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Identification of volatiles generated by potato tubers (*Solanum tuberosum* CV: Maris Piper) infected by *Erwinia carotovora*, *Bacillus polymyxa* and *Arthrobacter* sp.

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Bacteria were isolated from internal tissues of surface sterilized healthy tubers of *Solanum tuberosum* cv. Maris Piper (8 different isolates) and from tubers inoculated with *Erwinia carotovora* ssp. *carotovora* showing soft-rot symptoms (3 different isolates), and identified by fatty acid profiling. *Bacillus polymyxa* and an *Arthrobacter* sp. were isolated from both sources, *E. carotovora* only from the soft-rotted tubers. The volatile organic compounds (VOCs) generated by tubers inoculated with *E. carotovora*, *B. polymyxa* and the *Arthrobacter* sp. were identified. Inoculated tubers of cv. Maris Piper were incubated under controlled humidity (95% relative humidity) and temperature (10°C) to simulate typical storage conditions. *B. polymyxa and Arthrobacter* sp. did not cause symptoms, whilst *E. carotovora* caused limited soft-rot infections after 4 weeks at the low temperatures typically associated with potatoes in storage. The VOCs released to the headspace around these tubers were collected using an adsorbent system and analysed by Gas Chromatography-Mass Spectrometry (GC-MS). Twenty-two volatiles unique to *E. carotovora* infection of potato tubers were found, including 10 alkanes, four alkenes, two aldehydes, one sulphide, one ketone, one alcohol, one aromatic, one acid and one heterocyclic compound. *B. polymyxa* generated three unique volatiles: *N,N*-dimethylformamide, 1-pentadecene and 1-hexadecane. Only one volatile, 2,3-dihydrofuran, was unique to the *Arthrobacter* infection. Production of volatile nitrogen species from *E. carotovora*-infected tubers increased with time, whereas none were detected in the headspace above uninfected tubers. Further analysis using a modified GC-MS method established that ammonia, trimethylamine and several volatile sulphides were evolved from tubers infected by *E. carotovora*. No specific volatile was useful as a marker associated with any of the three bacterial species but in the case of *E. carotovora*-infected potato tubers a significant increase in the volume of compounds evolved was clearly observed. The results are discussed in relation to the use of sensors to detect VOCs evolved from infected tubers in order to provide an early warning system for the control of soft rot in potato stores.

Keywords: *Erwinia*, gas chromatography–mass spectrometry, potato tubers, soft rot, volatiles.

Introduction

The rotting of potato tubers in stores is a major problem for the potato industry. Bacterial soft rot (*Erwinia carotovora*) is probably the principal cause and the most serious in terms of crop losses. Fungal rots are also important. Infection by the late blight pathogen *Phytophthora infestans* is particularly significant, not just because of the damage it causes *per se*, but because it also appears to provide potential sites for secondary infection by *E. carotovora*.

E. carotovora is strongly pectolytic and, under favourable conditions, can rapidly turn an infected tuber into soft, wet, rotten tissue, which carries inoculum to healthy tubers and spreads the infection rapidly throughout a store (Bradbury, 1986). Early detection of the disease is vital if it is to be controlled and this could be achieved using vapour sensors that detect volatile organic compounds (VOCs) produced by infection.

Previous studies of potato tubers infected by *E. carotovora* have identified a range of VOCs evolved during the progression of the disease (Varns & Glynn, 1979; Waterer & Pritchard, 1984; Maga, 1994) which might be useful indicators of the onset of infection. Many low molecular weight volatiles, especially alcohols, were evolved during infection (Waterer & Pritchard, 1984); for example, ethanol, methanol and butan-1-ol were found to increase rapidly as infection progressed (Varns & Glynn, 1979). However, these volatiles tend

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not to be disease specific and are also evolved when no infecting organisms are present (Maga, 1994).

Knowledge of the volatiles produced from infected and healthy potato tubers is critical in the development of sensor systems capable of detecting the onset of disease in storage.

