14

Mass spectrometric gas release composition studies of living organisms

© A.G. Kuzmin,[1] Yu.A. Titov,[1] G.V. Mitina[2]

[1] Institute of Analytical Instrument Making, Russian Academy of Sciences, St. Petersburg, Russia
[2] All-Russian Institute of Plant Protection, Saint-Petersburg, Russia
e-mail: toplm@mail.ru

Received December 30, 2021
Revised December 30, 2021
Accepted March 1, 2022

The quantitative and qualitative composition of volatile organic compounds (VOCs) of human exhaled air, the air environment of various types of entomopathogenic fungi (EPF) of the genus *Lecanicillium*, used as an alternative to chemical pesticides to protect plants from pests, cabbage moth caterpillars, as well as spined soldier bug from the shield family, was studied using a quadrupole mass spectrometer. The main components found were acetone, acetic acid, and isoprene. These components in human exhalation are significant indicators for non-invasive disease diagnosis. For the first time, sulfur dioxide was found in the composition of VOC EPF, as one of the possible repellents. The caterpillars of cabbage moth secrete exclusively acetic acid.

Keywords: mass spectrometry, volatile organic compounds analysis, exhaled air, entomopathogenic fungi, *Mamestra brassicae L.*, *Podisus maculiventris S.*

DOI: 10.21883/TP.2022.07.54476.341-21

Introduction

It is known that metabolic reactions continuously take place in living organisms and cause the synthesis of necessary metabolic products in required amounts. Certain metabolic products — volatile organic compounds (VOC) - are also produced. These products are released during breathing of animals (including humans) and plants. Analysis of composition of gases released by a living organism allows to determine prevailing metabolic processes, evaluate the organism condition and to track their response to various external impacts.

The issue of composition analysis of air expired by human body is widely described in literature. Multiple reviews are dedicated to this issue [1–5]. The main physical analysis methods for expired air include: chromatography [6,7], combined gas chromatography mass-spectrometry [8,9], electrochemical sensors [10], laser spectroscopy [11] and diode laser spectroscopy [12]. However, all listed methods have significant disadvantages which did not allow till now to develop and launch into the medical market any commercial instrument meeting all medical diagnostics requirements. Main disadvantages of the listed methods may be listed. Chromatography and combined gas chromatography mass-spectrometry require complex and labour-intensive sample preparation procedure with expired air sample concentration on packings and subsequent thermal desorption in the chromatograph inlet system. One such analysis procedure takes at least 30 min. Electrochemical sensors are selective sensors designed only for one mixture component. They are very sensitive to high humidity which may exist in expired air, have low quick response (dozens seconds) and require additional time to return to operation after analysis. Laser spectroscopy is set to recording only individual components, i. e. is a selective method and requires the use of additional multipass cells to ensure optical path of several dozens meters.

Thus, development of polyselective composition analysis method is required to define the composition of air released by human body and other living bodies. The method shall be high sensitive and shall be able to analyze simultaneously all components o complex gas mixtures with high sensitivity and real-time quick response. Optimum method for high sensitive analysis of VOC composition is mass-spectroscopy.

1. Experiment

In order to study quantitative and qualitative composition of main VOC released by living organisms, direct gas mass-spectrometer analysis method was used. MC7-200 portable space-saving quadrupole mass-spectrometer with direct capillary insertion probe at ambient pressure and with electron impact ionization developed by the Institute for Analytical Instrumentation RAS was used as the analyzer. This spectrometer is widely used to analyze the composition of air expired by humans and animals and various gas media [13–16]. The mass-spectrometer allows to analyze VOC in mass number range 1−200 a.m.u., has resolution 1 a.m.u., sensitivity for individual components 50−500 ppb. Sample is introduced directly from atmosphere via a 3 m capillary warmed up to 50°C. Sample flow rate is 5−8 μl/s. Instrument weight is 13 kg.

The test objects included:

— air expired from mouth of the authors of this paper sampled by the mass-spectrometer capillary by non-invasive method near the mouth;

— air environment of *Lecanicillium* entomopathogenic fungus (EF) used as producers of environmentally safe

biological medical products for pest control of plants, and grown on culture medium in 20 ml glass chromatographic vials;

— air environment from a nursery with cabbage moth caterpillars *Mamestra brassicae L.*;

— air environment from a nursery with predatory shield bugs *Podisus maculiventris S.*

Registration and processing of mass-spectra was carried out in real time.

2. Results

2.1. Human expired air

Typical mass-spectrum of expiration of the experiment participants is shown in Figure 1.

