VOLATILE MONITORING AS A TECHNIQUE FOR DIFFERENTIATING BETWEEN *E. CAROTOVORA* AND *C. SEPEDONICUM* INFECTIONS IN STORED POTATOES[1]

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Abstract

The volatile production characteristics of ring rot (*Corynebacterium sepedonicum*) and soft rot (*Erwinia carotovora*) infected potatoes were investigated under controlled conditions in order to assess the monitoring of volatiles as a technique for differentiating between the infections in stored potatoes. Total volatile production/unit time was much greater from soft rot due to more rapid development after infection. Total volatile production during the period required for the development of the soft rot infection was similar to the output detected during the longer period required for the development of the ring rot infection. The volatile profiles of the two pathogens featured a number of commonly shared metabolites, but one compound was unique to the ring rot infections and two compounds were unique to the soft rot infections. There were also consistent disease-specific changes in the relative concentration of many of the metabolites detected in the volatile profiles.

Resumen

Las características de producción de sustancias volátiles en papa afectada con podredumbre anular (*Corynebacterium sepedonicum*) y pudrición blanda (*Erwinia carotovora*) fue estudiada bajo condiciones controladas con el fin de evaluar el registro de sustancias volátiles como método para diferenciar entre estas infecciones en papa almacenada. La producción total de sustancias volátiles por unidad de tiempo fue mucho mayor para la pudrición blanda, debido a un desarrollo más rápido después de la infección. La producción total de sustancias volátiles durante el tiempo que toma el desarrollo de la infección por la pudrición blanda fue similar a la detectada durante el período más largo que toma el desarrollo de la infección por podredumbre anular. Los perfiles para sustancias volátiles de los dos patógenos tuvieron un cierto número de metabolitos en común, pero un compuesto se presentó únicamente en el caso de la podredumbre anular y dos sólo con la pudrición blanda. Hubieron también cambios uniformes específicos en la concentra-

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ción relativa de muchos de los metabolitos en los perfiles de sustancias volátiles para cada enfermedad.

Introduction

The potato processing industry depends upon the year-round availability of supplies of high quality stored potatoes. Trends toward larger storages have necessitated proper monitoring of the storage to minimize losses due to storage diseases.

Varns and Glynn (7) suggested that prompt disease detection in potato storages might be achieved by monitoring the storage atmosphere for specific volatile metabolites arising from any host-pathogen interactions. In tests conducted under laboratory conditions and in commercial storages, they identified 3 compounds (acetone, ethanol, and 2-butanone) that consistently occurred at abnormally high concentrations in the headspace above potatoes that were infected with soft-rot bacteria [*Erwinia carotovora* (Jones) Bergey *et al.*]. The authors (9) were also able to follow the development of a soft rot infection by the monitoring of changes in the profiles of volatiles emanating from the potatoes.

Although prompt detection of any disease problem is a vital first step in the minimization of storage losses, the correct identification of the specific disease problem is also important. The disease management and control options available may vary considerably depending upon the characteristics of the pathogen, such as the potential for spread and sensitivity to temperature changes. Consequently, the value of volatile monitoring as a system for disease detection in storages would depend upon the identification of volatiles or volatile production patterns diagnostic of specific diseases.

In North America, bacterial soft rot (*E. carotovora*) is probably the most destructive disease of stored potatoes (1, 4). As much as 300,000 tonnes of potatoes may be lost annually in the United States to bacterial soft rot during storage (8). Under suitable conditions, the disease may cause rapid decay of infected tubers, with adjacent healthy potatoes becoming infected by the bacterial ooze produced by the decaying tubers (4).

Bacterial ring rot [*Corynebacterium sepedonicum* (Spieck. and Kott.) Skapt. and Burkh.] presents a serious threat to the crop in virtually every potato producing country (2, 6). During storage, contaminated tubers exhibit a characteristic slow internal breakdown of the vascular ring (5). During handling, the infected tubers may collapse, spreading a highly infectious bacterial ooze over adjacent tubers and the handling equipment. Ring rot is a particularly serious problem during the storage of seed potatoes due to the potential for extensive contamination of the seed stock resulting from the decay of a few scattered tubers (3).

