Ouellette, E., Raghavan, G.S.V. and Reeleder, R.D. 1990. Volatile profiles for disease detection in stored carrots. Can. Agric. Eng. 32:255-261. Volatile profiles emanating from stored carrots inoculated with Sclerotinia sclerotiorum (lib.) de Bary and Botrytis cinerea Pers. were monitored using a dynamic headspace analysis method to investigate their possible use in a system for detection of storage disease. Small containers filled with carrots were purged continuously with purified air and the metabolic volatiles adsorbed on porous polymer traps (Chromosorb 105). Subsequently, the volatiles were thermally desorbed and analyzed by gas chromatography. Comparisons between headspace volatile profiles revealed that at least one compound was specific to each infection. The identities of several normal carrot metabolites were determined by gas chromatography-mass spectrometry.

INTRODUCTION

Background

In the area of vegetable storage, much research effort has been devoted in determining optimum storage conditions and how these conditions may be maintained economically. As a result, large commercial storage facilities capable of preserving produce quality over longer periods are now common. Nevertheless, losses due to storage diseases incurred by Québec’s carrot storers remain high. Losses are estimated to range from 5 to 30 percent from year to year (personal communication, P. Sauriol, Agronomist, Le ministère de l’agriculture, des pêcheries et de l’alimentation du Québec). In 1984 specifically, a thorough survey carried out on major commercial holdings in Québec revealed that average losses were of the order of 20% corresponding to a farm value of about 2 million dollars. Since modern warehouses are larger and often loaded in bulk, the task of detecting disease outbreaks for rapid intervention is much greater and requires immediate attention from researchers.

Scope

The monitoring of headspace volatile metabolites in stored vegetables has been proposed as a technique to detect diseases (Varns and Glynn 1979). The concept is really a technological improvement of smelling the storage atmosphere. It assumes that the use of precise analytical instruments to follow changes in the volatile profiles produced by the crop during storage has the potential to replace the human nose and other existing detection methods advantageously. The ability to monitor headspace volatiles may: (a) allow early disease detection and identification (Varns and Glynn 1979); (b) give an objective measure of the level of infection; (c) become a source of permanent farm records which enable comparisons of crop performance from year to year; and (d) evolve as an integral component of an automatic storage control system (Schaper et al. 1984). Attempts to verify these assumptions have been reported on several agricultural crops such as peanuts and cereal grains (Abramson et al. 1980; Richard-Moulard et al. 1976; Lee et al. 1973).

Studies on potato volatiles

Major studies in this area have been conducted on stored potatoes. Changes in the volatile profiles of potatoes infected with different bacterial diseases were observed and some compounds identified as possible indicators of storage disease development (Waterer and Pritchard 1984a,b; Varns and Glynn 1979). These findings led to the development of a prototype system to sample and analyse headspace air in ventilated warehouses (Schaper et al. 1984).

Although headspace analysis techniques have been used to distinguish carrot cultivars, to determine their residual storage life (Rasekh and Kramer 1971), and in aroma analysis (Simon et al. 1980a,b), no experiments on stress detection in stored carrots by means of volatile monitoring were found in the literature.

Objectives

In this study, volatile monitoring was applied to the storage of carrots. The objectives were to: (a) Develop a method of collecting and analyzing headspace volatiles emanating from small lots of carrots; and (b) Determine whether this method can detect compounds that are specific to two important carrot storage diseases, Sclerotinia sclerotiorum (lib.) de Bary and Botrytis cinerea Pers., for possible use in a storage disease detection system.

MATERIALS AND METHODS

General

A dynamic headspace analysis technique (Numez et al. 1984) was utilized in order to simulate ventilated storage bins. Purified air (the purge gas) was continuously swept through small lots of carrots placed inside sealed containers. The emerging gas flow was passed through a suitable polymeric adsorbent and vented to the atmosphere. The headspace volatiles were removed from the gaseous effluent by adsorption onto the
trapping medium and subsequently thermally desorbed for examination by gas chromatography (GC).

