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VOLATILE ORGANIC COMPOUND EMISSIONS FROM *QUERCUS* GENUS UNDER ABIOTIC STRESSES

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Abstract

Volatile organic compounds (VOC) emitted by the plants constitute a sensitive signal of stress response. Quantitative relationships between volatile emissions and the stress severity have been demonstrated only for a few stresses. Among important stresses, heat stress can particularly significantly influence all the metabolic processes of the deciduous trees. We studied the effects of heat shock treatments on leaf photosynthesis and the emission of the volatile products of lipoxygenase pathway (LOX) and mono- and sesquiterpene emissions in Quercus genus (including here Q. robur, Q. petraea, Q. cerris, and Q. rubra) to gain quantitative insight into temperature stress elicited volatile emissions. Heat stress treatments ranged from mild that only weakly affected foliage photosynthesis to severe that almost completely inhibited photosynthesis. As well it have been demonstrated that all photosynthetic parameters are less affected for the plants which emit isoprenes due to the thermo-protector role of this VOC. Under non-stressed conditions, LOX emissions were close to detection limit, and terpene emissions were low. The emission of all metabolic compounds exhibit "breaking points" which are species specific. We suggest that the quantitative relationships between the stress strength and emissions observed in this study provide an important means to characterize the severity of cold and heat stresses.

Key words*: Volatile organic compounds, abiotic stress, Quercus genus, green leaf volatiles*

INTRODUCTION

Almost all plant species are emitters of different volatile organic compounds (VOC). It is known that plants emit more than 100,000 chemical products and at least 1700 of these are known to be volatile (Kesselmeier and Staudt, 1999). Plants emissions consist of a complex blend of chemically heterogeneous volatile isoprenoids. Often more than 20 different monoterpenes are emitted by a single species (Loreto et al., 2009; Niinemets and Reichstein, 2003). Isoprenoids are very different functionally and structurally and have diverse functions in photosynthesis, respiration, membrane fluidity and as well they play an important role in alleopathic and plant-pathogen interactions (Vranova et al., 2012). The studies devoted to plants mechanism show that all isoprenoids are synthesized *via* two different pathways from one common precursor, namely isopentenyl diphosphate. The mevalonate pathway (MVA) is localized in cytoplasm and is used to synthesize cytosolic and mitochondrial isoprenoids (sesquiterpenes, sterols). The second pathway, 2-C-methyl-*D*-erythritol 4-phosphate pathway (MEP) is responsible for monoterpene and diterpene production in plastid (Edreva et al., 2007). A vast array of VOC is involved in stress-dependent signalling within a single plant as well as communication between plants and between plants and insects (see for review (Dicke et al., 2009).

Lipoxygenase (LOX) pathway products are induced in a variety of plant species during different stress conditions in a process where free octadecanoid fatty acids are released from plant membranes by phospholipases. LOX activity produces 9- or 13-hydroperoxylinoleic or -linolenic acid or a mixture of both. Then, a hydroperoxide lyase catalyzes the breakdown

of 13-hydroperoxylinole(n)ic acid to a C6-compound, (Z)-3-hexenal, and a C12-product, 12-oxo-(Z)-9-dodecenoic acid. (Z)-3-hexenal can further give rise to (Z)-3-hexenol, (E)-2-hexenol, (E)-3-hexenol or (E)-2-hexenal in consequent reactions (Feussner and Wasternack, 2002; Matsui, 2006). The emissions of the green leaf volatile or LOX were presented in many studies and they are induced by different types of stress abiotic as heat and cold (Copolovici et al., 2012), flooding (Copolovici and Niinemets, 2010), drought (Rodriguez-Calcerrada et al., 2013) and biotic (Copolovici et al., 2011).

Quercus (oaks) are the largest genus in *Fagaceae* family which include deciduous trees and shrubs at around 400 species many of them being monoterpene and/or isoprene emitters. *Quercus* genus is dominating the forests with deciduous trees in temperate zones including Romania (Popa et al., 2013).

Our hypothesis has been that VOC emission from plants can be used to quantify the stress impact on the plant. As a consequence, different *Quercus* species leaves were exposed to heat stress and the VOC emissions were measured.

MATERIALS AND METHODS

Plant material

Seedlings of *Quercus robur, Q. petrea*, *Q. cerris*, and *Q. rubra* are from local origin (Arad National Forest Department ROMSILVA) and were grown in a local greenhouse in 5 L clay pots filled with a 1:1 mixture of commercial potting soil and sand. The plants were watered daily and fertilized once per month with a fertilizer containing microelements. In all experiments, we used similar-sized 2-yr-old seedlings with 20-25 leaves. The experiment was conducted with plants having fully mature leaves

Stress application

The procedure of heat stress application (5 minutes) has been used according to Frolec et al. (2008) and Copolovici et al. (2012). The temperatures were applied in sequence 25, 35, 47, 53, 56, and 58 °C. Individual plants were used for each treatment.

