Volatile Organic Compounds Emission from *Betula verrucosa* under Drought Stress

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Abstract

In the nature, plants are under a multitude of different stress factors. In response to biotic or abiotic stress plant elicited biogenic volatile organic compounds (BVOC). Plants emission patterns change both quantitatively and qualitatively as well in riposte to damage by biotic or abiotic stress. In the present work we focus our study to the emission of volatile organic compounds from *Betula verrucosa* under drought stress. Solid-phase micro extraction (SPME) technique have been used for trapping of the BVOC followed by GC-MS desorption. The results have been show that drought induced a high emission of lipoxygenase pathway products.

Introduction

During the past hundred years, there has been an increasing concern about the potential future impact of drought on global climate change. It is predicted that the warming effect of climate change will lead to significant changes in the frequency and quantity of precipitation (Saetersdal et al., 1998). Plants have frequently evolved in habitats where drought occurs, and so have developed multiple strategies to cope with drought stress. Tolerance strategies can be divided into resistance mechanisms, which enable plants to survive dehydration (drought stress causes stomata closure and reduced CO_2 diffusion into leaves, limiting photosynthesis), and avoidance mechanisms, which are growth habits that prevent the exposure of plant to osmotic stress (reduced normal growth and crop production in all species) (Chaves et al., 2011). However, plants have a variety of physiological and

biochemical responses to stress at cellular and whole organism levels. From them, we are focused on emission of volatile secondary metabolites.

Birch is a widespread tree in the temperate and boreal zones of Europe. The trees are fast growing tolerant to spring frost, low temperatures in general, and nutrient deficiency, though their ecological amplitude is limited by shade intolerance and short life span.

In the physiological conditions some plants emit BVOCs constitutively (Holopainen et al., 2010; Holopainen and Gershenzon, 2010).

Birch (*Betula* spp.) emits a variety of C_{10} monoterpenes, C_{15} sesquiterpenes, and also aliphatic and aromatic compounds even if they are not affected by stress factors (Pääkkönen et al., 1998; Vuorinen et al., 2007). The composition of the emitted volatile depends on abiotic or biotic stress factors. (Hakola et al., 1998; Pääkkönen et al., 1998; Schade and Goldstein, 2003; Schurgers et al., 2009; Vuorinen et al., 2005). In stressful conditions, birch could emit different quantities of BVOCs depending on the stress factors. For examples, applied heat stress at different day and night periods determined emission of isoprene, changing in sesquiterpenes and monoterpenes pattern. As well have been shown a high emission of green leaves volatiles (Z)-3-Hexenyl acetate (Ibrahim et al., 2010), (Laothawornkitkul et al., 2009). Some BVOCs, like β-ocimene and α-farnesene, are emitted in a higher quantity when birch is exposed to a pathogen (*Marssonina betulae*) (Vuorinen et al., 2007). Higher quantity of methylsalicilate, (Z)-ocimene, (E)-β-ocimene, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and linalool have been also detected after 72 h feeding by *Epirrita autumnata* larvae (Vuorinen et al., 2007).

In this work, we studied the emission of volatile organic compounds from *Betula verrucosa* under a long period of drought stress and after recovery.

Materials and methods

One year old seedlings of *Betula verrucosa*, provided by a local nursery (a generous gift from Arad Forestry Department) have been used. Plants have been cultivated in 3 liters pots filled with commercial soil (AGRO CS, Lucenic, Slovakia), fertilized with a universal fertilizer (Compo Gbh, Munster, Germany) and watered daily.

To study the response to drought stress three plants have been chose as control and three plants for drought experiment. The pots have been covered with plastic foils to exclude the influence of water evaporation from soil. Response to drought was followed for 11 days (up to relative soil water content was below 10 %) and then the seedlings was re-watered.

The water status of each pot was checked each day by measuring weight of the seedlings pots. Every day, to each control plant has been watered until full pot capacity.

The volatile organic compounds measurements have been performed using a custom-built system (Figure 1-for picture). The airflow in the measurement chamber was 1 L and all tubing in the system was made of Teflon. Light was provided by 2 fluorescent lamps at a level of light intensity at 300 μmol m^{-2} s^{-1}.

Figure 1. Custom-built system for measuring volatile organic compounds

Solid Phase Micro Extraction (SPME) was performed to analyze plant volatiles. 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 discussion

For identification of different mono and sesquiterpenes emitted by the plants, a solid phase microextraction followed by gas-chromatography mass spectrometer (GC-MS) analyses has been employed.

A mixture of different compounds (monoterpenes, LOX products and sesquiterpene) has been used to calibrate the GC-MS device. We used a method which has been already standardized for determinations of different terpenes and LOX by Copolovici et al. (2009). A typical chromatogram is presented in Figure 2.