The expired air generally contains three main components: acetone — C_3H_6O (weight 58), acetic acid — CH_3COOH (weight 60), isoprene — C_5H_8 (weights 53, 67, 68). The specified components concentrations are within 0.05−0.2 ppm. The content of the listed components varied within ±20−30% depending on health and individual features. The listed components are significant for non-invasive diagnostics of diseases in man. This was noted in some foreign publications [17,18]. However, it should be noted that for data acquisition, SIFT-MS type mass-spectrometers were used whose cost is an order of magnitude higher than that of MC7-200 and which have very high weight (weight 150 kg). Thus, due to small size and low cost, MC7-200 mass-spectrometer has an adequate sensitivity to obtain diagnostics-relevant data in terms of expired air composition.

2.2. Entomopathogenic fungi

EF are used as microbiological plant protection agents and are necessary alternative of chemical insecticides due to development of phytophag tolerance to insecticides and negative impact of residual quantities of insecticides on environment and health. Information available in literature on entomopathogenic fungus VOC composition and on their influence on arthropods is rather limited. Therefore, in-depth study of VOC composition will allow to evaluate the properties of various EF strains that influence insects behavior.

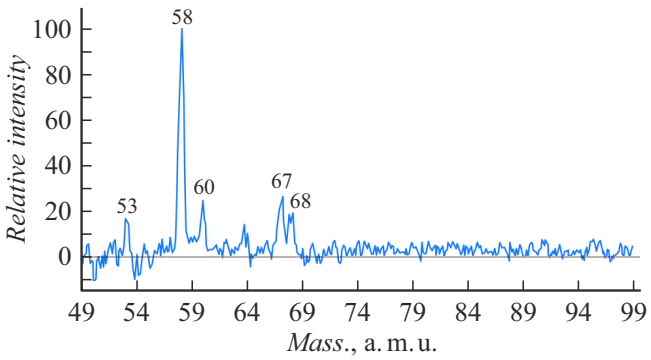

Figure 1. Mass-spectrum of human expired air.

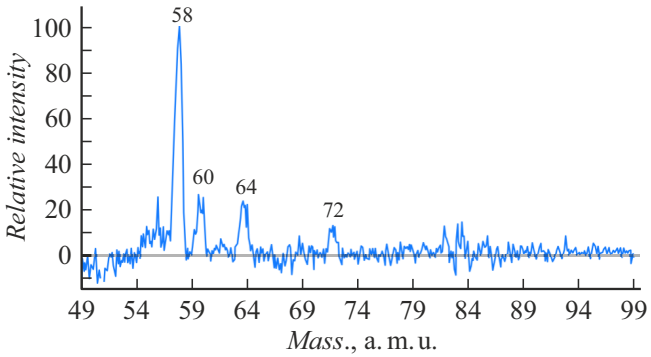

Figure 2. Mass-spectrum of Vl 21 *L. muscarium* strain.

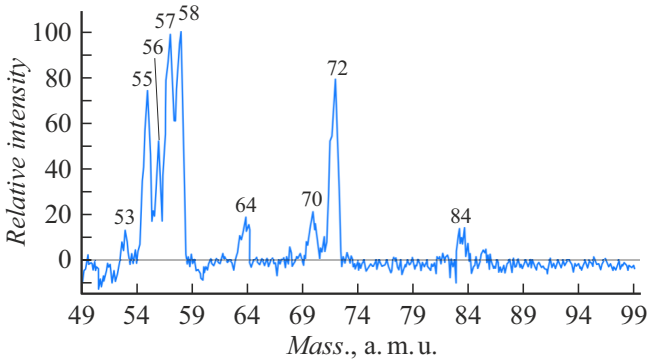

Figure 3. Mass-spectrum of Vl 5 *L. lecanii* strain.

Lecanicillium EF air environment mass-spectra showed high diversity. Due to this diversity, EF mycelium caused various behavioral response in insects: from repellency to attractiveness [19]. The main gas phase components detected over EF mycelium included acetone (weight 58), pentane (fragment weights 53, 55, 56, 57, 70, 72), acetic acid (weight 60). The listed components had the following concentrations: acetone 0.2−12 ppm, pentane up to 0.5 ppm, acetic acid up to 0.15 ppm. In some cases, acidic acid appeared only in several days after completion of active growth or was absent at all. Typical mass-spectra of Vl 21 *L. muscarium* and Vl 5 *L. lecanii* strains are shown in Figure 2, 3.