Gas and vapour detectors are generally nonspecific and can respond to a range of odours (Cowell *et al.*, 1994). Array-based systems or 'artifical noses' (Craven *et al.*, 1996) have been developed to combat this nonspecificity. Essentially, each sensor in an array produces an output in response to its gaseous environment. Measurement of the output from each sensor in the array on exposure to an odour results in a pattern unique to that odour (Brezmes *et al.*, 1997). Patterns are normally interpreted using neural network or pattern recognition algorithms (Getine *et al.*, 1997). Knowledge of the components that comprise an odour is essential in rationalizing the array design and choice of sensors to produce maximum sensitivity and minimum redundancy. This enables the construction of task-specific 'electronic noses' rather than more general arrays that are capable of broad odour classification tasks (Amrani *et al.*, 1997).

Identification of the volatiles generated by potato tubers infected by a variety of micro-organisms will enable the development of a sensor system, based upon the volatiles detected, for the early detection of disease in stored potatoes.

In this study, the typical storage conditions in which soft rots are known to cause the greatest problems were emulated, and the volatiles released were identified in relation to the progress of infection from initial inoculation to expression of characteristic soft-rot symptoms. Preliminary experiments used sensors to detect *E. carotovora* infections of potato tubers stored at 25°C (de Lacy Costello *et al.*, 1996).

Materials and methods

Micro-organisms

Erwinia carotovora ssp. *carotovora* isolate 312 was obtained from the National Collection of Plant Pathogenic Bacteria (Central Science Laboratory, York YO4 1LZ). Micro-organisms were isolated from uninfected and *E. carotovora*-infected potato tubers by excising small pieces of tuber or soft-rotted tissue, respectively, and plating onto potato dextrose agar (PDA: Oxoid Ltd, Basingstoke, RG24 8PW). Bacterial isolates were purified and identified initially using the Schaad identification scheme (Schaad, 1988). Twenty-nine isolates were subjected to further identification by fatty acid profiling (Central Science Laboratory, York, YO4 1LZ). Isolates identified as *B. polymyxa* and *Arthrobacter* sp. were selected for further work.

E. carotovora was grown on PDA, *B. polymyxa* and *Arthrobacter* sp. on asparagine agar (dextrose 40·0 g, asparagine 2·0 g, potassium dihydrogen phosphate 0·5 g, magnesium sulphate heptahydrate 0·25 g, thiamine dichloride 0·005 g, agar 15 g, in 1 L distilled water; Johnston & Booth, 1983), all at 19°C.

Bacterial cell suspensions were prepared by adding 10 mL of sterile distilled water to 4-day-old cultures, gently shaking to produce a dense suspension, then decanting into sterile universal bottles.

Controlled environment apparatus

A controlled environment apparatus (Fig. 1) (Harrison & Lowe, 1989) was used to simulate the temperature and humidity found in potato stores as closely as possible. It consisted of two controlled temperature water baths, one containing the sample jars with potatoes and the other containing the humidifying vessels. Blended air was passed from a gas cylinder (MG Gas Products, Reigate, RH2 9QE) into the humidifying vessels. The humidity of the resultant stream of gas was controlled by the temperature of the first water bath containing the humidifying vessels. The humidified air, at 95% relative humidity, then flowed into the sample jars in the second bath, which was maintained at 10°C.

Inoculation of potato tubers

Tubers of *Solanum tuberosum* cv. Maris Piper were obtained from Sutton Bridge Experimental Unit (Sutton