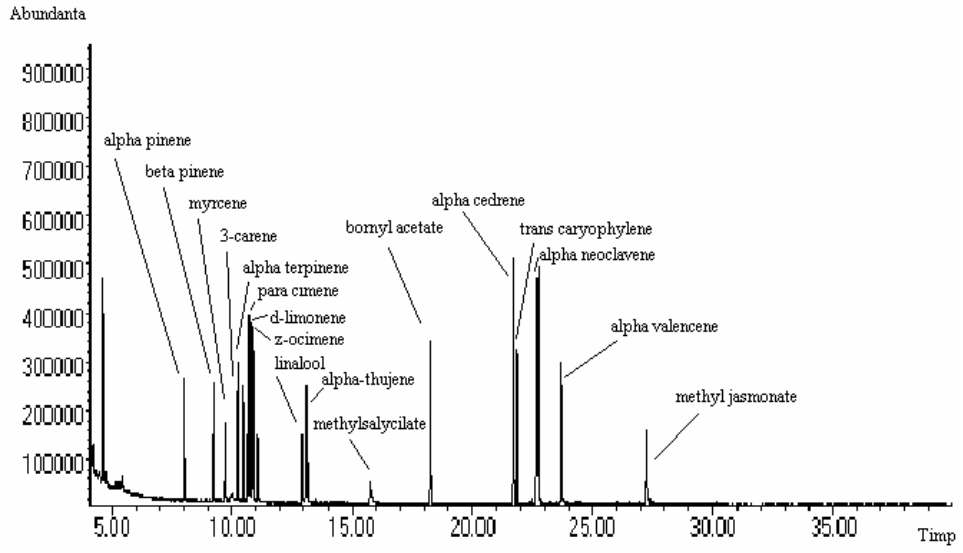

Figure 2. Typical chromatogram for standard mixture

The method provides a good separation of different compounds which could be found in stress plants emission. Table 1 presents analytical parameters of calibration curves for 17 compounds including detection and determination limits, correlation coefficients and range of

linearity. As well for all compounds Kovacs indexes have been calculated and they are in a good accordance to those presented in the literature.

Table 1 Analytical parameters obtained by the GC-MS method for all the tested BVOC standards

Substance	**Corr. coefficient**	**Det. limit (nmol/L)**	**Deter. Limit (nmol/L)**	**Target ion (m/z)**	**Qualifiers (m/z)**	**Kovacs Indices**
Alpha-pinene	0.999	28.91	43.35	93	92, 91	937
Betha-pinene	0.989	39.62	98.89	93	41, 69	979
myrcene	0.991	13.72	37.14	41	93, 69	992
3-carene	0.992	25.99	55.75	93	91, 77	1012
alpha terpinene	0.993	3.51	4.66	93	121, 91	1019
para-cimene	0.993	1.53	2.63	119	134, 91	1027
D-limonene	0.988	0.66	1.18	68	67, 93	1032
Z-ocimene	0.994	4.66	10.45	93	92, 91	1040
linalool	0.996	12.18	32.72	71	43, 81	1000
Alpha-tujene	0.995	2.90	7.39	81	110, 41	1008
methylsalycilate	0.998	1.37	3.14	120	92, 152	1097
bornyl acetate	0.999	1.02	1.63	95	43, 93	1089
Alpha-cedrene	0.999	1.55	3.23	119	93, 105	1020
trans-caryophyllene	0.999	1.00	2.41	41	69, 93	1026
alpha-neoclovene	0.999	1.11	2.50	189	161, 204	1059
Alpha-valencene	0.999	0.59	0.75	191	204, 105	1099
methyl jasmonate	1.000	1.72	4.08	83	41, 151	947

The method has been used to follow the response of *Betula Verrucosa* plants exposed to drought stress. We measured the emission of volatile secondary metabolites for plants exposed to drought for 11 days followed by two days of recovery.

Only two green leaves volatiles and two monoterpens have been increased emission due to drought stress: α – pinene, sabinene, 3-(*Z*)-hexene-1-ol, and 3-(*Z*)-hexene-1-ol-acetate. (Figure 3). The other compounds remained to a very low level at the detection limit of the device.

Both green leaves volatiles (GLV) emissions followed the same trend, increasing after one day and reached a maximum level after 4-5 day of drought (Figure 3). The GLV emission decreased drastically after the plants have been re-watered. The same trend have been found by Simpraga et al. (2011). GLV function as a fast and efficient airborne signaling way to pass stress information onto neighboring trees (Niinemets, 2010).

Lipoxygenase (LOX) pathway products are induced in a variety of plant species during different stress conditions in a process where free octadecanoid fatty acids (linoleic acid = 18:2 and linolenic acid = 18:3) are released from plant membranes by phospholipases. LOX activity produces 9- or 13-hydroperoxylinoleic or -linolenic acid or a mixture of both.