The purpose of this study was to compare the profiles of volatiles and the volatile production characteristics of ring rot (*Corynebacterium sepe-*

donicum) and soft rot (*Erwinia carotovora*) infections of potatoes under controlled conditions in order to assess volatile monitoring as a technique for differentiating between these infections in stored potatoes.

Materials and Methods

Sound Russet Burbank potatoes (stored 1 to 8 months at 10°C weighing 200 ± 5 g were washed, dipped for 30 minutes in a 1% solution of a quaternary ammonia disinfectant (Teramine: West Chemical Products, Montreal, Que.), and then rinsed in sterile water.

Nutrient-broth swirl cultures were inoculated with isolates of *E. carotovora*[3] and *C. sepedonicum*[4] bacteria. After 48 h of incubation at 21°C, the *E. carotovora* cultures reached a population of about 1.0×1.0^7 colony forming bodies (CFB)/ml, while the *C. sepedonicum* cultures contained about 3.0×10^5 CFB/ml. Each tuber was inoculated with 1 ml of one of the bacterial suspensions, after the cultures had been diluted to produce a uniform bacterial population (1.0×10^5 CFB/ml). A syringe was used to distribute the inoculum over 25 puncture points. Tubers which had been similarly wounded but not inoculated were used as controls.

Approximately 1 kg lots of the treated tubers were placed in gas-tight, low-porosity, polyethylene/polyvinyl chloride copolymer bags (3.2 L capacity, 0.08 mm thickness, Dow Chemical Canada, Toronto, Ont.). Filtered, volatile-free air was pumped into the bags to produce a headspace volume of 2.5 L. The bags were incubated at 22°C in the dark.

After each 24 h incubation period, the volatiles in a 1.8 L sample of the bag headspace were concentrated using a previously described sampling system (9) by adsorption on traps containing 180 mg Chromosorb 105 absorbent (Johns-Manville Co., Port Credit, Ont., 60/80 mesh). The traps were cooled to 1°C during collection of the volatiles in order to increase compound retention. After sample collection, the trap was introduced into the pre-heated (200°C) injector port of the gas chromatograph (GC). The heat of the injector port caused the instantaneous desorption of the volatiles from the trap. The carrier gas moving through the injector port carried the desorbed volatiles onto the analytical column of the flame-ionization detector-equipped GC. Analytical column characteristics and chromatographic conditions have been described elsewhere (9).

Immediately after the collection of each headspace sample, the incubation bags were completely evacuated. The fresh, filtered, volatile-free air for the next 24 h period was then metered into the bag.

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[3]Mixture of *E. carotovora* var. *carotovora* (Jones) Dye and *E. carotovora* var. *atroseptica* (Van Hall) Dye isolates provided by G. Platford, Manitoba Department of Agriculture, Winnipeg, Manitoba.

[4]Provided by G.A. Nelson, Agriculture Canada Research Station, Lethbridge, Alberta.

The bags were sampled until the pathogen had completely disrupted the tubers. Six replicates of each treatment (soft rot, ring rot, and wounded controls) were prepared. The experiment was conducted twice.

Results

Disease development occurred at substantially different rates in the soft rot and ring rot inoculated potatoes. The first visual indications of decay in the soft rot-inoculated tubers became apparent by day 3 of the incubation period, while the first signs of decay in the ring rot treatments were not observed until about day 7. About 9 days were required before the tubers were completely disrupted by the ring rot pathogen; it took the soft rot bacteria only 5 days to produce an equivalent degree of tissue damage. Both varieties of *E. carotovora* used in this study were reisolated from the soft rot inoculated treatments at the end of the incubation period. *Corynebacteria sepedonicum* bacteria were reisolated from all ring rot inoculated treatments at the end of the incubation period. All controls remained sound throughout the test period.

All quantitations for the volatile profiles were based upon the values obtained by the integration of the chromatogram peak areas. Based upon the data obtained from this technique of volatile adsorption and detection, the volatile characteristics resulting from the ring rot were considerably different from those of the soft rot. Many of these differences were likely related to the relative rates of disease development in the two infections.

In both the ring rot and soft rot infections, the daily total volatile outputs increased dramatically as the infections developed (Fig. 1). Elevated outputs of gases relative to the control were detected within 1-2 days in the soft rot infection but were not apparent until day 5 with the ring rot treatment. This difference corresponded with the rate of development of visual symptoms of decay in the two treatments.