**Experimental Setup**

The experimental set-up was designed to achieve comparative collection of headspace volatiles. Ten acrylic cylindrical containers having a capacity of 7.7 litres each and dimensions as shown in Fig. 1 were connected to a manifold via individual 3.2 mm O.D. Teflon tubings. Purified air originating from a pressurized cylinder was passed through a 6 m x 3.2 mm O.D. column of molecular sieve (type 5A; 60/80 mesh, Alltech Associates Inc.) to improve its purity and introduced into the manifold (Fig. 2). The manifold outlets were calibrated to supply each container with an equal rate of air flow. The air was then swept through the carrots inside the container and vented to the atmosphere after passage through parallel traps of porous polymer adsorbent. Because the setup was not totally volatile-free, a blank container was used to distinguish the extraneous volatiles from those emanating from the stored produce. The containers and the manifold were housed inside a controlled environment cabinet maintained at 3°C. This design is similar to that of Spence and Tucknott (1983).

**Trap construction and conditioning**

The traps located on the container lids were used to sample and concentrate the volatiles released by the stored carrots. They were constructed from stainless steel tubes 88 mm long by 3.2 mm O.D. and packed with 80 mg of 60/80 mesh Chromosorb 105. Batches of 20 traps were simultaneously prepared by screwing the tubes onto a manifold block supplied with a stream of oxygen-free Helium (200 ml/min; 10 ml/min per trap) and placed inside an oven. Prior to the beginning of the experiment, the traps were conditioned at 200°C for 24 hours. After each use, they were reconditioned for 12-16 hr at 170°C.

*Fig. 1. Construction details of one 7.7 liter container.*

*Fig. 2. Schematic of the experimental setup.*
Volatile collection

Root inoculation. Fungal inocula were used to produce diseases in carrot roots. They were prepared from sectioned roots that were first autoclaved in 500 ml Erlenmeyer flasks for 30 minutes at 100 kPa and 100°C. The sterile sections were then inoculated with a 5 mm diameter potato dextrose agar (PDA) disk of actively growing cultures of *S. sclerotiorum* (isolate #) or *B. cinerea* (isolate #) (Dhingra and Sinclair 1985).

Healthy carrots (*Daucus carota* L.) of the cultivar Charger, previously stored for 5 to 6 months at 1°C, were washed in sterile water. Nine of ten containers were filled with approximately 3 kg of carrots each. A carrot section (about 15 mm long) covered with *S. sclerotiorum* was inserted among the roots in three containers. *B. cinerea* inoculum was added in a similar fashion to three other containers. The control treatment consisted of three containers of non-inoculated roots. The tenth container was left empty to assess the quality of the purge air during the experiment.

Collection procedures. All containers were tightly closed, placed inside the controlled environment cabinet at 3°C, and connected to the manifold. The flow rate of air to each container was arbitrarily set to 20 ml/min. The trap screwed into the container lids were replaced every 4 days and the flow rates recalibrated to 20 ml/min. The removed traps were wrapped in aluminum foil to avoid contamination during handling and stored until analysis at 1°C. The experiment lasted 32 days and was repeated once.

In the second trial, two pieces of inoculum rather than one were used in the *B. cinerea* treatment to encourage more disease development. Once the experiment was completed, the degree of infection was assessed on the basis of a count of invaded roots.

GC analysis

Analyses were performed on a Hewlett-Packard 5980A Gas Chromatograph with a flame ionization detector. The unit was modified to allow direct introduction of the traps into the injector port for thermal desorption of the volatiles (Murray 1977). Each trap stored at 1°C was first brought to ambient temperature, purged with 10 ml/min of helium at 25°C for 1 min and then heated at the injector temperature for 6 min. The volatiles were recondensed on a short pre-column packed with 5% OV-101 on 60/80 mesh Chromosorb-W cooled through cryogenic cooling. Instant injection into the column was achieved by rapid heating of the cold zone with a probe maintained at 80°C. Separation of the volatiles was obtained with a 60 m x 0.75 mm I.D. glass column coated with Supelcowax 20M. Conditions of analysis are summarized in Table I. A Hewlett-Packard 3390A reporting integrator recorded the detector output.

