Environmental and Experimental Botany xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Leaf gas exchange and isoprene emission in poplar in response to long-term experimental night-time warming and summer drought in a forest-steppe ecosystem

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ARTICLE INFO

Keywords:
Climate change
Photosynthesis
Isoprene synthase gene expression
Temperature
Biogenic volatile organic compounds

ABSTRACT

Climate change projections forecast an average warming in mean temperature and an associated change in the distribution and intensity of rainfalls. At the manipulation experiment in Kiskunság, Hungary, we aimed to study the effect of the soil drought and night-time warming conditions projected for the coming decades on leaf gas exchange, including photosynthetic rates and isoprene emission rates and on isoprene synthase expression levels of *Populus alba* sprouts (root suckers). During the years 2010 and 2011, warming treatment increased the air temperature 0.35 °C on average whereas drought treatment reduced the soil moisture by 13% on average in relation to control plots. The results highlighted important seasonal differences. Photosynthesis was limited by stomatal closure in summer and also isoprene emission and isoprene synthase expression levels were lower in summer than in autumn for all treatments. As a consequence, a negative relationship was found between temperature, ranging from 25 °C to 35 °C, and isoprene emission throughout all the study period and treatments. Results also showed significant treatments effects on isoprene parameters but only in July 2011. Isoprene emission decreased with drought, and isoprene synthase expression levels decreased with night-time warming. A positive relationship was found between isoprene emission and leaf internal carbon in drought treated poplar plants, which contrasted with our findings for control and warming treated plants. These results indicated either a change in the regulation of pyruvate distribution between isoprene synthesis and respiration under drought or a utilization of alternative carbon sources for isoprene emission.

1. Introduction

Isoprene (2-methyl-1,3-butadiene) is the most abundant biogenic volatile organic compound (BVOC), a reactive hydrocarbon that contributes to the production of tropospheric ozone in the presence of nitrogen oxides (NOx) and sunlight (Hauglustaine et al., 2004) and to the formation and growth of aerosol particles in the atmosphere (Paasonen et al., 2013). It is emitted in large amounts by several tree species (Guenther et al., 1999; Kesselmeier and Staudt, 1999).

Isoprene is synthesized from dimethylallyl diphosphate (DMADP) through a reaction catalyzed by the enzyme isoprene synthase (ISPS) (Schnitzler et al., 1996; Silver and Fall, 1995) and it is emitted through stomata to the atmosphere (Sharkey et al., 2001). Given the importance of BVOC emissions for the photochemistry of the atmosphere, there is great interest in determining how global change factors affect isoprene synthesis and emission. Moreover, *in planta*, isoprene is considered to be an important molecule for ameliorating abiotic stresses by preventing the accumulation of reactive oxygen species and protecting cell membranes against lipid peroxidation (Loreto and Schnitzler, 2010).

Synthesis and emission of isoprene are affected by several environmental factors, including photosynthetically active radiation (PAR) and temperature, which are the main input parameters of all emission models (Guenther et al., 2006; Arneth et al., 2008; Monson et al., 2012). Therefore, the forecasted climate change scenario for the

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Abbreviations: A, photosynthesis; g_s, stomatal conductance; Ci, intercellular concentration of CO_2; ISPS, isoprene synthase; *PaISPS*, isoprene synthase from *Populus alba*; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; DOXP, 1,deoxy-D-xylulose-5 phosphate

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https://doi.org/10.1016/j.envexpbot.2018.04.005
Received 25 July 2017; Received in revised form 9 April 2018; Accepted 11 April 2018

Please cite this article as: Nogues, I., Environmental and Experimental Botany (2018), https://doi.org/10.1016/j.envexpbot.2018.04.005

year 2100 (IPCC, 2013), with predicted changes in the distribution of rainfalls, more frequent summer droughts and a 1.5–3.7 °C increase in temperature, are highly likely to alter emissions of isoprene.

Night-time warming is expected to increase soil temperature, plant respiration, and potentially also nutrient cycling and daytime photosynthesis (Turnbull et al., 2002; Xia et al., 2009) and therefore may influence total BVOC emissions. Though Ibrahim et al. (2010) reported an increase in terpene emissions as a consequence of night-time warming other studies have shown no changes (Nogués et al., 2012) and therefore more research is needed to understand how changes in night-time temperature will alter the emissions.

The impact of drought on isoprene emission has also been studied, since drought is a major limitation for plant growth worldwide. Nevertheless, drought effect on isoprene emission is controversial since it is largely dependent on the stress severity. There are several studies showing reduction of isoprene emission rates under severe drought stress (Delfine et al., 2005; Peñuelas and Staudt, 2010) possibly as a consequence of reduced photosynthetic activity and reduced carbon availability. However, Brilli et al. (2007) demonstrated that, under these conditions, the observed residual emission of isoprene can be attributed to alternative carbon sources entering the DOXP/MEP pathway from sources other than photosynthesis (Lichtenthaler et al., 1997). Also, Centritto et al. (2011) found that the entire metabolism associated to isoprenoid synthesis in black poplar may be up or down-regulated under long periods of drought or high temperatures. On the other hand, isoprene inhibition rarely occurs when drought is mild (Peñuelas and Staudt, 2010).