These measurements were the first to record sulfur gas (weight 64, 66) which is known as fumigator for insect-pest control [20,21], and hexyl acetate (weight 84). Especially high concentration of sulfur gas (41 ppm) was observed in Vl 29 *L. lecanii* strain (Figure 4) which showed high repellence for Orius predatory bugs. We have identified that sulfur gas causes fertility reduction in blood-sucking pest (aphis and thrips).

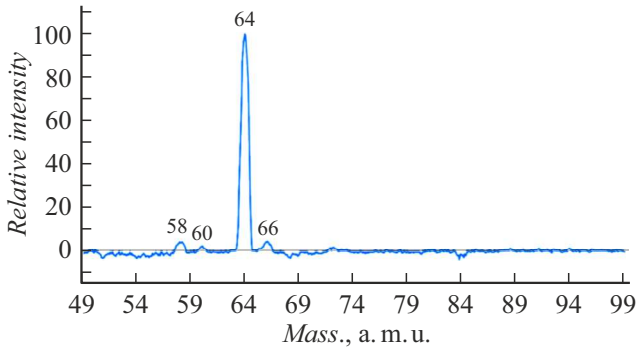

Figure 4. Mass-spectrum of Vl 29 *L. lecanii* strain.

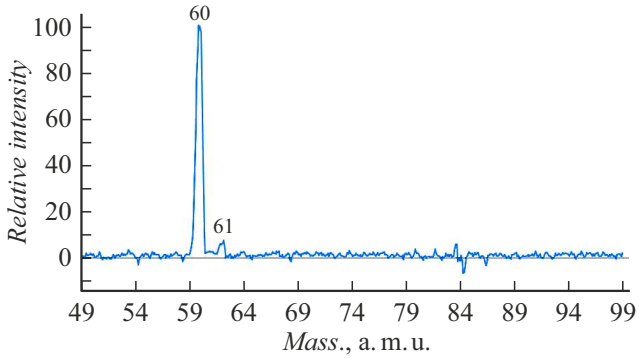

Figure 5. Air environment mass-spectrum of *Mamestra brassicae L.* cabbage moth caterpillars after feeding.

2.3. *Mamestra brassicae L.* cabbage moth caterpillars

For analysis, 3 cabbage moth caterpillars were transferred from the nursery into an individual 20 ml vial and were contained in it during 2 h. Then air composition in the vials was analyzed. The measurements were carried out in caterpillars before and after feeding (Figure 5). Acidic acid (weight 60.61) was the main VOC component with its concentration in caterpillars equal to 0.5 ppm before feeding and 1 ppm after feeding.

2.4. *Podisus maculiventris S.* predatory bugs

For analysis, 7 cabbage moth caterpillars were transferred into an individual sealed 20 ml vial and were contained in it during 3 h. Then air composition in the vials was analyzed. Measurements were carried out in bugs after feeding (Figure 6). Acetone and acetic acid were the main VOC components. In addition, components with weights 55 and 57 a.m.u. were detected and are not still identified.

Conclusions

The VOC analysis method used for air released by living organisms using quadrupole mass-spectrometer with direct capillary insertion probe enables to identify air components and measure their concentrations in real time. The experiments showed that the sensitivity of MC7-200 mass-spectrometer is sufficient for real-time recording of human expired air components important for non-invasive diagnostics of diseases. For the first time, sulfur gas was identified in *Lecanicillium* entomopathogenic fungi which influences behavioral response of blood-sucking insects, intensive acetic acid vapor release was recorded in cabbage moth caterpillars. The proposed direct analysis method and obtained information make it possible to study the metabolic processes in living organisms.

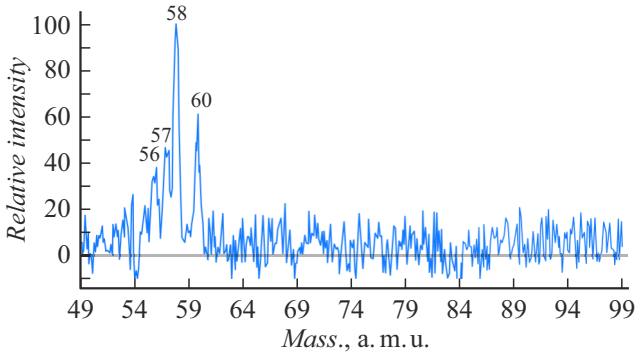

Figure 6. Air environment mass-spectrum of *Podisus maculiventris S* shield bugs.

