Volatile Organic Compounds Emitted by Plants Determination using New Gas Chromatography Mass-Spectrometry Methods

Lucian Copolovici[a,b], Astrid Kännaste[c], Andreea Pag[a], Daniel Tomescu[a], Adina Bodescu[a,b], Ülo Niinemets[c]

[a]*Institute of Research, Development, Innovation in Technical and Natural Sciences of "Aurel Vlaicu" University, 2 Elena Dragoi St., 310330, Arad, Romania*

[b]*Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, 2 Elena Dragoi St., 310330, Arad, Romania*

[c]*Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 1 Kreutzwaldi, 51014, Tartu, Estonia*

Corresponding author email: lucian.copolovici@emu.ee

Abstract

In the nature, plants emit numerous volatile organic compounds. Common plant volatiles include various green leaves volatiles, terpenes, phenylpropanoids and/or benzenoids. In the present paper it will be characterized thermal desorption (TD) and solid phase microextraction methods (SPME) for simultaneous determination of green leaves volatiles (GLVs), various mono- and sesquiterpenes in headspace of plants. The first method is based on preconcentration of VOCs on solid absorbents coupled with the gas chromatograpy mass-spectrometry coupled with thermal desorption system (GCMS-TD). For trapping the volatile organic compounds (VOC) we used a multibead tube filled with solid sorbents (Carbotrap© and Carbopack©). Different types of solid sorbents have been tested and characterised. The second method is based on adsorption of different volatile compounds on the fibres followed by GC-MS analyses. The fibres trapped and released volatile organic compounds with different numbers of carbons atoms. Both methods have been used for volatile organic compounds emitted by plants from *Betulaceae* family.

Introduction

Plants emit more than 100,000 chemical products and at least 1700 of these are known to be volatile (see for review (Loreto and Schnitzler, 2010)). A very large number of BVOC

from plants are synthesizing using a few common biosynthetic pathways. Generally, several classes of secondary metabolites are produced by the plants via: shikimate-phenylpropanoid pathway: salicylic acid, and hydroxycinnamates and their esters, lipoxygenase pathway: C6 aldehydes, alcohols, and their esters and terpenoid pathway: carotenoids and chlorophylls, plant hormones including gibberellins, abscisic acid, cytokinins and terpene and isoprene (Dudareva et al., 2006). A vast array of volatile compounds - terpenes (mono- and sesquiterpenes), lipoxygenate pathway compounds, ethylene, nitric oxide, methanol, ethanol are involved in stress-dependent signalling within a single plant as well as communication between plants and between plants and insects (Dicke and Loreto, 2010; Niinemets, 2010).

There are several methods for collecting those volatiles prior to analysis including washing of plant leaves with solvent (Griffiths et al., 1999), sorption of VOCs into liquid coatings (Pillonel et al., 2002), collecting the VOCs into coated capillary columns (Pillonel et al., 2002). In the last years solid phase microextraction (Bojko and Pawliszyn, 2014; Savelieva et al., 2014) and pre-concentration of the VOCs on solid adsorbents followed by thermal desorption has become a well-accepted methods (Krol et al., 2010; Zhang and Li, 2010; Jansen et al., 2011; Pandey and Kim, 2011; Miekisch et al., 2014). In the recent paper, Curtis et al (2014)have been determined the biogenic volatile organic compound (BVOC) emissions of nine urban tree species using preconcentration on adsorbent followed by GC-MS analysis. Samples have been collected in glass tubes filled with a multi-adsorbent bed composed of Tenax GR and Carboxen 1016. The same method has been used to characterized the emissions from polymeric materials from heritage collections and to understand how the different compounds might affect the stability of other heritage objects (Mitchell et al., 2014). Solid phase micro-extraction (SPME) technique has been used for measurement of halogenated, aromatic and oxygenated VOC in the snow pack (Kos et al., 2014). The same procedure has been proposed by collection and identification of the volatiles from Norway spruce (*Picea abies* (L.) Karst) seedlings (Kannaste et al., 2013).

