Volatile Metabolic Profiling for Discrimination of Potato Tubers Inoculated with Dry and Soft Rot Pathogens

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ABSTRACT

Volatile metabolites from 'Russet Burbank' potatoes inoculated with *Erwinia carotovora* ssp. *carotovora* (ECC), *Erwinia carotovora* ssp. *atroseptica* (ECA), and *Fusarium sambucinum* (FSA) were analyzed by sampling the headspace at 3 and 6 days after inoculation and then using a gas chromatograph/mass spectrometer (GC/MS) to identify the compounds. Non-wounded non-inoculated and wounded non-inoculated tubers served as checks. Compounds with an abundance of $\geq 10^5$ and with frequency of ≥ 3 out of 20 replicates (10 replicates x 2 incubation times) were subjected to further analysis. A total of 81 volatile metabolites were detected, of which 58 were specific to one or common to a few, but not to all inoculations/diseases. Acetic acid ethenyl ester was unique to ECA, while cyclohexene, diazene, and methoxy-(1,1-dimethyl-2-dihydroxy-ethyl)-amine were unique to ECC, and 2,5-norbornadiene and styrene were unique to FSA. Several metabolites were common only to tubers inoculated with ECC and ECA and were not detected in fungus-inoculated or in control tubers. High abundances of acetone and butane were detected in ECC- and ECA-inoculated tubers, respectively. The possible use of differences in volatile metabolic profiles to discriminate diseases of potato tubers in storage is discussed.

RESUMEN

Los metabolitos volátiles de la papa Russet Burbank, inoculados con *Erwinia carotovora* ssp. *carotovora* (ECC), *Erwinia carotovora* ssp. *atroseptica* (ECA) y *Fusarium sambucinum* (FSA) se analizaron por muestreo del espacio circundante 3 y 6 días después de la inoculación. Para identificar los compuestos se utilizó un cromatógrafo de gas/ espectómetro de masa (GC/MS). Sirvieron como testigos, tubérculos sin herir sin inocular y tubérculos heridos sin inocular. Los compuestos en cantidades $\geq 10^5$ y con una frecuencia ≥ 3 de 20 repeticiones (10 repeticiones x 2 periodos de incubación), fueron sujetos a análisis posteriores. Se detectó un total de 81 metabolitos volátiles, de los cuales 58 fueron específicos a una o comunes a unas pocas pero no a todas las inoculaciones/enfermedades. El ácido acético etenil ester fue exclusivo de ECA, mientras que el ciclohexeno, diazeno y metoxi-(1,1 dimetil-2-dihidroxi-etil)-amina fueron exclusivos de ECC, y el 2,5-norbordarieno y el estireno fueron exclusivos de FSA. Algunos metabolitos fueron comunes solamente en los tubérculos inoculados con ECC y ECA y no se detectaron en los inoculados con el hongo o en los testigos. Abundante acetona y butano se detectó en los tubérculos inoculados con ECC y ECA respectivamente. Se discute el posible uso de las diferencias de los perfiles volátiles metabólicos para distinguir las enfermedades en los tubérculos almacenados.

INTRODUCTION

Diseases are one of the major problems in potato storage. Some of the important diseases are soft rot (*Erwinia carotovora* ssp. *carotovora* (Jones) Bergey et al.), black leg

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(*Erwinia carotovora* ssp. *atroseptica* (van Hall) Dye), dry rot (*Fusarium sambucinum* Fuckel), leak (*Pythium ultimum* Trow), silver scurf (*Helminthosporium solani* Durieu & Mont.), pink rot (*Phytophthora erythroseptica* Pethbr.) and late blight (*Phytophthora infestans* (Mont.) de Bary) (Stevenson et al. 2001). Bacterial soft rot and black leg are probably the most serious diseases in terms of crop losses. Infections by the late blight and dry rot pathogens are also particularly significant, not only because of the damage they cause to potato tubers, but also because they provide potential avenues of entrance for secondary invasion by *E. carotovora* ssp. *carotovora* and *E. carotovora* ssp. *artroseptica*.

