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Drought reduced monoterpene emissions from the evergreen Mediterranean oak *Quercus ilex*: results from a throughfall displacement experiment

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Abstract. The effects of water limitations on the emission of biogenic volatile organic compounds are not well understood. Experimental approaches studying drought effects in natural conditions are still missing. To address this question, a throughfall displacement experiment was set up in a natural forest of *Quercus ilex*, an evergreen Mediterranean oak emitting monoterpenes. Mature trees were exposed in 2005 and 2006 either to an additional drought, to irrigation or to natural drought (untreated control). In both years, absolute monoterpene emission rates as well as the respective standard factors of the trees exposed to normal and additional drought strongly declined during the drought periods. Monoterpene emissions were lower in year 2006 than in year 2005 (factor 2) due to a more pronounced summer drought period in this respective year. We observed a significant difference between the irrigation and additional drought or control treatment: irrigated trees emitted 82% more monoterpenes during the drought period 2006 than the trees of the other treatments. However, no significant effect on monoterpene emission was observed between normal and additional drought treatments, despite a significant effect on leaf water potential and photochemical efficiency. During the development of drought, monoterpene emissions responded exponentially rather than linearly to decreasing leaf water potential. Emissions rapidly declined when the water potential dropped below -2 MPa and photosynthesis was persistently inhibited. Monoterpene synthase activities measured in vitro showed no clear reduction during the same period. From our results we conclude that drought significantly reduces monoterpene fluxes of Mediterranean Holm oak forest into the atmosphere due to a lack of primary substrates coming from photosynthetic processes.

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1 Introduction

Biogenic volatile organic compounds (BVOC) constitute a large family of molecules originating from many natural sources (Fuentes et al., 2000). The chemical breakdown of BVOC in the atmosphere affects air quality and climate forcing by influencing the formation and life time of greenhouse gases and air pollutants such as ozone and methane, as well as the formation and growth of secondary organic aerosols (Poisson et al., 2000; Monson and Holland, 2001). To assess these biosphere-atmosphere interactions precise quantifications of BVOC fluxes are essential. The major bulk of BVOC are isoprene and monoterpenes emitted by terrestrial vegetation. The quantity and qualitative composition of these emissions depends on the plant's capacity to produce these terpenoid compounds and on environmental factors constantly modulating this intrinsic capacity basal emission capacity and instantaneous emission rates (Kesselmeier and Staudt, 1999). Current approaches to predict BVOC emissions at

large scales use mainly the empirical model by Guenther et al. (1993), which describes the short-term influences of temperature and light on emissions and was later extended to account for seasonal effects on emissions (e.g. Sabillon and Cremades, 2001; Parra et al., 2004; Guenther et al., 2006).

Under Mediterranean conditions, water availability represents a major environmental constraint for plants, which experience severe drought stress episodes during summer and determines the annual pattern of vegetation activity together with air temperature and solar radiation (Di Castri et al., 1973). However, current BVOC emission models do not take into account water limitation as an emission-modulating factor. Indeed, the literature is still inconclusive on this subject. To our knowledge, the first study addressing this question showed no effect of drought on isoprene emission from young-potted *Quercus virginiana* plants (Tingey et al., 1981). Since, depending on studied plant species and applied protocols, BVOC emissions were found to be reduced (Bertin and Staudt, 1996; Brüggemann and Schnitzler, 2002; Staudt et al., 2008), enhanced (Ormeno et al., 2007; Staudt et al., 2008) or unchanged (Fang et al., 1996; Blanch et al., 2007) in response to water stress. However, under severe drought stress BVOC emissions were generally found to be reduced (Llusia and Penuelas, 1998; Pegoraro et al., 2004). This emission decrease is essentially explained by the reduced availability of primary carbon substrates necessary for BVOC biosynthesis (Loreto et al., 2001; Brüggemann and Schnitzler, 2002) or by a reduced activity of specific enzymes in the BVOC biosynthesis pathway (Fortunati et al., 2008).

