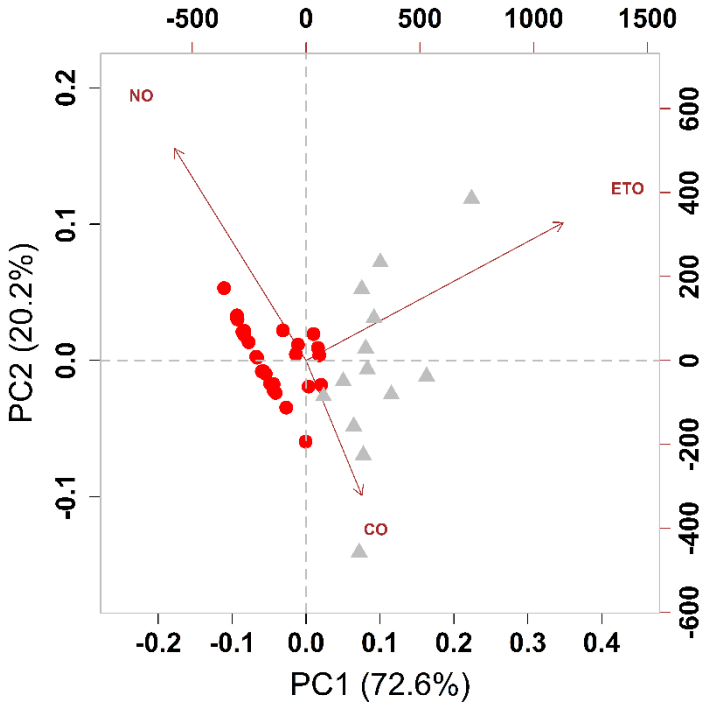


Fig. 4.28. Biplot for selected sensors (CO, ETO, NO) at time point ‘early detection’. Data points are as indicated in Fig. 4.27.

5. DISCUSSION

Identification of soft rot infection with the Lonestar FAIMS was achieved for samples 5 days post inoculation ("disease detection") and after allowing for rapid disease progression, by storing potato tubers at 25ºC in a humid environment. Discrimination between infected tubers and controls was also achieved for samples 48 and 24 hours post inoculation ("early disease detection"). The instrument yielded similar results in both cases, under the same experimental and data analysis conditions, thus indicating the potential of the technology not only for disease identification (at 5 days post inoculation) but also for early diagnostics (1 and 2 days post inoculation) in laboratory conditions. As explained earlier, it should be noted that the classification of results into two groups "standard" and "early" was aimed at answering two core objectives of the work. The first being if the technology could yield any result, and if so, how it could be benchmarked with current practices of identification employed by farmers (sensorial analysis). The second aim was to identify how early this identification could occur when the other approach failed. It has been shown that when no symptoms were identifiable by sensorial analysis (tactile, olfaction or visual inspection) of potato tubers. Once established that both 48 and 24 hours were equally suitable for the time point "early detection", the former was selected in order to facilitate soft rot determination by sensorial analysis at the termination of experimental work, as in the case of the Tiger PID instrument.

Another important consideration is the fundamental differences between FAIMS and PID technologies. Photoionization detection (PID) relies on the principle that the chemical substances of interest are in gaseous/vapour form below the ionization potential of the UV lamp employed (available only in few set values) will be ionized and consequently detected – without any selectivity. This implies that, unlike electronic noses and FAIMS, the Tiger PID device can then be only be employed to detect overall gas/vapour (below the ionization potential of the UV lamp) increase over time, which has been shown to be indicative of soft rot spread. Furthermore, commercial PID detectors can be found with specifications for both high sensitivity (to 0.5 ppb) and wide dynamic range (in the specific case of the Tiger instrument this ranges from 0.5 ppb to 20,000 ppm). This contrasts with the sensitivity and range of detection of the Lonestar FAIMS where excessive quantities of target chemicals may cause saturation of the sensor and consequently incorrect or unreliable readings, as occurred in earlier experimental work. In addition, FAIMS technology also suffers from a humidity intolerance, which is not as prevalent in other gas analysis technologies. On one side this implies that some compounds may not be identified because they cannot be properly ionized (and afterwards detected) and, on the other side, that humidity is an important parameter that can completely alter the results at the detector. For this reason, FAIMS requires continuous filtering of the inlet air flow. Hence the PID may be a more suitable solution for stores, whilst FAIMS as a very sensitive laboratory technology for soft rot disease identification.

In later work results also indicate that commercially available and cost-effective metal oxide and electrochemical gas sensing technologies are able to discriminate healthy controls from tubers infected with *P. carotovorum*. Detection of soft rot infection under selected laboratory conditions was achieved for samples at 5 and 2 days post inoculation, with similar results in both cases. In the former case, it was shown that the electronic noses employed are as good as current store practices for detecting soft rot symptoms. Furthermore, for the second time point it has been ascertained that, when no symptoms are identifiable by sensorial analysis, the instrument results in good discrimination between healthy controls and infected tubers and is therefore valuable for early detection of soft rot disease.

In the past forty years, the focus on potato soft rot in store has been placed on the identification and quantification of chemical compounds as possible biomarkers of disease presence and progression. Results of the current experimental work appear to suggest that recognition of soft rot, or for any other potato disease that may result in similar tissue breakdown and decay, is likely to be related to sensors detecting degradation of organic material, rather than specific compounds associated with the infection itself. Hence, these sensors may be useful in the detection of a range of potato storage pathogens that result in release of such general biomarkers of infection but may not be able be to specify either the pathogen causing the problem or the disease of interest. Moreover, while the research (with gas chromatography/mass spectrometry) on the chemical fingerprint associated to soft rot, commenced by Varns and Glyn (Varns & Glynn, 1979), may well offer a valuable quantitative analytical perspective, it suffers from the difficult task of dealing with the large number of compounds involved and their variation with environmental conditions that a crop produces prior and after harvest (Dixon et al. 2002; Fiehn 2002; Wilson & Wisniewski 1989). A corollary of the above consideration is that a sensor (or more) should be able either to measure effectively a total VOC increase or specific compounds.

