Development of a Sparging Technique for Volatile Emissions from Potato (Solanum tuberosum)

Elizabeth Berdis, Barbara Vieux Peterson, Neil C. Yorio,
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Elizabeth Berdis, Barbara Vieux Peterson, Neil C. Yorio, Jennifer Batten,
The Bionetics Corporation, Kennedy Space Center, Florida 32899
Raymond M. Wheeler, Biomedical Operations and Research Office,
NASA, Kennedy Space Center, FL 32899

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TABLE OF CONTENTS

ABSTRACT

The accumulation of volatile emissions from plants grown in tightly closed growth chambers may have allelopathic or phytotoxic properties. Whole air analysis of a closed chamber includes both biotic and abiotic volatile emissions. To investigate this complex mixture of volatile organic compounds, a method for characterization and quantification of biogenic emissions solely from plantlets has been developed. Volatile organic compounds from potato (*Solanum tuberosum* L. cv. Norland) were isolated, separated and identified using an in-line configuration consisting of a purge and trap concentrator with sparging vessels connected to a GC/MS system. Analyses identified plant volatile compounds: trans-caryophyllene, alpha-humulene, thiobismethane, hexanal, cis-3-hexen-1-ol, and cis-3-hexenyl acetate.

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

Controlled Ecological Life Support Systems (CELSS) studies are currently underway at NASA's Kennedy Space Center. These studies have centered on crop tests conducted inside a large, closed biomass production chamber to assess potential crops for bioregenerative life support systems. Several target crops, including white potatoes (*Solanum tuberosum* L. cv. Norland), have been grown hydroponically in the biomass production chamber (BPC) (Wheeler et al., 1993). The accumulation of volatile emissions from plants grown in tightly closed growth chambers may have allelopathic or phytotoxic properties. However, whole air analysis within the BPC includes both biotic and abiotic volatile emissions. Therefore, a method for characterization and quantification of emissions solely from plants has been developed.

Traditionally, isolation of plant volatiles has been accomplished by solvent extraction, steam distillation, and tissue maceration (Hamilton-Kemp and Andersen, 1984; Binder and Flath, 1989; Frolic et al., 1989; Yule et al., 1989). These methods are destructive in nature and the volatile organic compounds identified by these methods reflect those from damaged plant material rather than an intact plant. Plant tissue damage can cause alterations in the volatile emissions normally associated with the intact healthy plant (Tingey et al., 1991; Visser et al., 1978). These methods

also require an abundant supply of plant material, sample preparation and extraction with subsequent concentration. To eliminate some of these requirements, adsorbent tubes to trap and concentrate plant volatiles have been used (Buttery et al., 1987; Remboled et al., 1989). The requirement of additional extraction steps and sample transfers from the concentration step of plant emissions to the analytical instrument can result in sample loss and contamination (Walling, 1984).

The isolation of plant volatiles was explored using a modified method for analysis of volatile organic compounds in drinking water (Stephenson and Myron, 1990). Purging the plant as one would a water sample for volatile organic compounds allows for direct in-line analyses. This technique also provides for concentration of volatile plant compounds on the trap without further manipulation of plant tissue or adsorbent traps. Stress to the plantlet is minimized as minor damage of the plantlet occurs when it is inserted into the sparging tube. In addition, volatiles collected at ambient temperature reflect "normal" plant emissions. The sparging technique allows for entire plantlets to be analyzed without severing the leaves or roots, although individual plant leaves, roots or stems could be analyzed in this manner. For this study, leaves and roots from mature 65-day old plants were also sampled for volatile compounds. The compounds characterized in this study with potatoes have been detected in other crops (Table

1). The purge and trap method allowed the most "focused" sampling approach of emissions solely from plant material. There is minimal tissue damage as opposed to destructive sampling and extraction of tissue.