Over the 5 days required for the development of the infection, volatile production expressed as a daily average for the soft rot infection was more than 200 times greater than that of the ring rot treatment, while the average output from the ring rot treatment was not significantly different from the controls. Based upon the volatile production data for the number of days required for the full development of the infections (5 days for soft rot, 9 days for ring rot), the total quantity of volatiles produced over the entire incubation period by the two infections was not significantly different. This similarity might have been expected since it is the availability of metabolizable substrate that in the end determines the potential volatile output.

The pattern of increase in the total volatile production for the soft rot and ring rot infections showed a statistically significant fit to exponential regression equations (Fig. 1). The exponential rate of increase in the total volatile production may reflect the pattern of development of the pathogen pop-

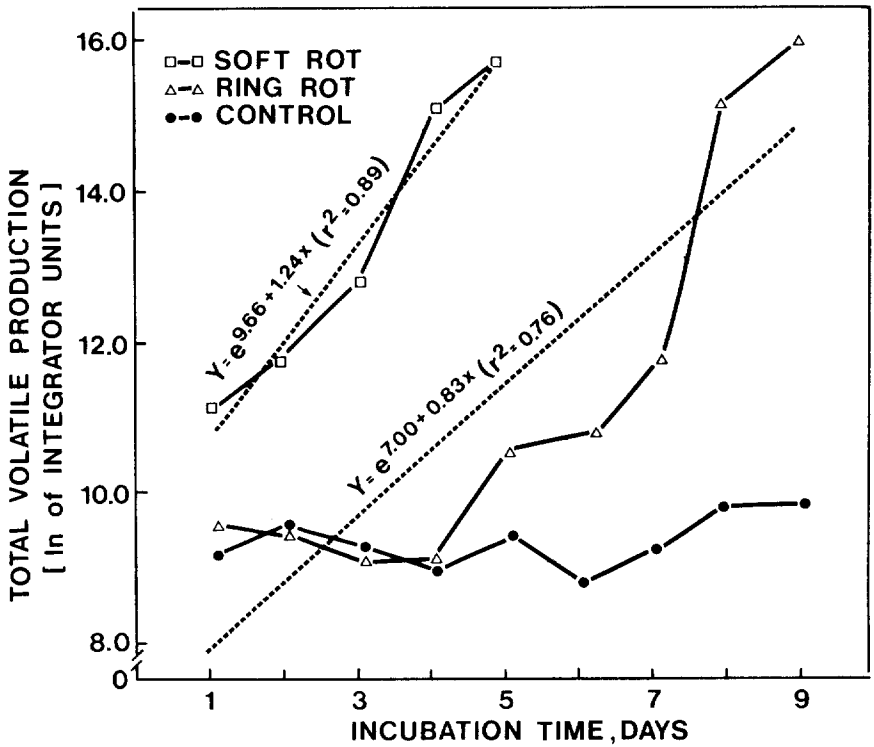

FIG. 1. Daily total volatile production and corresponding best-fit regression lines of non-inoculated, soft rot (*E. carotovora*), and ring rot (*C. sepedonicum*) inoculated Russet Burbank potatoes.

ulations, with the differences between the lines a function of the difference in generation times and relative metabolic activity of the two pathogens.

Schematic chromatograms representing the volatile profiles recorded at three stages during the incubation of the ring rot, soft rot, and control tubers demonstrate several factors which could differentiate among treatments (Fig. 2). Although there was considerable overlap in the compounds identified in the profiles of the three treatments (Table 1), the unidentified peak M (RT = 15.6 min) identified as a major component of the ring rot induced profile of volatiles ($\overline{X}$ percentage of total peak area = PTPA = 11%) was not detected in the profiles of the soft rot infected or control tubers. Conversely, acetaldehyde, propionaldehyde/acetone, and the unidentified peaks F, K, and N were not identified in the headspace samples from the ring rot infected potatoes but were relatively major components of the soft rot profiles. Three of the five compounds isolated in the soft rot profiles but not in the ring rot samples (acetaldehyde, propionaldehyde/acetone and peak F) were detected in the controls. Whether the failure to detect these baseline metabolites was related to the presence of the ring rot bacteria or was simply a function of ex-