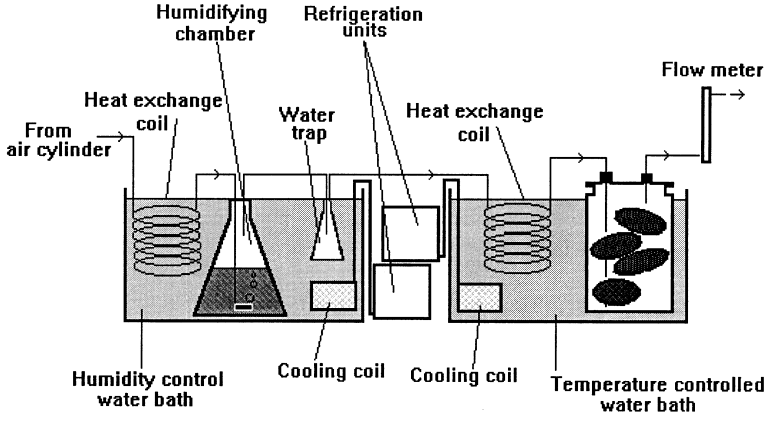

Figure 1 Schematic diagram of controlled environment apparatus

Bridge, Spalding, PE12 9YB). The tubers were washed in water to remove soil, then swabbed with 90% ethanol and dried in a laminar flow hood. Each tuber had a grid, consisting of two sets of four parallel lines orthogonal to each other and approximately 3 cm long by 1 cm deep, cut into the surface (skin) with a sterile scalpel before inoculation with either bacterial suspension (1 mL), or sterile distilled water for the control.

Inoculated tubers (approximately 0·9 kg per treatment) were placed in a sterile Quickfit jar (2 L) with a multiport lid. One port had a length of silicon tubing threaded through a thermometer adapter, which carried blended air to the bottom of the jar. A second port had a Quickfit tubing adapter connected to silicon tubing to allow air to leave the jar.

The jars were incubated for 12 h at 25°C, in the dark, before placing into the waterbath at 10°C and connecting to the humidified air supply, which was maintained at 50 mL min^{-1} per jar throughout the experiment. Such an air supply is typical of potato stores with forced ventilation (S. McDonald, Scottish Crop Research Institute, personal communication).

Adsorbent system for sampling volatiles

The adsorbent system consisted of lengths of deactivated glass-lined tubing (78 mm × 4 mm internal diameter) packed with a sandwich of Tenax TA (10 mg) (Jones Chromatography, Hengoed, CF8 8AU), Tenax GR (20 mg, 35/60 mesh) (Jones Chromatography) and Carbosieve III (20 mg, 60/80 mesh) (Supelco UK, Poole, BH12 4QV), contained by silanized glass wool plugs. The adsorbent tubes were conditioned at 250°C under a flow of nitrogen (100 mL min^{-1}) for 4 h prior to use. When not in use, the ends of the adsorbent tubes were sealed using Swagelok fittings and stored in a desiccated environment.

Volatile sampling and analysis

Volatiles were sampled after a period of four weeks. The sample was collected by passing air through the chamber containing the tubers for two hours, using the adsorbent system described. The adsorbent tubes were connected to the outlet of the sample jars via silicon rubber tubing with the Tenax TA end closest to the outlet. Volatiles were collected from each of the jars in turn at a constant flow rate of 50 mL min^{-1} and at 80% relative humidity. After collection, the adsorbent tubes were analysed using GC-MS as soon as possible. Where the analyses were not possible immediately, the tubes were capped and stored at −80°C. Volatiles from the control tubers (inoculated with sterile distilled water) were collected and analysed each time for comparison.

A Hewlett-Packard HP5890 series II GC coupled to a HP5971 Mass Selective Detector was used for vapour analysis. VOC fractionation was achieved using a Supelco SPB-1 Sulfur, fused silica capillary column (30 m, 0·32 mm i.d., 4 mm film thickness).

An improvised thermal desorption method was used to transfer the sample onto the GC column (P. R. H. Jones, UWE, Bristol, personal communication). A small loop (≈ 5 cm length) of the capillary column close to the injection end was placed in a beaker containing liquid nitrogen. The oven was held at ambient temperature and the injection port at 50°C. The glass liner of the injector port was removed and the adsorbent tube put in its place. The port temperature was then raised to 200°C as quickly as possible, to flash desorb the vapour onto the capillary column. After 10 min, the liquid nitrogen trap was removed. The oven was turned on to 40°C and held at 40°C for 150 s to allow air and water to pass through the system without damaging the mass selective detector (MSD) filament. The oven temperature was then programmed to rise from 40°C to 200°C at 4°C min^{-1} with a hold time of 5 min. The MSD was set up to record a scan range of 10–300 atomic mass units (amu).