EXPERIMENTAL

Plant Material. White potato (*S. tuberosum* cv. Norland) plantlets were aseptically cultured *in vitro* (Hussey and Stacey, 1981) on a modified MS media (Murashige and Skoog, 1968) containing 20 g/L sucrose, 0.7% Bacto-agar, and pH adjusted to 5.8 with 0.1 M KOH. The plantlets were maintained in 25 X 100 mm culture tubes capped with Magenta 2-way caps (Magenta Corp.) in a temperature controlled incubator at 25°C and 80-100 μmol $m^{-2}s^{-1}$ photosynthetic photon flux (PPF). The roots were washed with distilled water to remove excess agar that may have adhered to the stem and roots.

The cultured plantlets were transplanted at 4 - 6 weeks and grown in controlled environmental conditions (Wheeler et al., 1990). Leaves and roots were removed from 65-day old potato plants with fully expanded canopy leaves in the BPC. The leaves and roots were studied as a comparison to the younger whole plantlets (Table 4).

Collection of volatiles. An intact plantlet or plant material (i.e., leaves and roots) was placed into a 25-ml glass sparge vessel (20cm X 2cm sample placement section) connected to a purge and trap concentrator (Tekmar 2000/2016) (Figures 1 and 2). Helium was purged across the

plant material at 40 ml min^{-1} for 30 minutes. Volatile organic compounds were concentrated onto a VOCARB™ (Supelco) absorbent trap consisting of porous, graphite treated carbon mesh. The trap was held at an ambient temperature of 30°C. Plantlet headspace analysis was done at both ambient temperature and 100°C.

Desorption and gas chromatographic conditions. After the volatile organic compounds were concentrated onto the trap, the trap was heated to 250°C. The desorbed compounds were cryogenically focused at the gas chromatograph transfer line interface at -150°C. The volatile organic compounds were subsequently separated on a Hewlett Packard 5890 gas chromatograph with a non-polar, dimethylpolysiloxane column, 30 m x 0.596 mm with 3μm film thickness. The oven temperature was initially set at 10°C and programmed at 5°C min^{-1} to 200°C.

Mass Selective Detector. The 5970 Hewlett Packard Mass Selective Detector, MSD, with a jet separator was tuned to optimize for the lighter molecular weight compounds with 4-bromofluorobenzene (Stephenson and Myron, 1990). The scan range was set from 35 to 500 atomic mass units, AMU. Compounds with fragmentation ions or molecular weight of less than 35 AMU were not detected. The jet separator was set at 150°C and the heated GC/MS interface was set at 250°C.

Verification. Analytical standards were obtained from commercial sources (Table 2). Identification of the emitted

plant compounds was confirmed by comparing specific ion ratios in each mass spectrum and retention times of each standard component to those found in the sample (Table 3). Quantification was based on using fluorene as an internal standard in 5 ml of deionized water added to the sparge tube.

RESULTS AND DISCUSSION

Six compounds, thiobismethane, hexanal, cis-3-hexen-1-ol, cis-3-hexenyl acetate, trans-caryophyllene, and alpha-humulene, were identified by GC/MS as volatiles from potato plantlets, mature leaves and stems (Table 3). Cis 3-Hexen-1-ol was detected in small amounts which may be attributed to minor tissue damage caused by placing the plantlet into the sparge tube (Figure 4) or from the flow of helium passing over the plantlet during the concentration step of the analysis.

Sesquiterpenoids, trans-caryophyllene and alpha-humulene were detected from the potato leaf samples (Table 4, Figures 5 and 6). The intact plantlets also emitted these sesquiterpenoids but at a much lower concentration range on a dry weight basis (Table 4). This may be attributed to plant tissue maturity or damage. Plantlets were 28 to 56-days old and had immature leaves, whereas the leaf samples were taken from 65-day old plants grown in the BPC. Trans-caryophyllene and alpha-humulene were also detected from mature potato plants by whole air analysis of

an enclosed chamber (GC/MS analysis) and by adsorbent tubes with subsequent solvent extraction (GC/FID analysis) (unpublished data).

Hexanal and thiobismethane were not detected in the plantlets sampled at ambient temperature, but were found in the plantlets that were heated to 100°C during sampling (Table 4). The results suggest that these compounds are indicative of plant stress or tissue damage and may not reflect the "normal" emissions from intact plants. These findings are supported by the presence of hexanal and thiobismethane in the mature leaf samples (Table 4), in which leaves were removed from mature plants and subsequently cut into pieces before being placed into the sampling (sparge) vessels.