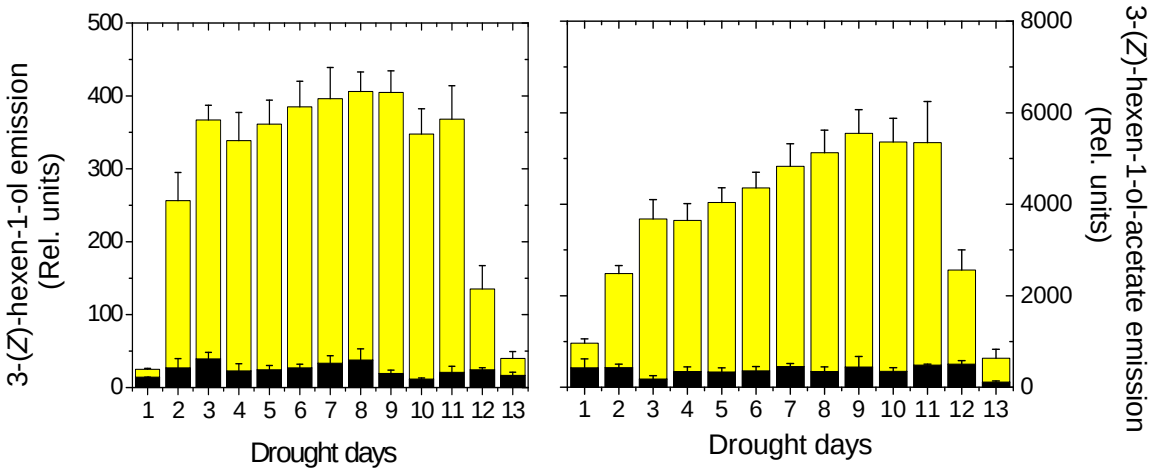

Figure 3. The emission of green leaves volatiles from *B. verrucosa*

A hydroperoxide lyase then 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 give rise to (*Z*)-3-hexenol, (*E*)-2-hexenol, (*E*)-3-hexenol or (*E*)-2-hexenal in consequent reactions (Copolovici et al., 2012; Liavonchanka and Feussner, 2006; Porta and Rocha-Sosa, 2002).

The emissions of α – pinene and sabinene increased after the first two days of drought stress treatment (Figure 4).

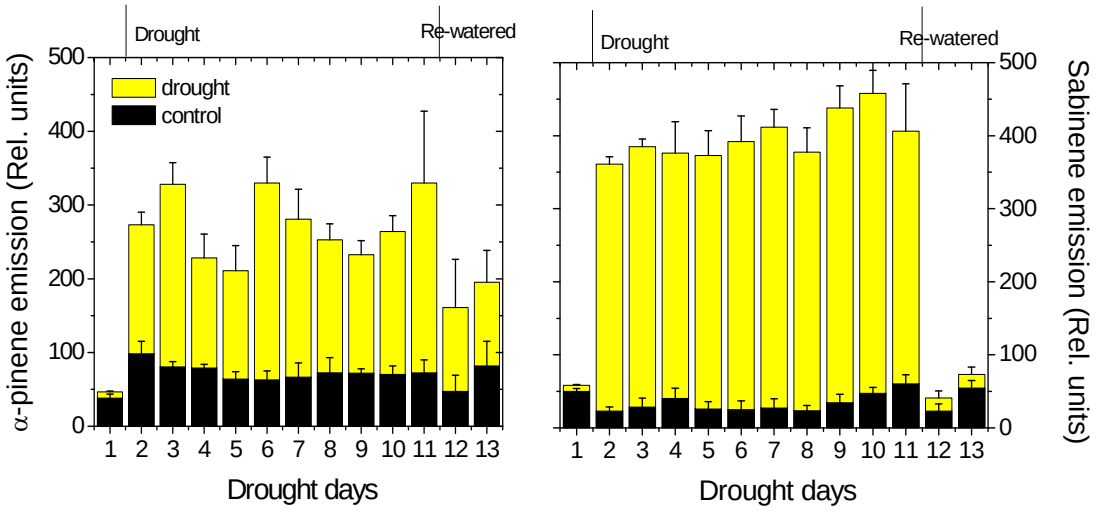

Figure 4. The emission of monoterpenes from *B. verrucosa*

The emission of α – pinene and sabinene were increasing after two days of drought followed by a decreasing in emissions. This type of trend has been found in other studies and could be due to the usage of non-specific storage pool(s) (Demarcke et al., 2010). Other hypothesis can be that monoterpenes could be stored in specialized organs in leaves and/or stems (Penuelas and Llusia, 2003).

Conclusions

We found that only some of terpenes and green leaves volatiles emission have been increased due to drought stress: α - pinene, sabinene, 3 - (Z)-hexen-1-ol and 3 - (Z)-hexen-1-ol-acetate.

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