In the present paper we used thermal desorption (TD) and solid phase microextraction methods (SPME) for simultaneous determination of volatile organic compounds emitted in stress conditions.

Material and methods

Whole plants were placed in a dynamic headspace sampling cuvette system consisting of two 3 L glass chambers similar to the system described in detail in Toome et al. (2010) and Copolovici et al. (Copolovici et al., 2011).

First method (TD) for VOC sampling determination used adsorbent cartridges which have been mounted at the outlets of each cuvette. The sampling have been performed with a flow rate of 200 ml min^{-1} for 15 min by using a constant flow air sample pump (1003-SKC, SKC Inc., Houston, TX, USA). In addition, a sample was taken from the air inlet prior to the cuvettes to estimate the background VOC concentrations. Multibed adsorbent cartridges were filled with different types of carbopacks and were optimized for trapping of all plant volatiles between C5-C15 (Copolovici et al., 2009). Adsorbent cartridges were analyzed for lipoxygenase (LOX) pathway products, mono-, homo- and sesquiterpene concentrations with a combined Shimadzu TD20 automated cartridge desorber and Shimadzu 2010Plus GC-MS instrument (Shimadzu Corporation, Kyoto, Japan) using a method detailed in (Toome et al., 2010). The identifications and quantifications of different compounds were done using authentic standards (Sigma-Aldrich, Taufkirchen, Germany). The background (blank) VOC concentrations were subtracted from the emission samples with the seedlings.

The second method for sampling has been used a solid-phase micro extraction (SPME) syringe. The fiber used for the absorption of the volatile components was polydimethylsiloxane/divinylbenzene (PDMS/DVB), thickness 65 µm Supelco Company (Bellefonte, PA, USA). The fiber was conditioned before use for 30 minutes, as recommended by the manufacturer.

Volatile organic compounds were captured by placing the coated fiber extraction phase in the measurement chamber, with the plant, for 10 minutes followed by desorption in gas chromatograph injector. All measurements of plant volatiles have been done at room temperature of 25 °C. Background air samples were collected from the empty chamber before the measurements and were subtracted from the emission samples of the plants. BVOC samples were analyzed using a gas-chromatograph Agilent Technology 7820A (Agilent Scientific, USA) coupled with mass spectrometer MSD 5975, using a method described previously (Copolovici et al., 2009). The compounds were identified based on NIST library and on the retention time of standard compounds and the concentration of alpha-pinene, sabinene, 3-(Z)-1-hexen-1-ol and 3-(Z)-1-hexen-1-ol acetate were calculated based on with external authentic standards consisting of known amount of those compounds.

Results and discussions

Using the first method we manage to quantify different lipoxygenase (LOX) pathway products, mono-, homo- and sesquiterpenes. An example with a chromatogram obtained for emission of an evergreen tree *Quercus ilex* has been shown in the figure 1.

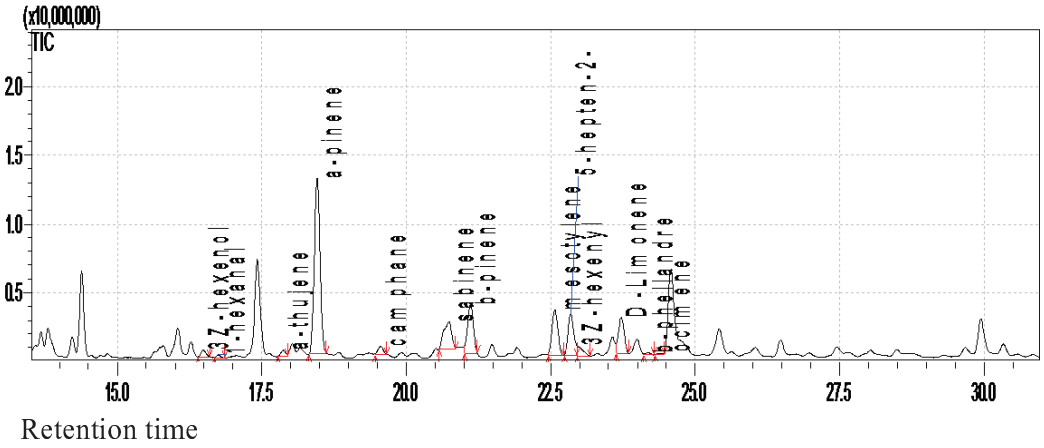

Figure 1 Typical chromatogram for emission of different volatile organic compounds from *Q. ilex* leaf