Spectral analysis was carried out using proprietary Hewlett Packard software (G1034C, V 01·05) running the NBS75K library. The spectrum obtained was matched as the peak average with the baseline measured by a baseline drop method (where baseline drop was not measured, the integrator took a tangent as the baseline). The integrator was used with a minimum peak area of 0·1% and a maximum of 250 peaks per spectrum. Spectra were matched with and without baseline subtraction. Poorly matched peaks were evaluated manually where possible.

Total volatile nitrogen determination

A total volatile nitrogen (TVN) analysis was performed on *E. carotovora*-infected and uninfected tubers that had been incubated for 7, 14 and 21 days at 20°C to obtain a quantitative measure of the amines released (Sagastizabal, 1996).

A sample of potato tuber (20 g) was homogenized with distilled water (200 mL) using a food processor for each analysis. The mixture was then transferred to a round-bottomed flask (1 L) containing distilled water

Table 1 Identification, by fatty acid profiling, of bacterial isolates from tubers infected by *Erwinia carotovora* and from uninfected tubers

Identity by fatty acid profiling	Number of isolates	
	Infected tubers	Uninfected tubers
Erwinia carotovora	5	0
Arthrobacter sp.	3	1
Bacillus polymyxa	3	4
B. pumilus	0	4
B. cereus	0	3
B. megaterium	0	3
Bacillus sp.	0	1
Agrobacterium sp.	0	1
Cytophagus-Flavobacterium complex	0	1

Table 2 Volatile profiles of potato[a] tubers inoculated with *E. carotovora*, *B. polymyxa*, *Arthrobacter* sp. or sterile distilled water

RT[b]	Volatile	*E. carotovora*	*B. polymyxa*	*Arthrobacter* sp.	SDW
12·75	Acetone	+	+	+	
13·01	2-Propenal	+			
13·11	Acetone	+			+
14·61	2-Methyl-propanal	+			+
15·16	2-Methyl-pentane		+	+	+
15·67	3-Methyl-pentane				+
15·62	2-Butanone	+			+
15·71	Acetic acid	+	+	+	+
16·06	1-Hexene	+			
16·21	Hexane		+	+	+
18·22	1-Butanol	+			+
18·56	But-1-ene		+	+	
18·67	Cyclohexane		+	+	+
18·71	2-methyl-1-pentene		+	+	+
18·8	(E)-2-Butene				+
18·94	2-Methylhexane	+	+	+	+
18·99	2-Pentanone	+			+
19·16	2,3-Dimethyl-pentane				+
19·31	3-Methyl-hexane		+	+	+
19·35	2,4-Dimethyl-1-heptene				+
19·63	2,3-Dihydrofuran			+	
19·69	1,2-Dimethyl-*cis*-cyclopentane			+	+
19·74	(E)-2-Butenal				+
20·46	Heptane	+	+	+	+
21·32	Dimethyldisulfide	+			
21·40	Methyl-cyclohexane	+	+	+	+
22·18	*N,N*-Dimethyl-formamide		+		
23·05	Toluene	+	+	+	+
23·99	Hexanal	+	+		+
25·05	Octane	+		+	+
25·61	Octene	+			
26·47	2-Methyl-octane	+			
27·47	Ethylbenzene	+			+
27·87	Xylene	+	+	+	+
28·14	3,4-Dihydro-2H-pyran				+
28·42	3-Methyl-octane	+			
28·74	1,2,3-Trimethyl-cyclohexane	+			
28·82	1,2-Dimethyl-benzene	+			+
29·12	1-Methyl-2-propyl-cyclo-pentane	+			
29·15	1-Heptene	+			
29·37	1-Ethyl-4-methyl-cyclohexane	+			
29·65	Nonane	+			+
30·44	2,4-Dimethyl-hexane	+			+
31·16	Propyl-cyclohexane	+			+
31·94	1-Ethyl-2-methyl-benzene	+			+
31·97	Phenol	+			+
32·59	2-Methyl-nonane	+			+
32·86	3-Methyl-nonane	+			+
33·29	Octanal	+			
33·40	Trimethyl benzene	+			+
33·56	1-Ethyl-3-metyl-benzene		+	+	+
33·75	2,2,3,4-Tetramethyl-pentane	+			
33·83	1,2-Undecadiene	+			
34·08	Decane	+			+
35·03	4-Methyl-decane	+			+
35·30	Limonene	+			+
35·35	1,3-Pentadiene				+
35·53	(2-Methyl-propyl)-cyclohexane	+			+
35·85	1-Methyl-3-propyl-benzene				+
36·07	3-Methyl-bicyclo-[3.2.1]-oct-2-ene	+			
36·15	2-Methyl-decane				+