This dichotomy (VOC or specific compounds) has been partially addressed in the previous study with FAIMS and PID where data indicated that soft rot can be detected regardless of the possible chemical emissions involved. A similar approach has been followed by de Lacy Costello *et al.* (de Lacy Costello et al. 2000), who claimed that the best biomarker for determination of soft rot inception is a general increase in VOC (volatile organic compounds) in the headspace over potato tubers, rather than in any specific chemical compound. As it has been shown in this piece of work, one of the sensors that appears responsive and disease specific is the one for ethanol/alcohols. However, no argument, investigation or experimental evidence was presented with regard to an increased need in sensitivity which would justify fabrication and deployment of custom-made sensors. Nevertheless, at least at given simulated experimental conditions, the authors show that in general metal-oxide and electrochemical gas sensing technology can be employed for detection of soft rot. At the current stage of the research is also less clear what are the processes involved in detection of carbon monoxide (with electrochemical gas sensors) associated to soft rot inception and spread. Finally, further and thorough experimental results with commercial sensors have shown that target compounds of chemical families could be potentially employed for soft rot monitoring either for different degree of VOC concentration (between controls and infected tubers) or as unique markers, as in the case of alcohols.

It should also be noted that all experimental results were obtained with a specific and widespread variety of potato tuber (Maris Piper). It is possible that other varieties of potatoes may produce different chemical signals as a result of the disease, which would require further investigation.

6. CONCLUSIONS

Past research on volatile profiling of soft rot has spanned a period of decades. The results showed no common consensus on specific biomarkers or approaches. Regardless of these contrasting views, experimental work for disease spread was always carried out by means of gas chromatography (GC) or gas chromatograph mass spectrometry (GC-MS). GC and GC-MS are well-established technologies and are used for VOC analysis due to a combination of high accuracy, selectivity, resolution and being the 'gold standard'. However, there are considerable drawbacks, including high costs of purchase and maintenance, the large number of variables involved for selection of parts prior to work, laborious manual processing of samples and complex data sets. These factors make this approach costly, impractical, time consuming and prone to errors. Therefore, GC and GCMS are less suitable for continuous monitoring of soft rot spread in controlled laboratory conditions, and even more inadequate to be applied in the challenging environmental conditions found in commercial potato stores.

However, there are a range of other technologies that could be deployed for potato storage. These appeared to offer new possibilities for determination and monitoring of soft rot (or more in general potato diseases). The original hypothesis was that these techniques could be employed to achieve early detection and management of the soft rot disease in a more practical and cost effective manner than GC/GC-MS, as done in the past. It is should be mentioned that these technologies are well established approaches in the other fields, whether in research or industry, and have been so over many years. The novelty in the current research resides in the application of these well-established technologies for detection of potato disease. Hence, when the hypothesis was originally formulated, a priori knowledge of chemicals involved in soft rot spread was deemed not necessary.

In this work, the hypothesis for gas analysis monitoring of disease spread was initially investigated with FAIMS (Field Asymmetric Ion Mobility Spectrometry). At this early stage of work, the need to accurately assess the technology emerged and a suitable experimental protocol for laboratory work was developed. The first objective of the protocol aimed to evaluate if the technology could successfully discriminate between controls and diseased tubers and how this could be benchmarked against sensorial analysis (tactile, olfaction or visual inspection), the common practice for identification of potato soft rot in store. The second objective aimed to evaluate if pre-symptomatic identification could be achieved when sensorial analysis proved ineffective.

The experimental outcome proved that early diagnostics via dynamic headspace sampling with FAIMS could be achieved both at symptomatic and pre-symptomatic stages. The results from the PID sensor substantiated and strengthened the original hypothesis that it was possible to employ gas analysis sensors for potato tubers disease monitoring at selected time points. Furthermore, both the Lonestar FAIMS and Tiger PID, as applied in this research, offered a considerable more practical and reliable engineering approach when compared to the more established techniques of GC/GC-MS, tools of previous experimental research.

Following these studies, further work was carried out with other main gas sensors technologies, usually used for industrial safety and environmental monitoring. The underlying motivation for choosing these techniques was to undertake a complete review of these different gas sensing technologies when applied to the detection of soft rot. In addition, if successful, how well did they work and if there were any drawbacks or trade-offs involved. This experimental work further

validated and supported the hypothesis that a range of different gas sensing technologies could be employed, at least in a controlled environment, for potato disease detection. This latter part of the research followed the same experimental method adopted and outlined in earlier chapters for both pre-symptomatic and symptomatic soft rot disease monitoring. However, unlike the early part of the work with FAIMS and PID, the larger number of sensors allowed some possibility to address selectivity to specific chemicals or chemical by means of readily available commercial detectors.

Further work (now in progress) will try to evaluate the possible deployment of the aforementioned technologies in commercial stores. In fact, in practical store conditions, ventilation, temperature and humidity can be modified depending on commercial use of the produce, store management practices and external environmental conditions. Variation of these conditions and effect on sensor response would probably be the most important aspect to be addressed in future work. Last, but not least, financial considerations will be taken into account in account. In fact, part, or all of these technologies, may provide to be technically feasible but financially prohibitive for any possible deployment in store.

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