It can be seen that in the normal condition, monoterpenes emission have been relatively high while green leaves volatiles (C6 aldehydes and alcohols) emission is very low.

Using the same technique we measured the emission rates from *Fuchsia magellanica* which emit sesquiterpenes in the physiological conditions.

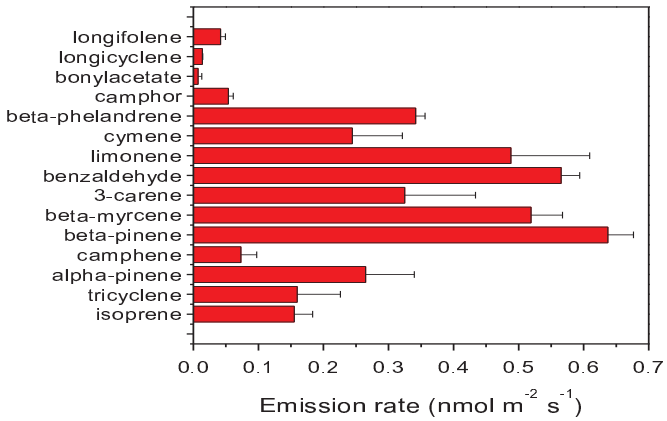

Figure 2 The emission rates of different terpenes from *Fuchsia magellanica* leaves

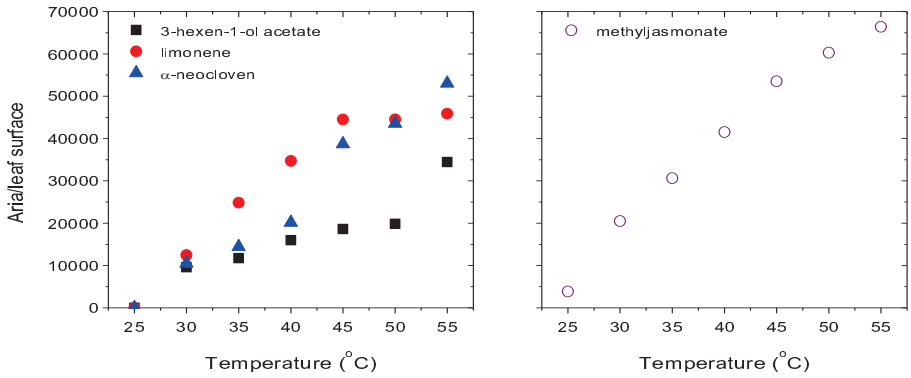

Figura 3 Relative emission rates for *F. excelsior* leaves function of temperatures

Using the SPME technique we have been shown that the emission of terpene from the leaves of *Fraxinus excelsior* increased with the temperature of the leaves.

Acknowledgement

Funding for this study has been provided by the Romanian National Authority for Scientific Research, CNCS – UEFISCDI (project number PN-II-RU-TE-2011-3-0022).

References

Bojko, B., Pawliszyn, J., 2014. *In vivo* and *ex vivo* SPME: a low invasive sampling and sample preparation tool in clinical bioanalysis. Bioanalysis 6, 1227-1239.

Copolovici, L., Kaennaste, A., Remmel, T., Vislap, V., Niinemets, U., 2011. Volatile emissions from *Alnus glutionosa* induced by herbivory are quantitatively related to the extent of damage. Journal of Chemical Ecology 37, 18-28.