The separated volatiles were reproduced as peaks on a chart (chromatogram) and recognized according to their retention time. The chromatograms were actual graphical displays of volatile profiles from stored carrots. The approach followed in this study was to discern the compounds (or the peaks) that were unique to carrot diseases. To achieve this, comparisons of the chromatograms obtained from non-inoculated and diseased carrots were made on the basis of a match of corresponding peaks. No quantitative analysis was performed.

<table>
<thead>
<tr>
<th>Sample introduction (injector port):</th>
</tr>
</thead>
<tbody>
<tr>
<td>temperature: 160°C</td>
</tr>
<tr>
<td>purge gas: Helium</td>
</tr>
<tr>
<td>purge gas rate: 10 ml/min</td>
</tr>
<tr>
<td>coolant: liquid Nitrogen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gases:</th>
</tr>
</thead>
<tbody>
<tr>
<td>carrier gas: Helium</td>
</tr>
<tr>
<td>carrier flow rate: 10 ml/min</td>
</tr>
<tr>
<td>make-up gas: Helium</td>
</tr>
<tr>
<td>make-up flow rate: 25 ml/min</td>
</tr>
<tr>
<td>air flow rate: 300 ml/min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature program:</th>
</tr>
</thead>
<tbody>
<tr>
<td>isothermal at 70°C for 5 min</td>
</tr>
<tr>
<td>70-160°C at 2.5°C/min, and</td>
</tr>
<tr>
<td>isothermal at 160°C for 4 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Detector:</th>
</tr>
</thead>
<tbody>
<tr>
<td>type: flame ionization</td>
</tr>
<tr>
<td>temperature: 220°C</td>
</tr>
</tbody>
</table>

GC-MS analysis

Analyses combining gas chromatography and mass spectrometry were carried out to identify the volatiles collected. The commercial thermal desorber attached to the GC-MS unit was designed to accept larger trap tubes than those described earlier. These tubes were made of stainless tubes 76.2 mm-long by 6.4 mm O.D., packed with 130 mg of a different polymeric material, 60/80 mesh Tenax GC. For GC-MS analysis, the adsorbed samples were transferred from the small traps to the larger ones. To achieve the transfer, the GC column was disconnected and a Tenax GC trap screwed onto a reducing union connected to the pre-column. The same unloading procedures described earlier were followed except that the pre-column was neither cooled nor heated but maintained at room temperature. The volatiles were simply re-adsorbed in the Tenax GC traps.

The GC-MS unit was a Varian 3700 gas chromatograph connected to a Finnigan-Mat 312 mass spectrometer with theINCONS Data System. Volatile separation was achieved with a shorter column but lined with the same coating, that is, a 30 m x 0.75 mm glass column coated with Supelcowax 10. Chromatographic conditions were different than those reported for the GC runs and are summarized in Table II along with those of the mass spectrometer. Details on the GC-MS unit are described elsewhere (Anonymous 1986). Identification of the compounds were tentative. Their spectra were compared with those present in the Finnigan Library (National Institute of Standards and Technology, Washington, DC).
Table II: Gas Chromatographic and Mass Spectrometry (GC-MS) conditions.