New infections occur not only in the field, but also at harvest, especially by *Erwinia* sp., *Phytophthora* spp., *F. sambucinum* and *P. ultimum*. During harvest, the movement of pathogen-contaminated tubers and soil increases the chance of tuber inoculation. Tuber wounding caused by harvesting and bin-piling equipment and delay in drying of tuber surface moisture soon after harvest can provide optimum conditions for pathogens to gain access into the tuber and to establish infection, leading to serious losses in storage (Burton 1989; Schoenemann 1991). Storage managers would like to know the disease potential of tubers they receive for storage, so that they could keep those with low disease potential for longer storage, and sell sooner those with higher disease potential. For lack of efficient disease-detection tools, they depend only on the presence of odors of decay to detect rotten tubers.

Plants produce more than 200,000 metabolites (Dixon et al. 2002; Fiehn 2002). These metabolites are classified as primary and secondary, where generally the former are essential for plant development. Some of the secondary metabolites are used for defense against abiotic and biotic stress, including pathogen attack (Fiehn 2002; Roessner et al. 2001). Even after harvest, the vegetative, flowering, and fruiting parts of plants generally stored continue to respire and to produce many volatile metabolites. The amount of volatiles produced increases with various biotic and abiotic stresses (Wilson and Wisniewski 1989). Efforts have been made to detect these volatiles by using gas chromatography (GC), and GC/mass spectrometry (GC/MS). Production of volatile compounds has been previously studied in potato tubers inoculated with bacteria and fungi (De Lacy Costello et al. 1999; Kushalappa et al. 2002; Lyew et al. 1999, 2001; Ouellette et al. 1990; Varns and Glynn 1979; Waterer and Pritchard 1984a, 1985). Various low molecular weight compounds produced by pathogen-infected potatoes have been identified using GC/MS, including dimethyl disulfide, 2-butanone, pentane, a furan ester, styrene, and phorone. (Varns and Glynn 1979; Waterer and Pritchard 1984a). De Lacy Costello et al. (1999) identified 57 volatile compounds from tubers inoculated with three bacterial pathogens, of which 22 were due to infection by *E. carotovora* ssp. *carotovora*. In another study, De Lacy Costello et al. (2001) detected 52 volatile organic compounds from potato tubers inoculated with *P. infestans* and *Fusarium coeruleum* (Lib. ex Sacc.) Booth. Whereas six volatile compounds were common in both species, four volatiles were specific to *P. infestans* and only two for *F. coeruleum*. The species of disease-specific volatiles produced, however, were quite inconsistent among experiments and among researchers (De Lacy Costello et al. 1999, 2001; Varns and Glynn 1979; Waterer and Pritchard 1984a, 1984b, 1985).This may be due to variations in host, pathogen, and environmental conditions affecting host and pathogen metabolism. Attempts have been made to trap volatiles at 10 C, a temperature level recommended for storage. However, at this temperature the amount of volatiles produced may be quite low because pathogens grow slowly (Lui and Kushalappa 2002). At higher temperatures a greater number and amount of volatiles can be expected, due to increased pathogen development. This paper presents differential metabolic profiles of potato tubers non-inoculated and inoculated with two bacterial pathogens (*E. carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroseptica*) and one fungal pathogen (*F. sambucinum*). The possible use of these differences to discriminate diseases of potato is discussed.

MATERIALS AND METHODS

Bacterial and Fungal Cultures

Erwinia carotovora ssp. *carotovora* (isolate 1028) and *E. carotovora* ssp. *atroseptica* (isolate 707) cultures were obtained from the Plant Diagnostic Lab., MAPAQ, Ste.-Foy, Quebec. The bacterial cultures were maintained in tryptic soy broth and glycerol and stored at -70 C. Bacterial cultures for inoculation were prepared by streaking a loop of bacteria on TSA plates and incubating at 22 C for 48 h.

The *F. sambucinum* (isolate 1068) culture was obtained from the Plant Diagnostic Lab., MAPAQ, Ste.-Foy, Quebec. The fungus was stored in sterile soil at 4 C. For the production of macroconidia, the fungus was cultured on half strength PDA and incubated at 22 C for 7 to 10 days.

