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

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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.

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, AHDB Potatoes, 2012). 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 1970 s (Varns and Glynn, 1979; Varns and Shaw, 1973) and 1980 s (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.,

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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. 2C). 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.

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.

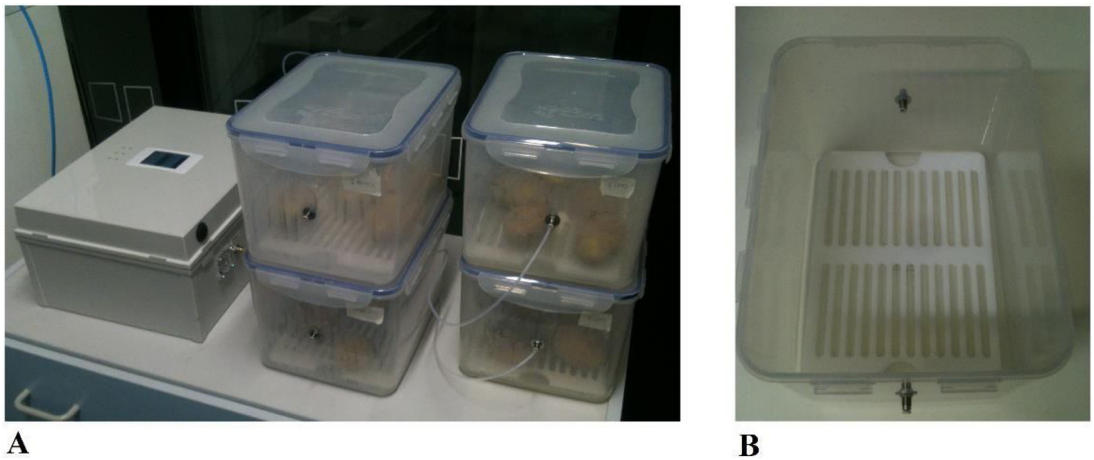

Fig. 1. Laboratory setup. (A) Environmental gas analyser connected to each of the four plastic containers via PTFE tubing. (B) Inside of each container with acrylic mesh on which tubers were suspended over water bath to maintain high humidity.