TABLE 1. — *Volatile compounds detected in headspace samples from non-inoculated, soft rot (*E. carotovora*), and ring rot (*C. sepedonicum*) inoculated Russet Burbank potatoes.*

Compound Identity	Designator Letter[1]	Retention Time (min)	Treatment		
			Soft rot	Ring rot	Control
Methanol	A	2.5	*[2]	*	*
Acetaldehyde	B	3.1	*		*
Ethanol	C	4.4	*	*	*
2-Propanol	D	5.9	*	*	
Propionaldehyde/ Acetone	E	6.5	*		*
Unidentified	F	7.1	*		*
1-Propanol	G	8.1	*	*	*
2-Butanol/ 2-Butanone/ Butyraldehyde/ 2-Methyl-1-Propanol	H	9.5-10.1	*	*	*
1-Butanol	I	11.8	*	*	*
Unidentified	J	12.7	*	*	*
Unidentified	K	14.1	*		
Acetoin	L	14.6	*	*	*
Unidentified	M	15.6		*	
Unidentified	N	16.3	*		
Unidentified	O	17.8	*	*	*

[1]Letters designate compounds in Fig. 2.
[2]* = compound detected in treatment.

perimental error could not be determined. All of the metabolites isolated in the profiles of the control treatments were detected in the samples from the soft rot infected tubers.

In addition to the differences between the disease profiles in terms of the presence or absence of peaks, there were also differences in the concentration of the individual compounds relative to each other. As a result of the soft rot infection, ethanol, peak F, methanol, and 1-butanol were the most important metabolites identified in the volatile profiles (Fig. 2). The same group of compounds dominated the headspace samples obtained from the controls. It appeared that the primary effect of the soft rot infection was a non-specific increase in the production the volatiles normally produced by healthy but wounded potatoes. There were, however, some disease-specific changes in the relative concentrations of some of the dominant peaks. In the profile of gases induced by the soft rot infection, ethanol (Peak C) had a PTPA value of 44%, which was significantly greater than the 34% recorded in the profiles of the controls.

Unlike the soft rot infection, the increased volatile output that occurred during the progression of the ring rot infection was not a non-specific increase in the volatiles produced by the non-inoculated tubers. Peak M and

the group of compounds that made up peak H dominated the profiles from ring rot infected potatoes but were either not detected or were found only in trace quantities in the profiles of the soft rot and control treatments (Fig. 2). Although the ring rot and soft rot infected tubers developed profiles of volatiles that were similar in terms of their volatile outputs and compound ranges, the relative importance of many of the individual compounds was clearly very different in the volatile profiles of the two diseases.

Discussion

The detection and differentiation between diseases in potato storages through the monitoring of volatile metabolite levels depends on the selection of diagnostic volatiles or volatile production patterns that are: a) as disease specific as possible (7); b) dependable under varying disease development and storage conditions.

In this study the most obvious difference between the ring rot and soft rot infections was the rate of increase in total volatile production/unit time as the infections developed. Over an equivalent time period, total volatile production by the rapidly developing soft rot infection was many times greater than the output from the slower developing ring rot infection. However, unlike the situation in these tests, in commercial storages tuber contamination and disease development do not necessarily occur simultaneously throughout a mass of potatoes nor are the environmental conditions ideally suited to the uniform development of the infections. Consequently, it is likely that any volatile production differences between two pathogens due to differences in the rates of disease development would be masked by the asynchronous and variable disease development that normally occurs in commercial storages. If the differences in the volatile production patterns of ring rot and soft rot infections that stemmed from the different rates of disease development were ignored, many aspects of the volatile profiles, such as the mean volatile production over the period of disease development and the volatile production versus the controls were relatively similar.

The profiles of volatiles induced by the two pathogens featured a number of commonly shared metabolites but each pathogen produced one or more unique compounds. Perhaps more importantly, there were consistent disease-specific changes observed in the relative ratios of several of the metabolites observed in the respective disease profiles. Because they are not dependent on synchronous, steady disease development, it is these types of differential factors that might have the greatest potential as a means of both detecting and distinguishing between soft rot and ring rot outbreaks in commercial storages.