Inoculum Production

Bacterial cell suspensions of *E. carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroseptica* were made from 48-h-old cultures. The concentration of the suspension was adjusted to 10^7 cells mL^{-1} (Kushalappa and Zulfiqar 2001). The suspension of macroconidia of *F. sambucinum* was prepared from 7- to 10-day-old cultures, by flooding the plates with distilled water, vortexing the suspension, and filtering through two layers of cheese cloth. The suspension was adjusted to 10^4 macroconidia mL^{-1} using a haemocytometer.

Tuber Wounding, Inoculation, and Incubation

Certified potato tubers, cv Russet Burbank, harvested from a single field were obtained from the Ministry of Agriculture, New Brunswick, and kept in a cold (maintained at 4 C and 90% RH) storage facility of the Horticultural Research Farm of the Macdonald campus of McGill University. Tubers appearing healthy and uniform in size (100-140 g) were selected and washed to remove excess soil, surface sterilized in 0.5% sodium hypochlorite solution for 15 min, and rinsed in several changes of distilled water. Ten holes were made on the surface of the tubers with a cork borer (5 mm in diameter x 3 mm deep). Each hole was inoculated with 20 µL of inoculum. The inoculated potato tubers were placed in 1-L septa bottles, one tuber per bottle, with 10 mL of water at the bottom to create a saturated atmosphere, and incubated at 20 C in the dark. Stainless steel supports were used to suspend the tubers above water level. After 24 h, the water was removed and the bottle was flushed with pure air from a cylinder, and again after every 24 h for 5 days. A magnetic stirrer was used to agitate headspace gas to establish equilibrium during sampling.

The experiment consisted of five treatments: a non-wounded and non-inoculated control (NCO), a wounded and non-inoculated control (WCO) and tubers inoculated with *E. carotovora* ssp. *carotovora* (ECC), *E. carotovora* ssp. *atroseptica* (ECA), or *F. sambucinum* (FSA). The volatiles were sampled at 3 and 6 days after inoculation (dai). The entire experiment was conducted 10 times.

Volatile Accumulation and Analysis

The head space gas was accumulated for 24 ± 2 h (two replicates for 3 h) in the dark at 20 C. Volatiles were sampled, after stirring for 1 min with a magnetic stirrer, and analyzed using a portable GC/MS system (HAPSITE-10122, INFICON, Syracuse, NY, USA). The HAPSITE was equipped with a hand-held gas-sampling probe attached to a terminal 15-cm stainless steel needle (Popper & Sons Inc., NY, USA). A built-in pump was used to sample a predetermined volume of air (100 mL) which passed through a tube containing 15 mg of carboxen to trap volatiles. The trap was heated at 225 C to momentarily desorb volatiles. Organic compound-free N_2 gas was used as a carrier gas at a flow rate of 3 mL min^{-1}. For compound separation, a GC capillary column (SPB-5 Supelco, Bellfonte, CA) 0.32 mm internal diameter, 30 m in length, coated with 1.0 µm film coating (INFICON part number:930-489-G8) was used. The GC column temperature was increased to and held at 50 C for 4 min and then increased by 3 C min^{-1} until it reached 200 C. The whole run took 54 min to complete. The GC was interphased with a mass spectrometer equipped with a quadrupole analyzer and the mass spectrum was scanned at the rate of 0.8 second per mass decade over a mass range of 46 to 300 *m/z*. The volatile compounds were tentatively identified using the NIST library (version 1.6, INFICON, Syracuse, NY). In addition, the spectrum of each compound, over replicates, was manually compared with that in the NIST. The amount of a metabolite, at a given retention time, was presented as mass-ion abundance (quadrupole detector output = related to compound concentration and can be obtained following establishment of a standard curve).

Data Analysis

The metabolites and their abundances were exported in to an EXCEL (Microsoft, Inc.) spreadsheet and sorted using the Pivot procedure to develop a list of compounds detected in this study. The metabolites that had mass-ion abundances $\geq 10^5$ and also occurred in more than three replicates out of 20 (10 replicates x two incubation times), in any one of the five inoculations/diseases were retained. For the compounds retained, an average abundance (for 20 replicates) and frequency of their occurrence over replicates was calculated for all inoculations/diseases (irrespective of abundance or frequency). Following this, the compounds were grouped according to their specificity to one or more inoculations/diseases.

