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BRIEF COMMUNICATION

Increase in isoprene and monoterpene emissions after re-watering of droughted *Quercus ilex* seedlings

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Abstract

We followed the diurnal cycles of isoprenoid emissions from *Quercus ilex* seedlings under drought and after re-watering. We found that *Quercus ilex*, generally considered a non-isoprene emitter, also emitted isoprene although at low rates. The emission rates of isoprene reached 0.37 ± 0.02 nmol m^{-2} s^{-1} in controls, 0.15 ± 0.03 nmol m^{-2} s^{-1} under drought and 0.35 ± 0.04 nmol m^{-2} s^{-1} after re-watering, while emission rates of monoterpenes reached 11.0 ± 3.0, 7.0 ± 1.0 and 23.0 ± 5.0 nmol m^{-2} s^{-1}, respectively. Emission rates recovered faster after re-watering than photosynthetic rate and followed diurnal changes in irradiance in controls and under drought, but in leaf temperature after re-watering.

Additional key words: light-dependent emissions, net photosynthetic rate, PTR-MS, stomatal conductance, temperature-dependent emissions, VOCs.

Plant volatile organic compounds (VOCs) include isoprene, terpenes, alkanes, alkenes, alcohols, esters, carbonyls and acids (Peñuelas and Llusià 2003). VOCs have many protective and ecological functions for the plant species that produce them and have important effects on the photochemistry and radiative properties of the atmosphere (Zimmerman *et al.* 1978, Kavouras *et al.* 1998, Peñuelas and Llusià 2003, Owen and Peñuelas 2005). Consequently there is great interest in determining the emission capacities of the different species and how environmental factors affect the volatile isoprenoid emissions (Peñuelas and Llusià 2001).

Oaks belong to the greatest VOC emitters (Harley *et al.* 1999). The holm oak (*Quercus ilex* L.), a widely distributed species in the Mediterranean basin, is considered a monoterpene and non-isoprene emitter (Kesselmeier and Staudt 1999, Loreto 2002) although Owen *et al.* (1997) reported small emission rates of isoprene. Variations in the emission of volatiles can also be triggered by changing environmental conditions (Peñuelas and Llusià 1999a, 2001, Niinemets *et al.* 2004). No specialized storage structures for monoterpenes have been found in its leaves or bark, and emissions appear to be mainly influenced by temperature, irradiance and water availability (Loreto *et al.* 1998, Llusià and Peñuelas 1998, 1999, 2000, Peñuelas and Llusià 1999a,b, Staudt *et al.* 2002).

In Mediterranean areas, drought driven changes in emission may be even more relevant under the future climate, since water availability in the Mediterranean region is likely to be reduced in the near future by the predicted increases in temperature and the consequent increases in evapotranspiration rates (Sabaté *et al.* 2002, Peñuelas *et al.* 2005). These changes in emissions may have important consequences in the formation of photochemical pollutants in this Mediterranean area (Filella and Peñuelas 2006).

The aim of this study was to describe the changes in isoprenoid emission rates induced by drought conditions and after subsequent re-watering together with physiological variables such as CO_2 and water exchange.

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Abbreviations: VOCs - volatile organic compounds; PTR-MS - proton-transfer-reaction mass spectrometer, P_N - net photosynthetic rate; g_s - stomatal conductance.

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We used two-year-old *Quercus ilex* L. plants grown in a nursery (Forestal Catalana, S.A., Breda, Spain) under typical Mediterranean environmental conditions (mean annual average temperature 16 ºC and mean annual precipitation 600 mm). They were grown in 2-dm^3 pots with a substrate composed of peat and sand (2:1), prior to being brought into the laboratory, where they were kept some days for an acclimation before starting the experiment. Control (well watered) plants were measured over one day at soil moisture of 25 % (volume of water per volume of dry soil; v/v), measured by time domain reflectometry (*Tektronix 1502C*, Beaverton, Oregon, USA) and left un-watered until soil water content reached 5 %. After one new day of measurements under these drought conditions, plants were re-watered until soil water content reached 25 % and measured again over another day. Three replicates were conducted, all at room temperature (25 ºC).

Intact leaves were clamped in a Parkinson leaf cuvette (*Std Broad 2.5*, *PP Systems*, Hitchin, England). The air entering the system was sampled at 4 m above ground from the outside of the building, filtered with glass wool to prevent any dust intake and passed through a polyethylene terephthalate (PET) recipient to buffer exterior CO_2 and VOC fluctuations. All tubing used was made of inert polytetrafluoroethylene (PTFE). A diurnal cycle of irradiance was programmed to simulate a typical sunny day, photosynthetic photon flux density (PPFD) ranging from 0 to 1500 µmol $m^{-2}\,s^{-1}$. The leaf temperature ranged between 25 and 32º C. A calibrated *Ciras-2* IRGA-porometer (*PP Systems*) was used for determining rates of CO_2 and H_2O exchange. Part of the air exiting the leaf cuvette flowed through a T-system to the PTR inlet.