However, almost all of these studies had been performed with young-potted plants (except Loreto et al., 2001). Therefore results cannot easily be extrapolated to natural conditions. Field-grown trees have large root zones due to a rather unrestricted root growth and therefore have access to large soil water reserves. Hence, field-grown mature trees have more time to adapt to water stress than potted plants, because water availability in the soil decreases more slowly reaching critical values only after extended drought periods. To date, field studies investigating drought effects on BVOC emissions from adult trees are scarce, although water stress is often cited as a hypothesis to explain seasonal and inter-annual emission patterns observed under field conditions (Bertin et al., 1997; Llusia and Penuelas, 2000; Nunez et al., 2002; Plaza et al., 2005). Field studies are lacking probably due to the difficulty to manipulate water availability without generating undesired side effects at the soil-atmosphere interface and due to natural variations in climate and plant phenotype that affect emissions and therefore may veil potential small effects of drought on BVOC emissions. Guenther et al. (1999) compared isoprene emission from two evergreen shrub species (*Berberis trifoliata* and *Condalia hookeri*) growing in irrigated and non-irrigated plots in a subtropical savanna. They observed a diminution of stomatal conductance and photosynthesis in the non-irrigated plots without significant changes in isoprene emission compared to the irrigated plots. In a comparable study within a mature *Quercus ilex* plantation in Southern France, Staudt et al. (2002) observed a 25% reduction of BVOC emission of water-stressed trees compared to control trees. Yet, this study was made in a row of planted trees, whose root zones were artificially restricted by a trench and covered by a soil roof, which may not reflect real drought conditions in natural forest ecosystems. At last, Gray et al. (2003) compared methyl butenol (MBO) emissions of *Pinus Ponderosa* mature trees on controlled and irrigated plots. The control plot sustained a natural drought period during the second year of the experiment and no effects were observed between both plots. As explanation, the authors suggested that the stress was not sufficiently severe to provoke stress symptoms. Recently, a study by Llusia et al. (2009) addressed this question on Mediterranean shrubland species in Catalonia (*Erica multiflora* L. and *Globularia alypum* L.). Plants were exposed to experimental warming plus drought of a ca. 20% relative decrease in soil moisture. Based on one measurement campaign per season, a significant negative effect of summer drought was observed on isoprenoid emissions from *Erica mutiflora*.

In the present study we describe the effects of different degrees of water-limitation on monoterpene (MT) emissions from mature *Quercus ilex* (L.) trees in a natural forest ecosystem. *Q. ilex* is considered as one of the strongest BVOC emitting species in the Mediterranean basin (Kesselmeier et al., 1997), where it is regularly exposed to severe summer drought. Its evergreen leaves produce large amounts of MT in a light-dependent process without storage in specific storage tissues or organs (Staudt and Bertin, 1998). The specific objectives of the present field study were to investigate at the leaf level (i) the effects of current drought conditions on the seasonal and inter-annual variation of MT emissions and (ii) whether a reduction of the summer precipitation as predicted by the IPCC (2007) for the end of the century in the Mediterranean basin can substantially alter seasonal drought effects on monoterpene emissions. For this purpose, foliar MT emissions, activity of MT synthases, photosynthetic parameters and plant and soil water status were monitored during two consecutive years on different plots with respect to water availability: an untreated control plot exposed to natural drought conditions, an irrigated plot and a plot equipped with a rainfall exclusion system simulating future drought conditions.

2 Materials and methods

2.1 Experimental sites

The study site is located 35 km NW of Montpellier (Southern France) in the Puechabon State Forest (43°44′29″ N, 3°35′45″ E; elevation 270 m) on a flat plateau of limestone that has been managed as a coppice for centuries (last clear cut in 1942). Soil texture is homogeneous in the 0–50 cm

layer (39.6% clay and 14.1% sand) and belongs to the silty clay loam part of the USDA (United States Department of Agriculture) textural triangle. Vegetation is dominated by *Q. ilex* trees forming a dense canopy (mean height: 5.5 m) that is mainly composed of current and one-year-old leaves. A previous leaf demography study showed that 22% of the leaves fall after 1 year and 69% after two years (average leaf life span: 23 months, Limousin, unpublished data). The climate is typical Mediterranean with cool and wet winters and warm and dry summers. Mean annual temperature is 13.5°C and mean annual precipitation is 872 mm. Rainfall happens largely during autumn and winter with about 75% of the total rain occurring between September and April.

At this site, a rain exclusion experiment was set up in late winter 2003 as part of the European MIND project (Mediterranean terrestrial ecosystems and INcreasing Drought: vulnerability assessment) to evaluate the impact of changes in water input as predicted for the current century (Mouillot et al., 2002; IPCC, 2007). A 20 m×20 m area was selected with uniform soil and canopy conditions and equipped with a scaffold providing access to the canopy. Within this area, two plots were selected: one for a drought treatment and one as control. In the "dry" plot, 17 cm wide PVC gutters hung 1 m height to intercept the rain throughfall under the canopy. As a consequence, the net precipitation input is reduced by 27% in the dry plot compared to the "control" plot (Limousin et al., 2008). In the control plot, the PVC gutters were installed with the convex side up to ensure that modifications in the microclimate were similar to those in the dry plot. Thus, the control plot presented natural drought conditions while the dry plot increased drought conditions as predicted for the end of the century.