RESULTS

Disease Severity of Inoculated Tubers

Visible symptoms of *E. carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroseptica*, including creamy soft tissue around inoculated sites, were apparent 3 days after inocula-

tion (dai). The lesions were >2 cm in diameter and coalesced with visible ooze at day 6. Symptoms of *F. sambucinum* infection developed more slowly and no visible symptoms were apparent at 3 dai, but white mycelial growth occurred around the site of inoculation with tissue discoloration of 3- to 5-mm radius beyond the wound at 6 dai. The WCO tubers, inoculated with sterile distilled water, showed a slight browning at the inoculation site, while NCO tubers remained unchanged throughout the 6-day incubation period. No other diseases were found on any of the tubers used in this study.

Volatile Metabolic Profiles

The number of volatile compounds detected in headspace samples varied between inoculations/diseases, incubation times, and blocks. More than 1,000 volatile metabolites were detected in potato tubers inoculated with water or with three different pathogens; however, only a total of 81 volatile metabolites were relatively consistent, occurred in more than three replicates (out of 20 replicates) with abundance (= quadrupole detector output = amount of compound) $\geq 10^5$ in at least one of the inoculations/diseases. Out of 81 compounds, 78 and 79 were detected at 3 and 6 dai, respectively. Two compounds detected in day 3 were not detected in day 6, and three new compounds were detected on day 6 (details not shown here).

Inoculation/Disease-specific Volatile Metabolites

Out of a total of 81 relatively consistent volatile metabolites detected in this study, 58 volatiles were specific to one or more inoculations/diseases, meaning they were not present in all the inoculations/diseases (Table 1). Out of 58 inoculation/disease-specific volatile metabolites, 10 were unique to a single inoculation/disease, 21 compounds were common only to ECC and ECA, and two compounds were common only to NCO and WCO. Apart from these, five and 20 metabolites were common to different combinations of 3 and 4 inoculations/diseases, respectively. All these metabolites can be qualitatively used in the discrimination of inoculations/diseases. The metabolite acetic acid ethenyl ester was unique to ECA, while cyclohexene, diazene, and methoxy-(1,1-dimethyl-2-hydroxyethyl)-amine were unique to ECC, and 2,5-norbornadiene and styrene were unique to FSA. Acetonitrile, benzeneacetaldehyde and ethene were produced only in NCO tubers, while 1,3-cyclopentadiene was detected only in WCO tubers. Tubers inoculated with two subspecies of *E. carotovora*, ECA and ECC, produced 13 disease-discriminatory compounds.

The average abundance varied between inoculation/disease-discriminatory compounds. A maximum mass-ion abundance of 67×10^6 of acetone was detected from tubers inoculated with ECC; however, this compound also occurred in ECA-inoculated tubers, though not in other inoculations. Disulfide was also produced in high abundance, but was common to all the three pathogens and occurred in very low abundance in WCO. Quantitative differences in these and in other metabolites can be used to discriminate different combinations of inoculations/diseases.

DISCUSSION

In this study we have identified several volatile metabolite markers using GC/MS to discriminate five inoculations/diseases of potato tubers. The qualitative criteria, inoculation/disease-specific volatile compounds, can be used to discriminate all the five inoculations/diseases studied here. ECA- and ECC-inoculated tubers produced several compounds that could be used in the discrimination of these inoculations/diseases from the others studied here. Several combinations of presence and/or amounts of different compounds could be used to discriminate several groups of inoculations/diseases (Table 1).

Some of the inoculation/disease-specific compounds reported here also have been reported from diseased potatoes by other researchers. Styrene, which was specific to tubers inoculated with FSA in our study, has been reported from tubers inoculated with *F. coeruleum* and *P. infestans* (De Lacy Costello et al. 2001). We have detected three compounds specific to ECC and one to ECA. De Lacy Costello et al. (1999) reported 22 volatiles unique to ECC, as compared to *Bacillus polymyxa* (Praz.) Mig. and *Arthrobacter* sp., though many were produced in trace amounts in non-inoculated tubers, by endophytic bacteria. Disulfide, though not specific to an inoculation, was the most abundant compound in tubers inoculated with ECC and ECA, and it was low in FSA-inoculated and WCO tubers, but not detected NCO potato tubers. Disulfide also has been reported from potato inoculated with ECC, but not in non-inoculated tubers (De Lacy Costello et al. 1999; Ouellette et al. 1990). It also has been reported in tubers inoculated with *Fusarium roseum* Link. Fr. em. Snyder & Hansen