We used a highly sensitive proton-transfer-reaction mass spectrometer (PTR-MS-FTD, *Ionicon Analytik*, Innsbruck, Austria) as described by Lindinger *et al.* (1998). The PTR-MS drift tube was operated at 0.21 kPa and 40 ºC, with a drift field of 600 V cm^{-1}. The parent ion signal was maintained at around 3×10^6 counts s^{-1} during the measurements. We measured isoprene (m69) and monoterpenes (m137). The quantification of isoprene and monoterpenes was based on the use of 3 times replicated calibration standards of isoprene and α-pinene (*Sigma-Aldrich*, Barcelona, Spain).

For volatile isoprenoid determination and quantification, both the air entering and exiting the leaf cuvette were analysed by (PTR-MS), at alternate 5-min intervals, and continuously monitored with flow meters. The difference between the concentration of volatile isoprenoids before and after passing through the cuvette (with leaves and without leaves), along with the flow rates, was used to calculate the volatile isoprenoid exchange (Peñuelas *et al.* 2007). Leaves were excised after emission sampling to measure leaf area (*LI 3100*, leaf area meter, *Li-Cor*, Lincoln, NE, USA). Leaves were then dried in an oven at 70 °C until constant weight (usually for 72 h) to determine dry mass.

We used one way *ANOVA*, and Bonferroni post-hoc tests to compare the leaf emissions in the three different treatments. Regression and correlation analyses were also conducted for the studied variables. In all cases we used *Statistica* (*Statsoft Inc.*, Tulsa, OK, USA) programme package.

Q. ilex seedlings emitted isoprene in all three replicates both under control watered conditions and in the three replicate measurements of the diurnal cycle during drought/re-watering experiment (Fig. 1 shows one of those replicates). The emission rates of isoprene reached 0.37 ± 0.02 nmol $m^{-2}\,s^{-1}$ in control conditions, 0.15 ± 0.03 nmol $m^{-2}\,s^{-1}$ in drought conditions and 0.35 ± 0.04 nmol $m^{-2}\,s^{-1}$ in re-watering conditions (significantly different at, $P < 0.01$, $n = 3$). Those of monoterpenes reached 11.0 ± 3.0, 7.0 ± 1.0, and 23.0 ± 5.0 nmol $m^{-2}\,s^{-1}$, respectively. *Q. ilex* was thus emitting low but significant amounts of isoprene, and the emissions ranged between 1.5 and 3.4 % of monoterpene emissions. These emissions of isoprene were slightly higher than those reported in the previous study (Owen *et al.* 1997).

There was a clear diurnal cycle in emissions of both isoprene and monoterpenes. Both emissions were stimulated during day and declined in the dark. As in recent physiological studies of oaks (Schnitzler *et al.* 2004) these isoprenoid emissions by *Q. ilex* were largely associated to net photosynthetic rate (P_N) and stomatal conductance (g_s) which presented two peaks, one in the morning and another one in the afternoon when irradiance and temperature started to decrease (Fig. 1). The concurrently performed measurements of photosynthesis revealed that *Q. ilex* leaves lost about 0.7 - 1.5 ‰ of the assimilated carbon *via* isoprenoid emission; lower than reported under field conditions in several other studies (Sharkey and Loreto 1993, Kesselmeier and Staudt 1999, Peñuelas and Llusià 1999a,b, 2001, Llusià and Peñuelas 2000).

The relative water content of *Q. ilex* leaves in the control, drought and re-watering treatments were 93.0 ± 2.2, 77.1 ± 4.0 and 91.8 ± 0.3 %, respectively (significantly different, $P < 0.01$). The early morning values of P_N and g_s were high even under drought since these plants take advantage of a short window of activity in the mornings after the night water status recovery. P_N and g_s in the afternoon followed the changes in water availability rapidly; they increased by more than twice the day after re-watering (Fig. 1). The emissions of isoprene and monoterpenes increased even more, *ca*. 3 times, after re-watering the droughted plants (Fig. 1). They responded immediately to changes in leaf temperature but not to changes in P_N or g_s as occurred in controls and under drought. This is another manifestation of the uncoupling between volatile isoprenoid emissions and photosynthesis described in several previous studies that showed that short term drought caused substantial reduction in photosynthesis, whereas isoprene emissions were either not inhibited or only slightly reduced (Tingey *et al.* 1981, Sharkey and Loreto 1993, Fang *et al.* 1996, Funk *et al.* 2005). The changes in the emissions' dependence on temperature after re-watering (Fig. 1) indicated that the temperature was the factor driving increases in the emission rates, probably as a result of enhanced isoprenoid synthesis, diffusivity and volatility (Peñuelas and Llusià 2001). Our results also indicate that emissions recovered to previous unstressed values faster than P_N or g_s which is in agreement with the recent results reported for isoprene emissions in *Populus alba* by Brilli *et al.* (2007). In addition, our results show very high terpene emission rates just after the re-watering of plants. During the recovery, the emission rates reached values in the higher limit or above the normal range reported for this species in this Mediterranean region (Llusià and Peñuelas 2000, Peñuelas *et al.* 2007). Further studies are necessary to figure out the mechanisms ruling this high emission rates temporally uncoupled of photosynthesis and highly responsive to temperature.