Additional measurements were made on 10-year old *Q. ilex* saplings, which were watered weekly (50 L m^{-2}) during the experimental period. This plantation is located in Montpellier, close to the institute (43°36′ N, 5°53′ E), on a deep clay soil with good water availability. At this site annual mean temperature is 14.0°C and annual mean precipitation 789 mm.

2.2 Experimental protocol

At Puechabon two field campaigns were conducted: from April to September 2005 and from May to December 2006. Measurements were made on three adult trees of each treatment. Soil water storage, predawn leaf water potential, leaf MT emissions, leaf CO_2/H_2O gas exchange and chlorophyll fluorescence were determined once a week under sun-lit conditions to minimize weather effects on MT emissions. In addition in 2006, enzyme activities of MT synthases (mono-TPS) were determined from the same leaves used for emission measurements.

In May 2005 (Day of the year: DOY 122-144) a mass outbreak of Gypsy moth larvae (*Lymantria dispar*) occurred at the Puechabon forest. The herbivory attack was stopped by a treatment with *Bacillus thuringiensis*. Caterpillar larvae fed exclusively on young developing leaves. Therefore, measurements were performed on one-year-old leaves in 2005 and on current-year leaves in 2006. In order to check the potential effect of leaf age on monoterpene emissions, an additional experiment was conducted in 2007 in which one-year-old leaves were compared with current-year leaves on three trees in the control plot. On each tree, three leaves per leaf age class were assayed for monoterpene emission and the means were used for statistical analysis (Appendix Fig. A1).

In the irrigated plot, leaf gas exchange, MT emission and mono-TPS activity were monitored every other week from May to December 2006 on current leaves of three trees. In addition, predawn leaf water potential was measured 3 times during the summer period to check the irrigation efficiency. Minimum values were about −0.5 MPa suggesting that the trees never underwent water limitation.

2.3 Plant and soil water status

Predawn leaf water potentials (ψ_{pd}) were determined by a Scholander-type pressure chamber (PMS 1000, PMS Inst., Corvallis, OR, USA). Measurements were started one hour before sunrise and completed by dawn on two leaves of each tree. If the difference between them was greater than 0.2 MPa, a third leaf was measured.

Soil water storage (SWS) was measured at 20 cm interval from 10 cm to 450 cm in depth using a neutron moisture gauge (CPN503 Campbell Pacific Int., Martinez, USA). In parallel it was evaluated with a soil water balance model (Rambal, 1993; Grote et al., 2009). The model simulates on a daily basis: transpiration, evapotranspiration and deep drainage, SWS and ψ_{pd}. The evapotranspiration includes the evapotranspiration from soil under the plant canopy and the evapotranspiration of intercepted rain water. Comparison of measured against simulated SWS showed very good agreement, standard error of estimate being lower than 7% (n=35) (Rambal et al., 2003).

2.4 Gas exchange measurements and monoterpene collection

CO_2/H_2O gas exchange measurements and MT trapping were done concomitantly in situ with a Li-Cor LI-6400 gas-exchange system (Clear Chamber bottom 6400-08, LI-COR Lincoln, Nebraska, USA). An ozone scrubber (eight layers of MnO_2-coated copper nets) was placed in the air inlet of the system to avoid decomposition of ozone sensitive MT species (Calogirou et al., 1996). Leaf temperature was measured before each measurement with an infrared thermometer and was hold constant at this temperature during MT trapping by means of the Li-Cor 6400 chamber air-conditioning system. Leaf temperature and incident light were recorded during MT trapping.

Leaf gas exchange was measured at a constant flow rate inside the chamber (0.576 L min^{-1}) and values were recorded when the photosynthesis reached steady state. Afterwards, MT were trapped by directing a fraction of chamber air (10 min at a flow rate of 0.1 L min^{-1}) through adsorption tubes (200 mg Tenax TA, Chrompack, Middelburg, The Netherlands) by means of a mass-flow controlled BVOC sampler. Adsorption tubes were stored at −4°C until analysis. Recorded gas exchange rates were corrected afterwards for the enclosed leaf area as determined by a leaf area meter (Delta-T devices Ltd., Cambridge, United Kingdom). Seasonal variation of stomatal conductance, transpiration and leaf intercellular carbon concentration are shown in Appendix Fig. A2.