Ethylene, a volatile organic compound that is known to be emitted from damaged or stressed plants, has been monitored by gas chromatography with a photoionization detector in the BPC potato crop studies (Wheeler et al., 1993). The scan range on the mass spectrometer was set for detection of 35-500 AMU, atomic mass units. Ethylene with a molecular weight of 28 was not detected during this study.

CONCLUSION

The method used in this study allows for sampling of volatile organic compounds from intact plants without extraneous, abiotic contamination. The in-line analysis

prevents sample loss, allows for concentrated sampling over a period of time, and reduces the amount of plant material needed. Also, by minimizing plant stress in this technique, natural emissions from undamaged plant tissue may be more accurately quantified.

Future use of this system could incorporate studies of volatiles emitted during the plant's life cycle, using modified growth chambers. Plants could be subjected to stressful conditions (i.e., nutrient deprivation, water loss, high light, etc.) with subsequent monitoring of the volatiles that are emitted during these times. The study of *in vitro* plantlets could allow for screening of various crop plants for characterization of volatile emissions. A correlation between plant volatiles and stress may afford an early injury detection system (before morphological changes are seen) for plants grown in enclosed chambers.

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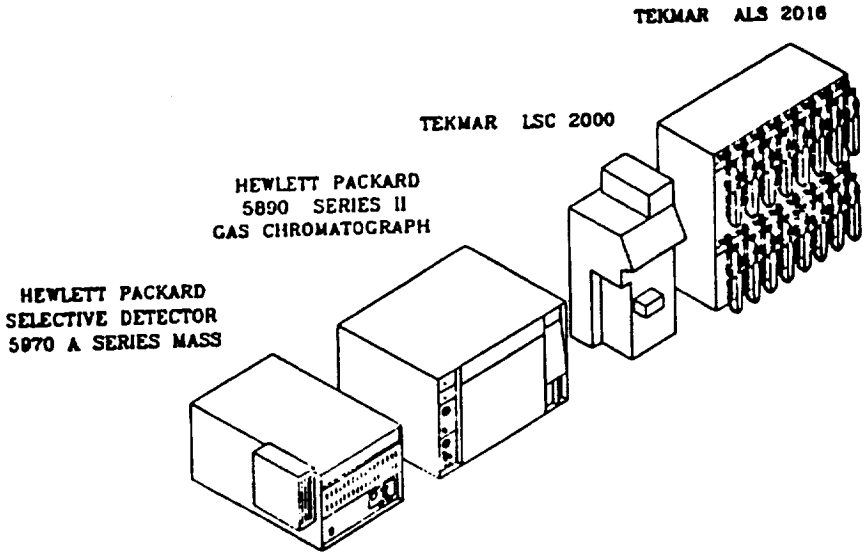