Table 2 (continued)

36·42	3-Methyl-decane	+			+
36·55	5,6-Dimethyl-decane	+			
37·26	1-Methyl-4-(1-methyl-ethyl-benzene	+			+
37·65	Nonanol	+		+	
37·77	3-Methyl-1-heptene	+			
40·16	2,9-Dimethyl-decane				+
41·40	Napthalene	+			
41·71	Decanal	+	+	+	+
41·88	2-Phenoxy-ethanol	+	+	+	+
42·21	Dodecane				+
43·97	1-Pentadecene		+		
45·74	Long chain aliphatic (Heptadecane?)	+			
46·16	Butanoic acid	+			
46·33	1(3H)*iso*benzo-furanone	+			+
49·22	Long chain aliphatic	+			
49·29	Hexacosane	+		+	
51·1	Phytol		+		+
52·57	3,4-Dimethyl-1-decene	+			+
52·63	3-Methylnonane	+			+
57·35	1-Hexacosanol	+			+
57·89	1-Eicosanol				+
58·35	((Dodecyloxy)methyl)-oxirane				+
58·91	3,5,24-Trimethyl-tetracontane				+
58·95	1-Chloro-tetradecane		+		+

[a] The tubers were incubated at 10°C and 95% relative humidity under a constant 50 mL min^{-1} flow of air for four weeks prior to sampling.
[b] RT: Retention time of compound in minutes.

(100 mL). Magnesium oxide (2 g, S.L.R, Fisons, Loughborough, LE11 0RG) was then added to the flask with a drop of silicon oil antifoaming agent (Fisons) and several antibumping granules. Boric acid (0·65 M, 50 mL, BDH/Merck, Lutterworth, LE17 4XN) was added to a conical flask with 6 drops of indicator solution prepared by adding methyl red (0·2 g) and methylene blue (0·1 g) dissolved in ethanol (99·75 v/v, 100 mL). The solution containing the homogenized potato was then heated using a Bunsen burner so that it boiled after 10 min. It was then distilled for 25 min at the same rate of heating, ensuring that the receiver tube from the condenser was immersed in the boric acid solution. After distillation, the condenser and delivery tube were rinsed with distilled water and the distillate was collected in the conical flask. Presence of volatile nitrogen compounds, such as amines, would produce a colour change from purple to green. The distillate was then titrated to a clear end point against a standardized solution of sulphuric acid (0·1 N, BDH/Merck). The equation used for the calculation of TVN (in mg of nitrogen per 100 g of tuber) was:

$$TVN = (Vs - Vb) \times 14 \times (10/M) \times (N/0{\cdot}1)$$

where Vs = sample titre, in mL, of standardized acid, Vb = blank titre, in mL, of standardized acid, M = mass in grams of potato tissue, and N = normality of standardized acid.

Modified GC-MS method for detection of volatile amines and other volatiles

Gaseous amine standards of ammonia, methylamine, ethylamine, dimethylamine, diethylamine and triethylamine (0·01% v/v, Aldrich, Gillingham, SP8 4XT) were sampled as headspace (20 mL, Hamilton gas tight syringe), and injected into the GC-MS to optimize the column parameters.