Gas Chromatography:

Sample introduction (CDS 320 concentrator):
- temperature: 250°
- purge gas: Helium
- purge gas rate: 30 ml/min

Gases:
- carrier gas: Helium
- carrier flow rate: 3 ml/min

Temperature program:
- isothermal at 50°C for 5 min,
- and 50-200°C at 10 ml/min

Mass Spectrometry:

Ion source:
- ionizing voltage: 70EV
- accelerating voltage: 3kV
- ionizing multiplier: 2kV

Mass scan: 40 to 400; 2 sec/scan
Resolution: 1000

Temperature:
- ion source: 250°C
- capillary interface: 250°C

RESULTS AND DISCUSSION

Volatile collection method
The experimental setup designed for this study permitted the simultaneous collection of headspace volatiles from nine samples. The samples were small lots of carrots enclosed in vented containers to simulate ventilated storage bins. This arrangement produced comparative volatile profiles from carrots stored in controlled conditions.

The basic component of the headspace analysis technique was the trap. It facilitates pre-concentration of the volatile compounds. Figure 3 shows a typical background pertinent to this study. Chromatogram A is the background of a clean trap before volatile collection (i.e., after reconditioning). Chromatogram B is the background after a 96 hr collection period. Each peak on the chromatogram consists of a volatile compound that has been differentiated by the analytical method. The compounds that produced the chromatogram were volatile metabolites produced by the sample under study, volatiles released by the materials utilized in the experimental setup, or volatiles produced following thermal or oxidative breakdown of the porous polymer during the analysis procedure (Murray 1977). Impurities in the purge gas were also trapped. These compounds from external sources were classified as the background of the system.

In studies of this nature, it is important to maintain the background signal to the lowest possible level. To minimize it, in addition to using a trapping medium that gives acceptably low and consistent backgrounds (Murray 1977), the experimental setup should be built only with materials which are, for practical purposes, chemically inert (i.e., glass, stainless steel, etc) and purged with gas of high purity. Varns and Glynn (1979) found it necessary to clean the equipment components they used by placing them in a vacuum oven to reduce the background.

Using the volatile collection method described here, at least 30 compounds in the headspace above small lots of stored carrots were detected. The technique appeared to provide improved separation and detection capability over other techniques, since only 12 compounds were reported in a previous study on stored carrot (Rasekh and Kramer 1971). Long collection periods (4 days) resulted in the generation of more

Fig. 3. Two chromatograms representing the background of the volatiles collection system.
A: Background of a clean trap. 1: Acetone; B: Background for the carrot experiment. 1: Acetone; 2: Dichloro methane; 3: Ethanol; 4: 2- methyl-2-propenic acid, methyl ester

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peaks by allowing the accumulation of trace components such that the detection limit of the GC analysis was reached. Nevertheless, the volatiles found almost certainly represent only a small fraction of the number of metabolites produced by the crops (Waterer and Pritchard 1985).

Disease specific responses

In this study, special attention has been focused on volatiles which could be considered as disease indicators in stored carrots. The criteria for the selection of volatiles to be used in a disease detection strategy have been discussed (Varns and Glynn 1979; Waterer and Pritchard 1984a,b). Compounds showing greater potential were those specifically produced by diseased samples and not by healthy ones. They were recognized by comparison of the chromatograms obtained from inoculated and non-inoculated treatments.

Comparisons of the carrot volatile profiles revealed that four compounds were of significant importance. Compounds c and d, represented as peaks in the chromatograms shown in Fig. 4, were only detected in the headspace of carrots infected with *S. sclerotiorum* and *B. cinerea*, respectively. Compound c, identified as dichloro benzene, appeared 16-20 days after the beginning of the experiment and remained detectable until the end. Compound d, whose identity was not determined, was detected right from the first analysis but faded away and completely disappeared after 24 days in the first trial and after 12 days in the second trial. These production sequences confirmed visual observations indicating that *B. cinerea* did not stay metabolically active in the environment provided in the experiment, while conditions were more favorable to the growth of *S. sclerotiorum*. The difference in the time of appearance (or disappearance) of the compounds of interest between the two trials were due to an accidental rise in the cabinet temperature (up to 30°C) that occurred during the first few days of trial 1. Compounds a and b (Fig. 4) were found in all profiles. The former was part of the background and was identified as 1,3,5-tris(methylene)-cycliheptane. On the other hand, b represents a volatile identified as methyl(1-methylenyl)-benzene and evolved from inoculated and non-inoculated roots. Both compounds served as reference marks on the chromatograms.