TABLE 1—*The abundance, frequency, and specificity of volatile metabolites*[1] *detected in potato tubers inoculated with water or pathogens (*E. carotovora *ssp.* atroseptica, E. carotovora *ssp.* carotovora, F. sambucinum*).*

Metabolites	ECA[2]	ECC	FSA	NCO	WCO	Specificity
Acetic acid ethenyl ester	21(5)	-	-	-	-	A
Cyclohexene, 1-methyl-4-(1-methylethenyl)-	-	8(3)	-	-	-	C
Diazene, dimethyl-	-	2338(6)	-	-	-	C
Methoxy-(1,1-dimethyl-2-hydroxy-ethyl)-amine	-	15(10)	-	-	-	C
2,5-Norbornadiene	-	-	1(4)	-	-	F
Styrene	-	-	18(7)	-	-	F
Acetonitrile, dichloro-	-	-	-	2(4)	-	N
Benzeneacetaldehyde, à-phenyl-	-	-	-	1(6)	-	N
Ethene, fluoro-	-	-	-	20(8)	-	N
1,3-Cyclopentadiene,	-	-	-	-	4(4)	W
1,2-Dimethoxy-ethene	284(7)	407(4)	-	-	-	AC
1-Butanol	437(4)	840(3)	-	-	-	AC
1-Butanol, 2-methyl-	479(16)	645(16)	-	-	-	AC
1-Butanol, 2-methyl-, acetate	69(1)	11(3)	-	-	-	AC
1-Butanol, 3-methyl-, acetate	121(3)	147(3)	-	-	-	AC
1-Pentanol	160(10)	550(5)	-	-	-	AC
1-Propanol	58(5)	24(4)	-	-	-	AC
2-Butanone, 3-hydroxy-	237(8)	427(8)	-	-	-	AC
Acetic acid, 2-methylpropyl ester	38(6)	32(8)	-	-	-	AC
Acetic acid, methyl ester	11(5)	30(7)	-	-	-	AC
Acetone	6708(11)	3633(6)	-	-	-	AC
Borane-methyl sulfide complex	134(3)	56(3)	-	-	-	AC
Butanoic acid, 2-methyl-	412(3)	967(5)	-	-	-	AC
Butanoic acid, ethyl ester	73(4)	51(5)	-	-	-	AC
Cyclobutanone, 2,2,3-trimethyl-	3(1)	1(3)	-	-	-	AC
Dimethyl trisulfide	33(2)	26(3)	-	-	-	AC
Ethanol	177(10)	174(13)	-	-	-	AC
Ethyl Acetate	199(6)	134(9)	-	-	-	AC
Methyl ethyl disulphide	28(3)	14(1)	-	-	-	AC
Pentanoic acid, methyl ester	441(5)	340(4)	-	-	-	AC
Thiirane	8(6)	14(5)	-	-	-	AC
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	-	-	9(3)	18(2)	-	FN
Propylene oxide	-	-	48(3)	3(1)	-	FN
DL-3,4-Dimethyl-3,4-hexanediol	1(1)	1(1)	2(4)	-	-	ACF
Butane, 2,2-dimethyl-	1(3)	1(2)	-	-	1(1)	ACW
Cyclohexene, 4-methylene-1-(1-methylethyl)-	-	10(1)	9(3)	7(3)	-	CFN
1,4-Cyclohexadiene,	-	16(3)		13(4)	19(3)	CNW
Trichloroethylene	-	-	4(2)	15(3)	18(6)	FNW
Azetidine	2(3)	1(2)	1(1)	1(1)	-	ACFN
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	1(3)	3(1)	4(3)	5(3)	-	ACFN
Bicyclo[4.1.0]hept-4-en-3-ol,	6(6)	24(5)	10(6)	11(5)	-	ACFN
Cyclohexene, 1-methyl-5-(1-methylethenyl)-	4(2)	2(1)	6(4)	2(2)	-	ACFN
Hydrazine, methyl-	4(1)	5(1)	3(3)	7(2)	-	ACFN
Disulfide, dimethyl	3985(9)	653(12)	56(2)	-	1(3)	ACFW
1,2,4-Benzenetricarboxylic acid,	1(1)	1(1)	1(3)	-	1(1)	ACFW
1-Undecene	172(7)	190(11)	37(4)	-	51(1)	ACFW
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-	27(2)	6(5)	2(1)	-	65(1)	ACFW
Methyl nitrate	7(2)	10(3)	2(2)	-	13(1)	ACFW
2-Propanamine, 2-methyl-	11(1)	7(2)	-	4(3)	3(1)	ACNW
Benzene, 1,3-dimethyl-	6(2)	12(1)	-	8(3)	5(1)	ACNW
Dimethyl ether	655(5)	31(1)	-	9(3)	13(4)	ACNW
Benzene, 1,4-dichloro-	16(2)	-	22(4)	188(5)	125(6)	AFNW
Benzenemethanol, à,4-dimethyl-	8(2)	-	2(3)	3(2)	2(2)	AFNW
Methylene Chloride	2(2)	-	2(6)	7(9)	4(7)	AFNW
Benzene, 1,2-dimethyl-	-	1(3)	1(2)	7(9)	6(5)	CFNW
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-,	-	30(3)	23(2)	24(5)	40(4)	CFNW
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	-	49(2)	11(2)	93(6)	39(4)	CFNW
Disulfide, methyl propyl	-	1(1)	1(3)	2(1)	4(1)	CFNW