Figure 1 GC/MS sparging system for analysis of volatile emissions

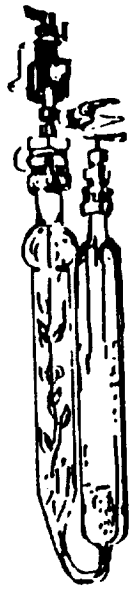

Figure 2 Sparge tube containing *Solanum tuberosum* cv. Norland

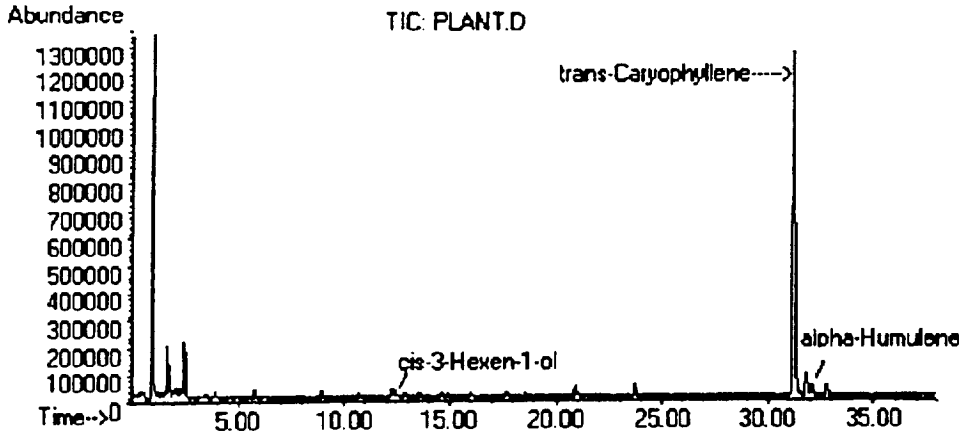

Figure 3. Total ion chromatogram from GC/MS analysis of potato plantlet with 0.098 grams dry weight.

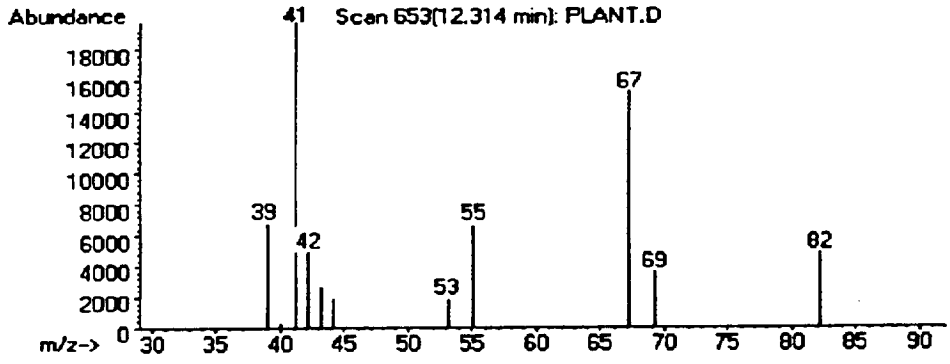

Figure 4. Mass spectrum of cis-3-Hexen-1-ol from analysis of whole potato plantlet

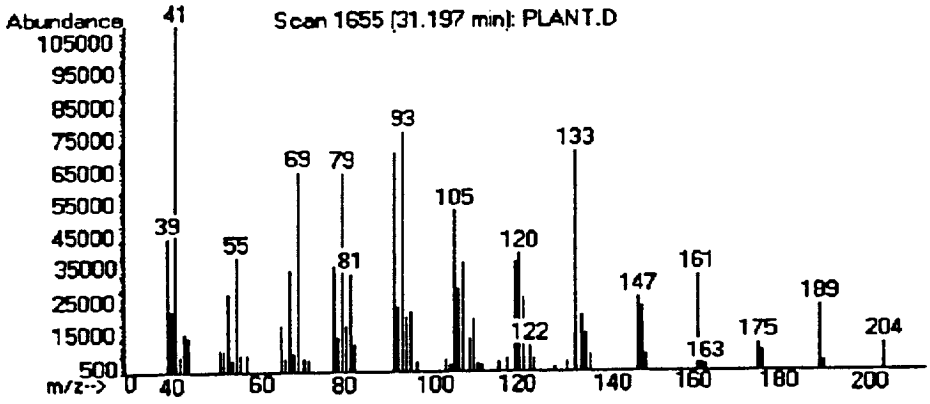

Figure 5. Mass spectrum of trans-Caryophyllene from analysis of whole potato plantlet.