Here is an example of how a disease monitoring system might operate. Compounds a and b would keep the same function and act as reference gases. The role of compound a

![Fig. 4. Complete and truncated chromatograms illustrating disease-specific responses from inoculated carrot roots. A: Complete chromatogram of healthy carrot roots (control); B: Truncated chromatograms of diseased roots. B.1: Control; B.2: *S. sclerotiorum*; B.3: *B. cinerea*. Compounds: a: 1,3,5-tris(methylene)-cycliheptane; b: methyl(1-methylenyl)-benzene; c: dichloro benzene; d: unidentified.](image)
would be the one of an internal standard to check for leaks or other defects in the volatile collection apparatus. Compound b, a normal metabolite of stored carrots, would be used to normalize changing storage conditions related to, for example, management practices and external climatic influences on the storage atmosphere (Schaper et al. 1984; Varns and Glynn 1979). Finally, the concentration of the disease specific gases, compounds c and d, would indicate the degree of disease infection. The data collected would be processed and presented in terms of gas concentration ratios (Schaper et al. 1984; Varns and Glynn 1979).

The damage caused by the fungi as assessed after the completion of the experiment is reported in Table III. In the control treatment, some roots showed signs of decay caused by *Alternaria dauci* and *Penicillium* Link. Minor secondary invasions by *Erwinia* were also noticed. Carrot dormancy was broken as indicated by the growth of adventitious roots and leaflets. Handling of the carrots at room temperature for several hours prior to the experiment and their storage at 3°C (instead of the recommended 1°C), combined with the fact that the carrots utilized were approaching the end of their storage life, may have triggered this reaction. It is believed that the invasion of unwanted microorganisms was minimal and did not cause noticeable shifts in the volatile profiles. Visual inspections revealed that the interruption of the dormancy affected all nine samples equally. Data in Table III also show that variation in disease progression among the replicates within the same treatment was minimal.

**Table III: Number and percentage of healthy and diseased carrot roots per treatment after the completion of each trial.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Healthy</th>
<th>Diseased</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39</td>
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<td>0</td>
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<td>0</td>
<td>36</td>
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<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>S. sclerotiorum</td>
<td>102</td>
<td>94</td>
<td>13</td>
<td>115</td>
</tr>
<tr>
<td>B. cinerea</td>
<td>31</td>
<td>30</td>
<td>82</td>
<td>113</td>
</tr>
</tbody>
</table>

1 roots invaded by other microorganisms.
2 trial number.
3 replicate number.
4 standard deviation.
5 percentage of the total number of carrots in a given treatment.

**Other carrot volatiles**

The normal metabolic volatiles detected in the headspace of stored carrots are listed in Fig. 5. Of the 20 volatiles identified by GC-MS analysis, none have been reported in aroma studies (Simon et al. 1982, 1980a, b; Salunkhe and Do 1976; Buttery et al. 1968). However, in these studies, the volatile constituents were obtained by destructive methods (e.g. steam distillation-extraction; Buttery et al. 1968) of freshly harvested carrots and not from intact roots stored in normal atmosphere for several months. Identification was tentative and not confirmed with standard reference components.

CONCLUSIONS

Based on the results observed during the course of this study, it may be concluded that:

1. The technique of headspace analysis developed allowed the comparative collection of headspace volatiles emanating from nine ventilated samples simultaneously;

2. One metabolic volatile was unique to each carrot fungal infection. A compound, identified as dichloro benzene, was only detected above roots inoculated with *S. sclerotiorum*. The identity of the compound responsible for the specific response of roots inoculated with *B. cinerea* could not be determined.

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REFERENCES