TABLE 1—*Continued.*

Metabolites	ECA[2]	ECC	FSA	NCO	WCO	Specificity
1-Butanamine, N,N-dimethyl-	8(2)	6(4)	4(8)	11(4)	3(3)	ACFNW
2-Butanone	1573(9)	1137(11)	18(2)	62(1)	7(1)	ACFNW
2-Cyclopenten-1-one,	1(3)	6(3)	2(4)	1(2)	4(2)	ACFNW
3-Carene	155(7)	1(7)	1(10)	1(11)	1(10)	ACFNW
4,6-Octadiyn-3-one, 2-methyl-	2(6)	2(5)	2(6)	1(2)	1(2)	ACFNW
á-Myrcene	8(3)	17(7)	7(4)	6(3)	29(3)	ACFNW
à-Phellandrene	1(1)	3(3)	3(5)	2(3)	4(4)	ACFNW
á-Pinene	30(3)	265(5)	128(9)	212(9)	211(5)	ACFNW
Benzene, 1,2-dichloro-	37(3)	70(5)	20(1)	40(1)	40(1)	ACFNW
Benzene, 1,3-dichloro-	20(2)	57(2)	13(2)	141(2)	86(3)	ACFNW
Benzene, 1-methyl-3-(1-methylethyl)-	24(1)	1(1)	21(4)	53(3)	70(2)	ACFNW
Benzene, 1-methyl-4-(1-methylethyl)-	3(1)	56(5)	39(4)	35(4)	97(3)	ACFNW
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	13(3)	5(1)	2(3)	9(9)	14(3)	ACFNW
Butane, 1-methoxy-3-methyl-	83(8)	4090(19)	27(4)	12(5)	29(2)	ACFNW
Chloroform	602(13)	294(12)	132(20)	212(20)	225(20)	ACFNW
Disulfide, dipropyl	26(6)	30(1)	14(4)	248(3)	123(3)	ACFNW
Ethylbenzene	1(1)	2(2)	2(3)	3(8)	3(6)	ACFNW
Formic acid	21(5)	6(2)	14(4)	7(3)	8(2)	ACFNW
Hydrazine, methyl-, oxalate (1:1)	58(10)	76(9)	9(8)	5(10)	10(10)	ACFNW
Ketone,1,3-cycloheptadien-1-ylmethyl	4(5)	40(2)	4(4)	6(3)	7(2)	ACFNW
Limonene	2(2)	39(11)	42(7)	55(8)	51(4)	ACFNW
p-Xylene	5(3)	16(4)	3(5)	10(6)	8(10)	ACFNW
Trichloromonofluoromethane	8(1)	5(1)	2(4)	10(9)	5(10)	ACFNW

[1]Volatile metabolites: Average (for 20 reps) mass ion abundance x 10^4 (the quadrupole analyzer output of mass ions); the frequency in parenthesis is compound occurrence out of 20 replicates (10 replicates x 2 incubation times); "-" are compounds not detected.
[2]ECA = A = *E. carotovora* ssp. *atroseptica*; ECC = C = *E. carotovora* ssp. *carotovora*; FSA = F = *F. sambucinum*; NCO = N = non-wounded non-inoculated control; WCO = W= wounded non-inoculated control; Specificity = compound specificity to one or more inoculations/diseases.