Although the extrapolation of these results to field conditions is never straightforward, they show that *Q. ilex* is able to emit isoprene and that there are complex responses of isoprene and monoterpene emissions to drought cycles. The emission of volatile organic compounds from vegetation is particularly sensitive to temperature (*e.g.* Peñuelas and Llusià 2001, 2003, Guenther *et al.* 1995, Filella *et al.* 2007), but the effects of drought that accompany high temperatures must also be considered to estimate the consequences of climate change on isoprenoid emissions, especially because of the

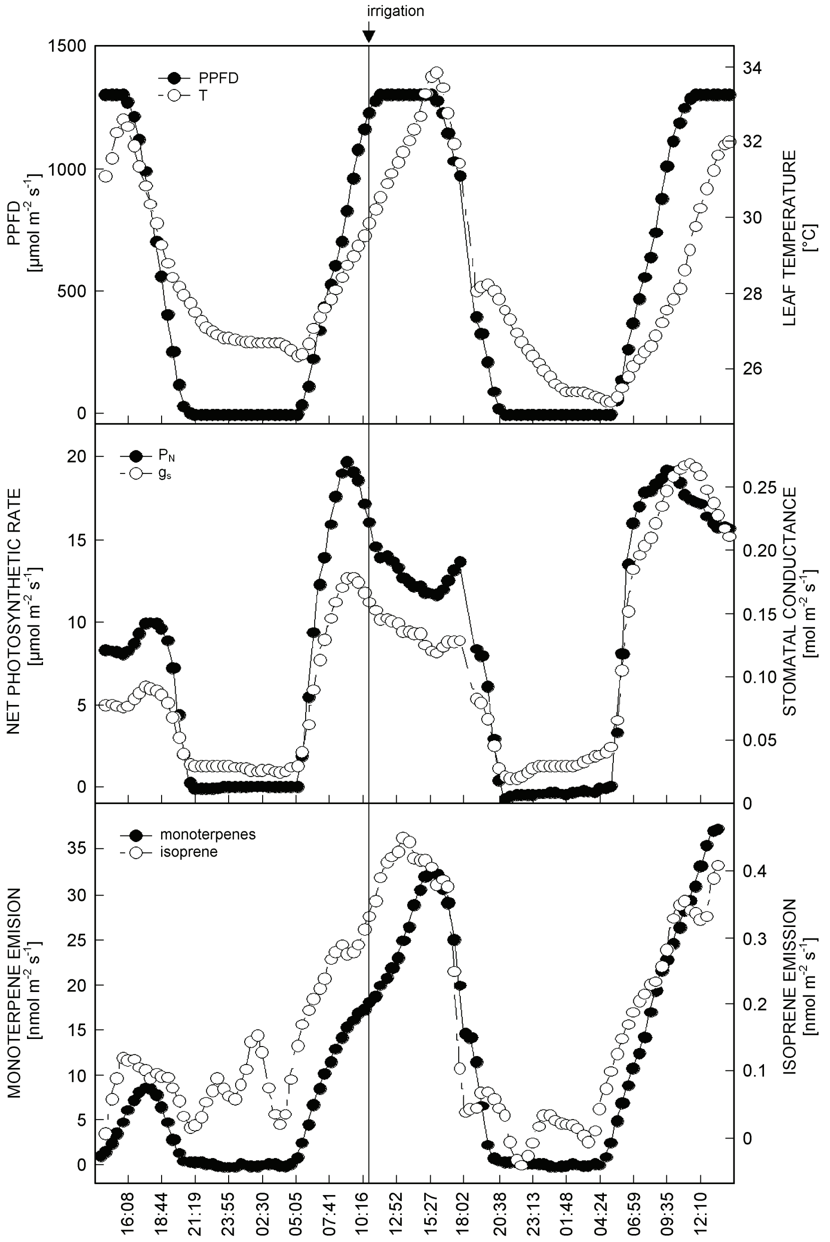

Fig. 1. Daily time course dynamics of PFD, leaf temperature, net photosynthetic rates, stomatal conductance and isoprene and monoterpene emissions in a *Quercus ilex* potted seedling submitted to drought and re-watering. *Vertical line* indicates the moment of re-watering. Replicated three times (see text).

antagonist effect of the two factors on VOC emissions. Future work should therefore investigate the interactive effect of both stresses on vegetation emissions in order to improve current generation of emissions models (Monson *et al.* 2007).

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