Solid phase microextraction (SPME) polydimethylsiloxane/divinylbenzene fibres (65 mm, partially cross-linked, Supelco UK, Poole, BH12 4QH) were conditioned thermally prior to sample exposure by insertion into the GC injector port (270°C, 30 min). The fibre was exposed to the sample headspace and the entrained analytes thermally desorbed in the injector port (270°C, 4 min) under splitless conditions.

Standards of the amines used (0·01% v/v) were purged onto the entrapment systems to determine their efficiency, using charcoal-purified air.

SPME passive sampling was conducted above the headspace of potato samples (approx. 5 g) sealed in deactivated HP headspace sample vials (20 mL) for 5 min. Dynamic sampling was used with Chromosorb 103 and Tenax adsorbents, manufactured as before. Samples of potato tuber (20 g) sealed in deactivated chambers (20 mL) were purged with blended air (200 mL min^{-1}, for 10 min). GC-MS operating conditions were as before except that the detector was turned on earlier to detect amines.

Results

Micro-organisms

Fatty acid profiling of the 11 bacterial isolates from *E. carotovora*-infected potato tubers revealed that five

were *E. carotovora*, three were *Arthrobacter* spp. and three were *B. polymyxa* (Table 1).

Fifty-six isolates were collected from uninfected tubers. Preliminary identification using the Schaad scheme and visual comparison of morphological characteristics of cultures reduced this number to 18 for fatty acid profiling (Table 1).

Volatile profiles from potato tubers inoculated with bacteria

E. carotovora

Fifty-seven volatiles were identified as being associated with *E. carotovora*-infected potato tubers at four weeks after inoculation (Table 2). Of these, 22 volatiles were identified solely with the infected tubers, whereas 17 were also found with uninfected tubers. The volatile profile contained 25 alkanes, 10 aromatic species, seven alkenes, five aldehydes, three ketones, three alcohols, two acids, one heterocycle and one sulphide.

Uninfected tubers generated 36 different volatiles, of which five were found to be unique. These were 3,4-dihydro-2H-pyran, 1-methyl-3-propylbenzene, 2-methyl decane, 2,9-dimethyldecane and 2,9-dimethyldodecane.

B. polymyxa *and* Arthrobacter *sp.*

Of the 22 volatiles associated with *B. polymyxa*-inoculated potato tubers (Table 2), three (*N,N*-dimethylformamide, 1-pentadecene and 1-hexadecane) were not obtained from uninoculated control tubers. *Arthrobacter* sp. produced 20 volatiles (Table 2), of which one (2,3-dihydrofuran) was unique to tubers inoculated with this species.

Total volatile nitrogen determination

Evidence of amine production was obtained from the results of TVN analysis. A colour change from purple to green was observed in the solution containing the indicators on distillation of *E. carotovora*-infected potato tubers, but there was no colour change on distillation of healthy tubers.

Calculation of TVN showed that no volatile nitrogen was present in fresh tubers but that there was a steady increase in the amount of volatile nitrogen species present in inoculated tubers (Table 3).

Table 3 Total volatile nitrogen (TVN, in mg of nitrogen per 100 g of tuber) analysis for uninfected and *E. carotovora*-infected potato tubers incubated at 20°C postinoculation

	TVN[a]	
	Titre 1	Titre 2
Uninfected potato tuber	0·0	0·0
Infected tuber: 7 days	35·0	40·6
Infected tuber: 14 days	77·7	91·7
Infected tuber: 21 days	109·9	114·8

[a] Table contains repeat titrations for each time interval.

Further identification of amines and other volatiles

The presence of amines and sulphides in the headspace of *E. carotovora*-infected tubers that had been cultured at 20°C was determined by the modified GC-MS method. Ammonia, trimethylamine, dimethylsulphide, dimethyldisulphide and dimethyltrisulphide were detected in significant amounts along with acetone, ethanol and butan-1-ol.