2.5 Chlorophyll fluorescence measurements

The fluorescence parameter *Fv/Fm* is a measure of the PSII (photosystem II) photochemical trapping efficiency under dark-adapted conditions (Maxwell and Johnson, 2000): in darkness, all PSII reaction centres are in an "open state" and therefore, the rate of photochemistry is not limited. PSII fluorescence was determined during dawn on three dark-adapted leaves per tree with a PAM 2000 fluorometer (Walz, Effeltrich, Germany) and calculated as following *Fv/Fm* (=$(F_M - F_0)/F_M$). Minimum fluorescence F_0 depends on the size of PSII chlorophyll antenna and on the functional integrity of PSII reaction centres. It was measured under weak red modulated irradiance. The maximum fluorescence F_M appeared when all PSII reaction centres are closed due to complete reduction of primary electron acceptors (QA) under a saturated pulse of white light.

2.6 Monoterpenes analysis

The adsorption tubes were analysed by gas chromatography with flame ionisation detector (GC/FID) using a Chrompack CP9003 GC equipped with a Chrompack TCT4002 thermo-desorber (all Varian Inc., Palo Alto, USA). Occasionally, parallel MT analysis was made with a GC/MS system (Varian CP3800/Saturn2000 MS plus a Perkin-Elmer Turbomatrix thermo-desorber) using similar analytical conditions as for the GC/FID instrument (see Staudt and Lhoutellier, 2007).

Peaks were identified by comparing their mass spectra and retention times with those from authentic standards analysed under the same conditions. All GC systems were calibrated using authentic standards (Fluka Chemie, Buchs, Switzerland; Roth, Karlsruhe, Germany) diluted in methanol. 0.5 to 3 μL of standard solution was injected on the head of the adsorption trap through a T-fitting equipped with a septum and purged with 300 mL pure N_2 at a flow rate of 50 mL min^{-1}. Precision as determined by repeated measurements of standards at realistic concentrations was within 5% for all considered MT.

2.7 Monoterpene synthase activity

After measurements the enclosed leaf was frozen in liquid N_2 and stored at −80°C until mono-TPS analysis as described in Fischbach et al. (2000). 250 mg of frozen leaves were homogenised in liquid N_2, suspended in 6 mL extraction buffer (700 mM Mops/HCl pH 7.3 containing 1.5% (v/v) PEG 1500, 1% (w/v) PVP-30, 8.3% (w/v) Dowex 1x2, 20 mM $MgCl_2$, antioxidants [200 mM ascorbic acid, 50 mM β-mercaptoethanol]) and stirred on ice for 20 min. After centrifugation (18 000 g for 20 min), 2.5 mL of supernatant was desalted on PD-10 columns (GE Healthcare, Freiburg, Germany) with 3.5 mL assay buffer (50 mM KPi, pH 7.3, with 10% (v/v) glycerol and antioxidants (10 mM Na-ascorbate, Na-disulfite, DTT, each). Assays were run in gas-tight 2 mL vials with 91 μL of protein extracts, 4 μL $MgCl_2$ (final conc. 20 mM) and 5 μL of GDP (final conc. 250 μM) for 60 min at 40°C. Reactions were terminated by removing the reaction mixture and washing the gas-tight vials with 100 μL assay buffer. GC/FID analysis was performed according to Fischbach et al. (2000).

2.8 Data treatments

Monoterpene emission rates were calculated as the difference between the MT concentration in the chamber enclosing a shoot and MT concentration in an empty chamber multiplied by the inlet air flow and related to projected leaf area.

To factor out immediate effects of actual light and temperature intensities, emission rates were normalised to standard temperature (30°C) and light conditions (photosynthetic photon flux density (PPFD) 1000 μmol m^{-2} s^{-1}) according to the algorithm of Guenther et al. (1993) using the empirical parameters determined for *Q. ilex* by Staudt and Bertin (1998). Leaf temperature and incident light intensity during MT trapping were recorded and used for the determination of MT standard emission factors (E_S). A potential error in the normalisation could result from the use of inappropriate parameter values, although the applied parameters have been developed specifically for *Q. ilex* emissions over a wide range of PPFD and temperature conditions (α=0.0041; C_{L1}=1.04; C_{T1}=87.62 J mol^{-1}; C_{T2}=188 200 J mol^{-1}; T_M=317 K; T_S=303 K). Hereafter emission rate (E) refers to the measured emission under given environmental conditions and standard emission factor (E_S) to normalised emission rates.