(Ouellette et al. 1990). We have detected 13 metabolites to discriminate ECC and ECA, with three and one unique compounds, respectively. Waterer and Pritchard (1985) have detected 3-hydroxy-2-butanone in ECA and not in ECC, whereas we have detected this compound in both ECC and ECA. In our study we have detected many alcohols, esters, and ketones only in ECC- and ECA-inoculated tubers. These compounds were observed in high abundance and thus have high potential to be detected under practical storage conditions. High abundance of ethanol, acetone, and 2-butanone has been reported from ECA-infected tubers as compared to non-inoculated (Varns and Glynn 1979). In our study, all the three compounds were detected in ECC and ECA, though 2-butanone was also detected in relatively very low abundance in other inoculations/diseases. Ethylene is generally detected in diseased potato tubers, but was not detected here because mass ions < 46, which constitute the mass spectra of ethylene, were not quantified in our study due to error in this range of mass ions associated with detection of such volatiles in high moisture headspace. As well, low molecular weight volatiles are generally not disease/spoilage-specific (Marsili 1999; Toivonen 1997).

Though we detected many metabolites that are inoculation/disease discriminatory, they were not consistent in all 10 replicates. Such variations have been reported by others (De Lacy Costello 1999; Toivonen 1997; Waterer and Pritchard 1985). Variability in metabolite production is obvious because of variability associated with host, pathogen, and environmental interactions in disease development (Dixon et al. 2002; Fiehn 2002; Roessner et al. 2001). Some volatiles that were present at 3 dai were not present at 6 dai. Volatiles react with each other and make different compounds, or fruits and vegetables can reabsorb volatiles (Hamilton-Kemp et al. 1996). In our study the headspace relative humidity was very high with some condensation on the bottle and tuber surface. Although we used a stirrer to establish equilibrium, the volatile solubility in water can influence the types of volatiles sampled (Toivonen 1997). A dynamic headspace sampling, which was not done in our study, may reduce such problems. Potato tubers were surface sterilized before inoculation; however,

some saprophytic microbial contamination is possible and may be responsible for some inconsistencies.

The method developed in this study to discriminate potato inoculation/diseases can be used by storage managers to assess the quality of tuber samples collected either before harvest, at harvest, or during storage, after validation under commercial conditions. This knowledge base can be used to decide the storability of tubers originating from a lot or field. Potato samples can be collected on a regular basis to monitor tuber quality in "real time." Samples can be incubated for 24 h in closed containers at room temperature to trap and to analyze volatiles using GC/MS. Samples collected from hot spots in storage may enable monitoring of potential spoilage. This knowledge base of disease potential could be used in making intelligent management decisions, such as how long the product can be stored or which lot could be kept for longer storage. Since the variability is high among replicates, analysis of larger samples or a larger number of samples is warranted for accurate diagnosis of diseases. However, use of combinations of compounds, rather than a single marker compound, can enable disease discrimination with fewer samples.

Many factors other than those studied can influence volatile dynamics (Toivonen 1997). In our study, the experimental units were confined and the volatiles were sampled separately for each disease to avoid cross-contamination. Though many diseases are important in storage, disease outbreaks often include only one disease. The disease-specific volatile markers could be used to discriminate diseases even when more than one disease is present; however, their validity must be tested. One of the most important variables that affects wider application of this tool would be the effect of cultivars. If there is significant cultivar effect, then separate models must be developed for each cultivar or it may be possible to group cultivars that have similar discriminatory compounds. We identified metabolites specific only to five inoculations/diseases. More diseases, however, must be studied and modeled for practical applications.

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