Discussion

B. polymyxa is pectolytic and commonly found in soils, although it is not considered to be an active plant pathogen (Bradbury, 1986). It is frequently associated with plants and can cause rots, although normally this is only observed at high temperatures and/or where there is a lack of oxygen. Other *Bacillus* spp., such as *B. megaterium* and *B. cereus*, have been isolated previously from potato tubers, but did not cause obvious symptoms (Hollis, 1951).

It is interesting that both *B. polymyxa* and the *Arthrobacter* sp. were also isolated from internal tissues of surface sterilized tubers that appeared healthy. These bacteria, together with the other isolates listed in Table 1, are therefore likely to be endophytes (Gunson & Spencer-Phillips, 1994) that may contribute to the profile of volatiles emitted following *E. carotovora* infection. For this reason GC-MS analysis was used to analyse volatiles from potato tubers inoculated with *E. carotovora*, *B. polymyxa* and the *Arthrobacter* sp.

It should be noted that the GC-MS method used did not detect some volatiles, such as methanol, ethanol and acetaldehyde, which elute before the water peak has passed through the system. Preliminary work and that of other workers showed ethanol, methanol and acetaldehyde to be present with both infected and uninfected potato tubers (Waterer & Pritchard, 1984). For this reason, they were not considered to be significant as potential marker volatiles for a soft-rot detection system, even though ethanol was previously identified as one of the most significant vapours when *E. carotovora* infection occurs. Concentrations were shown to rise by as much as 2000 times with infection (Waterer & Pritchard, 1984), whilst other alcohols such as propan-1-ol remain at relatively constant levels. Other volatiles are less well documented.

The concentration of VOCs evolved from tubers infected by *E. carotovora*, *B. polymyxa* and *Arthrobacter* sp. was very low at 10°C, in contrast to higher temperatures, necessitating preconcentration of the samples taken. This methodology enabled sufficient VOCs to be collected for analysis. A qualitative study was aimed at ascertaining whether marker volatiles could be identified for the three infecting species. In parallel studies at room temperature, *E. carotovora*-infected tubers rapidly softened and became macerated. Progress of disease was far slower at 10°C resulting in only small isolated areas of rot after 4 weeks incubation. Relatively few volatiles were identified after tubers were

inoculated with *B. polymyxa* and *Arthrobacter* sp. No evidence of infection or damage was visible on the tubers after four weeks, suggesting that the bacteria were not pathogenic under the conditions employed in this study.

No single compound could be used as a marker for a specific bacterial infection of tubers but it appears that an overall increase in volatiles, as described by other workers (Waterer & Pritchard, 1984), could suffice to indicate the presence of *E. carotovora* in a stored crop of potatoes and, qualitatively, the balance of volatile components, coupled with the significant increase in total volatile organics, should permit differentiation between the presence or absence of disease in stores. In a parallel study, infections by *Fusarium coeruleum* and *P. infestans* were observed to progress far more slowly than that by *E. carotovora,* and did not appear to generate volatiles at the same rate of production (R. Wheatley *et al*., Scottish Crop Research Institute, personal communication).

The presence of volatile amines in the headspace above rotting potatoes has not been previously reported, although it would seem likely that the micro-organisms would decarboxylate amino acids to form amines. Most of these amines would remain within the tuber as protonated salts because of the relatively low pH.

Tubers inoculated with *E. carotovora* and incubated at 20°C for one week produced volatile amines, while the control tubers produced no volatile amines, or negligible amounts. Ammonia, trimethylamine, dimethylsulphide, dimethyldisulphide and dimethyltrisulphide were detected in significant amounts by a modified GC-MS method. Some potato stores are still maintained at ambient temperatures and therefore the detection of these volatile amines and sulphides could give valuable information about the onset of soft rot in such stores.

In an early warning system to detect the onset of *E. carotovora* infection, sensors responsive to a broad range of volatiles, such as amines, sulphides, alcohols, hydrocarbons and aldehydes, are required. The array of sensors may be capable of detecting the significant increase in volatiles as a primary indicator of disease and, when coupled with pattern recognition software, could identify the causative agent.

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