Funding

The study was performed in the Institute for Analytical Instrumentation RAS as part of State Assignment 075-00780-19-02.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] E.V. Stepanov. Tr. IOFAN, **61**, 5 (2005) (in Russian).
[2] F. Di Francescoa, R. Fuocob, M.G. Trivellaa, A. Ceccarinib. Microchem. J., **79**, 405 (2005). DOI: 10.1016/j.microc.2004.10.008
[3] B. Buszewski, M. Kesy, T. Ligor, A. Amann. Biomed. Chromatogr., **21**, 553 (2007). DOI: 10.1002/bmc.835
[4] C. Lourenco, C. Turner. Metabolites, **4**, 465 (2014). DOI: 10.3390/metabo4020465
[5] V.L. Vaks, E.G. Domracheva, E.A. Sobakinskaya, M.B. Chernyaeva. UFN, **184** (7), 739 (2014) (in Russian). DOI: 10.3367/UFNr.0184.201407d.0739
[6] M. Phillips. Sci. Am., **267** (1), 74 (1992). DOI: 10.1038/scientificamerican0792-74
[7] M. Phillips, J. Herrera, S. Krishnan, M. Zain, J. Greenberg, R.N. Cataneo. J. Chromatogr. B, **729** (1–2), 75 (1999). DOI: 10.1016/S0378-4347(99)00127-9
[8] A.M. Leone, L.E. Gustafsson, P.L. Francis, M.G. Persson, N.P. Wiklund, S. Moncada. Biochem. Biophys. Res. Commun., **201** (2), 883 (1994). DOI: 10.1006/bbrc.1994.1784

[9] E.I. Savelieva, O.P. Gavrilova, T.Yu. Gagkaeva. Ekologicheskaya khimiya, **23** (2), 110 (2014).
[10] H.J. Vreman, J.J. Mahoney, D.K. Stevenson. Atmos. Environ., **27** (14), 2193 (1993). DOI: 10.1016/0960-1686(93)90049-5
[11] A.V. Chugunov, V.I. Novoderezhkin, T.V. Razumikhina, V.Ya. Panchenko. Proc. SPIE, **1922**, 18 (1992).
[12] E.V. Stepanov, V.A. Milyaev. Kvant. elektronika, **32** (11), 987 (2002) (in Russian).
DOI: 10.1070/QE2002v032n11ABEH002333
[13] A.G. Kuzmin, E.I. Tkachenko, L.S. Oreshko, Yu.A. Titov, A.S. Balabanov. Meditsinskiy akademicheskiy zhurnal, **16** (4), 106 (2016) (in Russian).
[14] V.V. Manoilov, A.G. Kuzmin, U.A. Titov. J. Anal. Chem., **71** (14) 1301 (2016). DOI: 10.1134/S1061934816140094
[15] A.N. Shevchenko, A.G. Kuzmin, Yu.A. Titov. Nauchnoe priborostroenie **28**, (2) 62 (2018) (in Russian). DOI: 10.18358/np-28-2-i6268
[16] L.V. Novikov, V.V. Manoilov, A.G. Kuzmin, Yu.A. Titov, I.V. Zarutsky, A.O. Nefedov, A.V. Nefedova, A.I. Arseniev. Nauchnoe priborostroenie **30**, (4), 94 (2020) (in Russian). DOI: 10.18358/np-30-4-i94105
[17] T.P.J. Blaikie, J.A. Edge, Gus Hancock, D. Lunn, C. Megson, R. Peverall, G. Richmond, G.A.D. Ritchie, D. Taylor. J. Breath Res., **8** (4) 2014. DOI: 10.1088/1752-7155/8/4/046010
[18] K. Dryahina, V. Pospíšilová, K. Sovová, V. Shestivska, J. Kubišta, A. Spesyvyi, F. Pehal, J. Turzíková, J. Votruba, P. Španěl. J. Breath Res., **8**, 037109 (2014).
DOI: 10.1088/1752-7155/8/3/037109
[19] G.V. Mitina, E.A. Stepanycheva, A.A. Choglokova. Vestnik zashchity rasteniy, **103** (4), 265 (2020) (in Russian).
DOI: 10.31993/2308-6459-2020-103-4-13466
[20] J. Riudavets, M.J. Pons, R. Gabarra, C. Castañé, O. Alomar, L.F. Vega, S. Guri. J. Stored Prod. Res., **57**, 58 (2014). DOI: 10.1016/j.jspr.2013.12.003
[21] Y.B. Liu. J. Econ. Entomol., **112** (2), 597 (2019).
DOI: 10.1093/jee/toy373