Varns and Glynn (7) also noted disease-specific changes in the relative importance of the dominant metabolites in the volatile profiles of soft rot infected tubers. However, with the exception of ethanol, the dominant peaks

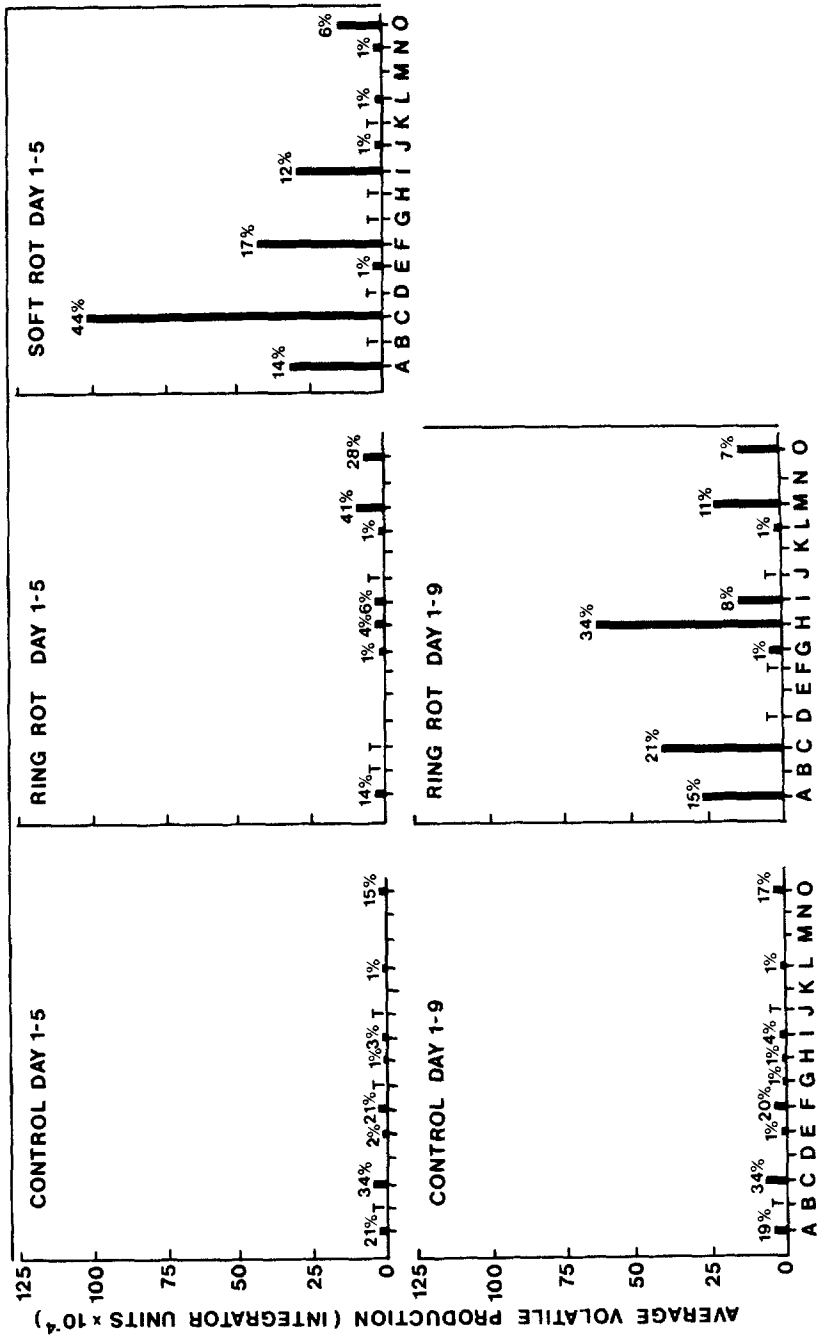

FIG. 2. Average peak area and percentage of total peak area (PTPA) values for non-inoculated, soft rot (*E. carotovora*), and ring rot (*C. sepedonicum*) inoculated Russet Burbank potatoes. The letter T indicates compounds with average PTPA values of less than 1%. Chromatogram peaks are identified in Table 1.

in the soft rot-induced gases they recorded were completely different from the dominant peaks in this study.

These differences between the two studies may have been related to differences in the test conditions and/or variability related to the volatile collection, detection, and identification procedures. The potential impact of these factors must be established before the monitoring of changes in the relative concentrations of the volatile metabolites may be utilized as a reliable technique for the detection of disease development in potato storages.

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