Copolovici, L., Kännaste, A., Niinemets, U., 2009. Gas chromatography-mass spectrometry method fordetermination of monoterpene and sesquiterpene emissions from stressed plants. Studia Universitatis Babes-Bolyai Chemia 54, 329-339.

Curtis, A.J., Helmig, D., Baroch, C., Daly, R., Davis, S., 2014. Biogenic volatile organic compound emissions from nine tree species used in an urban tree-planting program. Atmospheric Environment 95, 634-643.

Dicke, M., Loreto, F., 2010. Induced plant volatiles: from genes to climate change. Trends in Plant Science 15, 115-117.

Dudareva, N., Negre, F., Nagegowda, D.A., Orlova, I., 2006. Plant volatiles: Recent advances and future perspectives. Critical Reviews in Plant Sciences 25, 417-440.

Griffiths, D.W., Robertson, G.W., Birch, A.N.E., Brennan, R.M., 1999. Evaluation of thermal desorption and solvent elution combined with polymer entrainment for the analysis of volatiles released by leaves from midge (*Dasineura tetensi*) resistant and susceptible blackcurrant (*Ribes nigrum L.*) cultivars. Phytochemical Analysis 10, 328-334.

Jansen, R.M.C., Wildt, J., Kappers, I.F., Bouwmeester, H.J., Hofstee, J.W., van Henten, E.J., 2011. Detection of Diseased Plants by Analysis of Volatile Organic Compound Emission. Annual Review of Phytopathology, Vol 49, pp. 157-174.

Kannaste, A., Zhao, T., Lindstrom, A., Stattin, E., Langstrom, B., Borg-Karlson, A.K., 2013. Odors of Norway spruce (*Picea abies L.*) seedlings: differences due to age and chemotype. Trees-Structure and Function 27, 149-159.

Kos, G., Kanthasami, V., Adechina, N., Ariya, P.A., 2014. Volatile organic compounds in Arctic snow: concentrations and implications for atmospheric processes. Environmental Science-Processes & Impacts 16, 2592-2603.

Krol, S., Zabiegala, B., Namiesnik, J., 2010. Monitoring VOCs in atmospheric air II. Sample collection and preparation. Trac-Trends in Analytical Chemistry 29, 1101-1112.

Loreto, F., Schnitzler, J.-P., 2010. Abiotic stresses and induced BVOCs. Trends in Plant Science 15, 154-166.

Miekisch, W., Trefz, P., Bergmann, A., Schubert, J.K., 2014. Microextraction techniques in breath biomarker analysis. Bioanalysis 6, 1275-1291.

Mitchell, G., Higgitt, C., Gibson, L.T., 2014. Emissions from polymeric materials: Characterised by thermal desorption-gas chromatography. Polymer Degradation and Stability 107, 328-340.

Niinemets, Ü., 2010. Mild versus severe stress and BVOCs: thresholds, priming and consequences. Trends in Plant Science 15, 145-153.

Pandey, S.K., Kim, K.-H., 2011. Human body-odor components and their determination. Trac-Trends in Analytical Chemistry 30, 784-796.

Pillonel, L., Bossett, J.O., Tabacchi, R., 2002. Rapid preconcentration and enrichment techniques for the analysis of food volatile. A review. Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology 35, 1-14.

Savelieva, E.I., Gavrilova, O.P., Gagkaeva, T.Y., 2014. Using solid-phase microextraction combined with gas chromatography-mass spectrometry for the study of the volatile products of biosynthesis released by plants and microorganisms. Journal of Analytical Chemistry 69, 609-615.

Toome, M., Randjärv, P., Copolovici, L., Niinemets, Ü., Heinsoo, K., Luik, A., Noe, S.M., 2010. Leaf rust induced volatile organic compounds signalling in willow during the infection. Planta 232, 235-243.

Zhang, Z., Li, G., 2010. A review of advances and new developments in the analysis of biological volatile organic compounds. Microchemical Journal 95, 127-139.