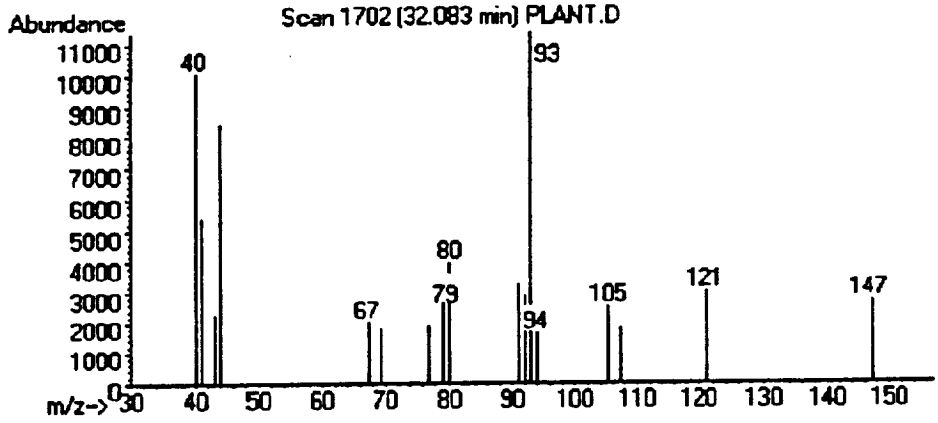

Figure 6. Mass spectrum of alpha-Humulene from analysis of whole potato plantlet.

Table 1. Previously described plant compounds.

Compound	Plant Source	Reference
Caryophyllene, trans-	Mentha	Bicchi et al., 1989
	Pineapple guava	Binder & Flath, 1989
	Pear	Miller et al., 1989
	Tomato	Buttery et al., 1987
	Celery	Wassenhove et al.,1990
	Wheat	Buttery et al., 1985
Humulene, alpha-	Mentha	Bicchi et al., 1989
	Pineapple guava	Binder & Flath, 1989
	Pear	Miller et al., 1989
	Tomato	Buttery et al., 1987
	Celery	Wassenhove et al.,1990
Hexanal	Chickpea	Rembold et al., 1989
	Tomato	Buttery et al., 1987
	Celery	Wassenhove et al.,1990
	Naranjilla	Brunke et al., 1989
	Scorzonera	MacLeod & Ames, 1991
3-Hexen-1-ol, cis-	Red clover	Buttery et al., 1984
	Tomato	Buttery et al., 1987
	Potato	Visser et al., 1979
3-Hexenyl acetate, cis-	Mentha	Bicchi et al., 1989
	Red clover	Buttery et al., 1984
Thiobismethane	Amaranth	Connick et al., 1989
	Scorzonera	MacLeod & Ames, 1991

Table 2. List of standards with quantification detection limits (QDL) and vendor.

Compound	QDL[a] (ug/m3)	Vendor
Caryophyllene, trans-	0.32	Sigma
Humulene, alpha-	0.98	Sigma
Hexanal	0.85	Aldrich
3-Hexen-1-ol, cis	115.00	Aldrich
Dimethyldisulfide	0.21	Aldrich

[a]Quantifiable detectable limits

Table 3. Volatile compounds identified in *S. tuberosum* cv. Norland using sparging technique.

Compound	Retention Time (min)	MW[a]	(m/e)[b]
Thiobismethane[d]	8.24	62	47,62,40,41,61,35
Hexanal[c]	24.54	100	44,41,43,39,60
3-Hexen-1-ol, cis-[c]	27.90	100	41,67,82,55,69
3-Hexenyl acetate, cis-[d]	30.90	142	43,67,41,39,82
Caryophyllene, trans-[c]	54.16	204	41,69,93,133,79
Humulene, alpha-[c]	55.70	204	93,80,121,41,147

[a]MW - Molecular Weight
[b]m/e - mass to electron charge ratio
[c]Tentatively identified by comparison to standard
[d]Tentatively identified by spectral matching

Table 4. Concentration ranges of volatiles emitted from *Solanum tuberosum* cv. Norland using sparging technique.

Compound	Concentration Range (μg/g/min)[a]			
	Plantlet[b] Ambient	Plantlet[b] 100°C	Roots[c]	Leaves[c]
Caryophyllene, trans-	0.01-0.54	0.17-6.14	----	4.48-7.05
Humulene, alpha-	0.03-0.60	----	----	0.38-0.79
Hexanal	----	8.30-10.13	----	4.56-6.73
Thiobismethane	----	0.50-35.52	0.07-0.10	0.03-0.08
3-Hexen-1-ol, cis-	2.52-5.55	1.62-13.31	----	0.29-4.87
3-Hexenyl acetate, cis-	0.54-5.77	----	----	----

[a]μg compound/g dry wt./min. purge time
[b]*in vitro* grown plantlets (4-8 weeks old)
[c]Mature plants (65-day old after transplanting)
----,not detected

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