Isoprene and green leaves volatile measurements

After stabilization following the stress application, the leaves subject to stress were mounted in the clipping cuvette of a commercial gas-exchange system (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany). VOC sampling was performed on adsorbent cartridges via the outlets of each cuvette every day 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). Adsorbent cartridges were analyzed for isoprene and lipoxygenase pathway products concentrations using a Shimadzu TD20 automated cartridge desorber and Shimadzu 2010Plus GC-MS instrument (Shimadzu Corporation, Kyoto, Japan) according to the GC-MS method detailed in our previous studies (Copolovici et al., 2009; Toome et al., 2010). The background (blank) VOC concentrations were subtracted from the emission samples with the seedlings.

RESULTS AND DISCUSSIONS

The emission of isoprene from *Quercus* genus plants have been shown in many other papers (Monson et al., 2013). *Quercus robur, Q. petrea*, and *Q. rubra* have been demonstrated to be isoprene emitters while *Q. cerris* is not emitting isoprene. The standard isoprene emission (at temperature 30^{o}C and 1000 nmol m^{-2} s^{-1} light) have been found at 23.54 nmol m^{-2} s^{-1} for *Q. robur*, 25.76 nmol m^{-2} s^{-1} *Q. rubra* and 26.45 nmol m^{-2} s^{-1} *Q. petrea*. The temperature dependences for isoprene emission from different *Quercus* species have been fitted using a hyperbolic function based on Guenther algorithm (Guenther et al., 1991; Guenther et al., 1993).

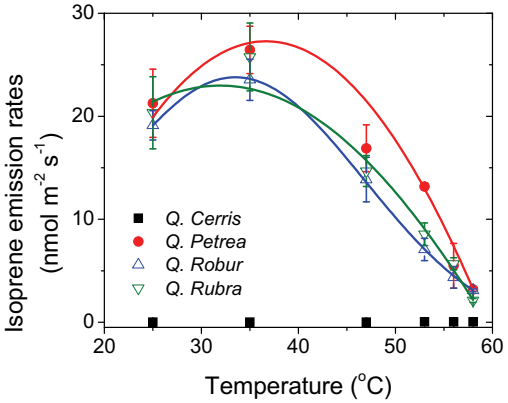

Figure 1. Changing in isoprene emission rate function on temperature for different *Quercus* species

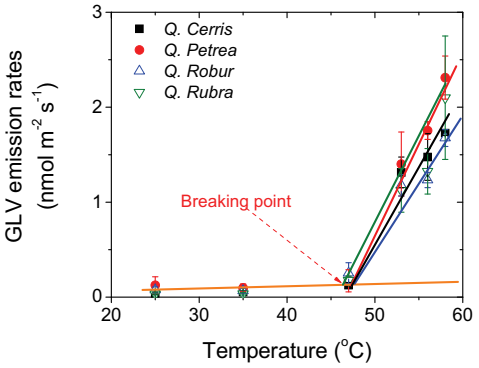

Figure 2. Changing in green leaf volatiles (GLV) emission rate function on temperature for different *Quercus* species

An Arrhenius type response was used for the isoprene emission function on temperature.
This function describes a curve with an optimum at usually 30 °C. The supraoptimal temperatures for photosynthetic processes of isoprene biosynthesis in the *Quercus* leaves could be limited by the availability of isoprenoid precursors. The same type of response have been found by Staudt and Lhoutellier (2011) for temperature dependence of monoterpene emission in *Quercus coccifera* based on metabolic investigation made by Rasulov et al. (2010).
The green leaf volatiles (GLV) have been emitted usually by plants due to different type of stress (see (Niinemets, 2010)). In case of the temperature stress, there are a burst of green leaf volatiles (GLV) in the first minutes after the stress application (Brilli et al., 2011; Copolovici et al., 2012). The GLV found in the samples were 1-hexanol, (*Z*)-3-hexenol, (*Z*)-2-hexenal, and (*Z*)-3-hexenyl acetate. In our determinations we have been shown that the emission of GLV function of temperature has breaking points which are at around 47 °C (Figure 2).
After that the GLV emission rates increased with increasing the temperature. Quantitative relationships between the GLV emission rate and stress severity have been previously shown for other abiotic stresses such as flooding ozone (Beauchamp et al., 2005), antibiotic concentrations (Opris et al., 2013) and flooding (Copolovici and Niinemets, 2010).

CONCLUSIONS

The sum of green leaf volatiles was quantitatively associated with the dose of the treatment Thus, monitoring volatile emissions is a promising tool for quantitative evaluation of abiotic stress.

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