ISPS gene expression has been reported not to vary during the first phases of drought and to decrease under severe drought stress (Fortunati et al., 2008). (Fortunati et al., 2008) also reported of decreases in ISPS activity before isoprene emission during drought development indicating that the main control of isoprene emission may take place at a transcriptional or post-transcriptional level under moderate drought stress.

This study focuses on the impacts of two field-manipulated environmental factors, namely night-time warming and more severe summer drought on isoprene emissions and isoprene synthase expression levels of white poplar (*Populus alba*). Though the effects of these changing climatic factors on the emission of BVOC have been already studied for different shrub and tree species, this is the first time that white poplar isoprene emission is assessed at ecosystem level *in situ* in a forest-steppe transitional zone and the first time that also isoprene synthase expression levels are determined under field conditions.

Given the existing uncertainties on this topic, the present study provides new data that helps us better understand: 1) the effect of long-term *in situ* manipulation of night-time warming and increased drought on isoprene emission and *ISPS* gene expression levels from *Populus alba*; 2) seasonal variability of isoprene emission and of the responses to manipulated warming and drought; 3) and help unravel the physiological mechanisms underlying the above-mentioned responses.

2. Material and methods

2.1. The experimental manipulation

The Hungarian experimental site Kiskunság is one of the seven experimental sites of the INCREASE EU-funded infrastructure project. This site is located in the Kiskunság National Park (46°53′N 19°23′E) (Kröel-Dulay et al., 2015). It represents a forest-steppe transitional biome between temperate deciduous forests and continental steppes in Europe. The dominant species are the deciduous clonal *Populus alba* and the perennial C3 bunchgrass *Festuca vaginata*. Other important species are the C3 grass *Stipa borysthenica* and the C4 grass *Cynodon dactylon*.

The coarse sand soil is very poor in organic matter (< 1%), and has extreme heat and water regime, with soil temperature (in 5 cm depth) ranging from −17 °C to +43 °C and soil moisture content (V%/V%; 0–20 cm) ranging from 2% to 16% during 2010–2011.

The experimental site included nine plots (three replicates for each treatment, size 4 × 5 m) arranged in a random pattern. In three plots, air temperature was increased (0.4 °C increase in mean annual temperature) by covering the plots with a reflective material at night throughout the year (Kröel-Dulay et al., 2015). In three other plots, drought was simulated by covering the plots with a transparent waterproof material during rain events in May and June excluding 22% of the mean annual precipitation (Kröel-Dulay et al., 2015). Three untreated plots served as control. The experiment was built in 2001, and the treatments started in 2002. Drought was repeated in each summer (May and June), and warming treatment was running continuously.

Due to the transitional (grassland-shrubland-forest) character of Kiskunság, this ecosystem is expected to be sensitive to predicted changes in climate, thus being an ideal target system for climate change studies. Treatments and relative measurements (leaf gas exchange, isoprene emission rates and isoprene synthase expression levels) were carried out on *Populus alba* basal shoots, which are generally referred to as sprouts or suckers. The age of these poplar root suckers are not known, and can range from a few years to several decades. Their height ranged from 50 cm to 120 cm, and there were about 5–15 root suckers per plot (20 m^2) (see picture in Supplementary material).

The technique used to manipulate the environmental conditions was characterized by a passive night-time warming technique and an automated covering system to make summer drought more severe (Beier et al., 2004). The night-time warming was induced throughout the year by covering vegetation and soil with aluminium curtains at night, simulating global warming. The curtains run along two rails placed on a light scaffolding 1.5 m tall. The warming plots were open during the day and automatically covered when light intensity at sunset was lower than 4 W m^{-2}. Severe summer drought was induced by covering the plots with transparent waterproof plastic curtains during rain events. A rain sensor activated a mechanism to cover plots with the curtains whenever it rained and remove them when the rain stopped. We continuously measured air temperature at 20 cm height (one sensor per plot) with a PT-100 resistance sensor-based thermometer. Soil moisture content (V/V%) was also continuously monitored in the 0–20 cm soil depth with a Campbell Scientific CS616 Water Content Reflectometer (one sensor per plot).

Three campaigns of measurements were carried out in total (Table 1). Mean air temperature and mean photosynthetic photon flux density (PPFD) in sampling dates are shown in Table 1. Air temperature and soil moisture values for each treatment throughout the study period are shown in Fig. 1. The temperature average increase by warming and the soil moisture reduction average by drought are shown in Table 2. The effects of warming on soil moisture and of drought on air temperature are not shown because they were negligible (Fig. 1). In the long term, the overall increase in Mean Anual Temperature in warming plots was 0.4 °C (Kröel-Dulay et al., 2015).

Rainfall during the years 2010 and 2011 was 1025 mm and 498 mm respectively. The 32% and the 25% of it was intercepted in years 2010 and 2011 respectively by the rain exclusion device in the drought treatment that was active during May and June. When considering only the spring season, drought curtains excluded the 69.79% and the 74.96% of these spring totals in years 2010 and 2011, respectively. In the long term, the overall decrease in mean annual precipitation was

Table 1
Campaign dates and mean air temperature (°C) and mean maximum photosynthetic photon flux density (PPFD) in sampling dates.

Campaign dates	Temperature T (°C)	PPFD (μmol m^{-2}s^{-1})
192nd-194th 2010	29.00 ± 0.17	1992 ± 13.5
242nd-245th 2010	10.51 ± 0.05	1133 ± 17.9
189th-193rd 2011	27.13 ± 0.12	1685 ± 64.8