A student-test (Sigma stat) was used to check significant differences between the emissions of 1-year-old and current-year leaves in 2007. No significant differences were found in either E or E_s (P=0.3096 and P=0.152, Appendix Fig. A1).

Non-parametric MANOVAs (Multivariate ANOVA, adonis procedure, library vegan, R©, Vienna, Austria; see R guideline, 2008 and Anderson, 2001) were applied separately on the 2006 and 2005 data of E_S, mono-TPS activity, assimilation rate, ψ_{pd} and F_V/F_M to test the effects of the different

treatments (control and dry in 2005, and control, dry and irrigated in 2006).

In addition, to characterise the period for which the treatments had an impact on E_S in 2006, data were subdivided into three periods defined according to a drought index based on the relative soil water content (see below). We fitted linear models on the data of each period assuming (or not) a treatment effect (Table 1). As an example, the reference model (1) took into account only the seasonality, without any treatment effect, to explain the variation of E_S while the model 8 assumed a treatment effect for the three tested periods. All the intermediates were tested (models 2–7). The inference of each model was determined by the value of the AIC index (Akaike Information Criterion; see Burnham and Anderson, 2004). Because the data set of each period was unbalanced, the AIC index was corrected for the number of data per period as follows: $AICc=AIC+[(2k(k+1)/(n-k)]$, where $AICc$ is the corrected AIC, n the number of data and k the degree of freedom of each period. The model with the lowest $AICc$ is considered as the best to explain the data set (procedure AIC lm, library nlme, R©, Vienna, Austria. See R guideline, 2008).

Table 1. Results of the AICc (Akaike Information Criterion corrected) comparison of the linear models. 8 tested models differed with the applied treatment effect during periods, which were defined as begin of drought (DOY 144–167), drought (DOY 173–221), and post-drought (DOY 230–333) in 2006. Applied treatment effects were control, dry and irrigated treatment. 1 and 0 refers to whether or not a treatment effect was applied on the data of a given period. The best model fitting the data is that with the lowest AICc. See M&M for further explanations.

Models	Periods in 2006			AICc
	Begin of drought	Drought	Post-drought	
1	0	0	0	468
2	1	0	0	481
3	0	1	0	454
4	1	1	0	475
5	0	0	1	494
6	1	0	1	518
7	0	1	1	495
8	1	1	1	537

3 Results

3.1 Composition of monoterpene emission

The foliar emission of monoterpenes from all measured *Q. ilex* trees was dominated by five compounds: α-pinene, β-pinene, sabinene, myrcene and limonene. Other terpenoid compounds were found occasionally at trace concentrations, namely α-thujene, camphene, α-terpinene, p-cymene, (Z) - and (E)-β-ocimene and γ-terpinene. Therefore, only the five main monoterpenes are considered for in the present analysis. The emissions of most trees were dominated by α-pinene and β-pinene (means of 45 and 28%, respectively) except for two trees whose emissions were characterized by a high percentage of limonene (>40%). For a given tree, the emission composition found on different leaves were always very similar regardless of daytime, season, leaf age and year of measurement (data not shown). Therefore, E and E_S could be presented as the sum of the five main MT. The monoterpene pattern obtained by in vitro assays of mono-TPS activity were similar to those found in the emission measurements (linear regression, r^2=0.73, t-test, P=0.985, data not shown). The consistent high limonene fraction in the emission of leaves of two trees is reflected by similar pattern of mono-TPS activity (r^2=0.98).

3.2 Seasonal trends of monoterpene emission rates

Contrary to the monoterpene emission pattern, the emission rates (E) showed a pronounced inter- and intra-annual variability (Fig. 1). In 2005, E on the dry and control plots followed the seasonal temperature and light profile, except two time periods. In spring under mild weather conditions (DOY 95–126), extremely low E values were followed by a sudden increase of MT emission in late May (DOY 140). From mid-June (DOY 179) to September (DOY 242) E decreased dramatically while temperature and light conditions were almost stable during this time period. In 2006 on the same plots, E of the current leaves increased in June (DOY 150–167) but did not reach the same level as the preceding year (factor 2 approximately; see Fig. 1e and 1f). During summer, E remained globally low with some fluctuations and finally dropped in autumn (DOY 263–333) together with temperatures and light intensities close to zero. On the irrigated plot (Fig. 2), E increased in late spring (from DOY 144 to 185) reaching an annual maximum during summer (DOY 185 to 242) and strongly decreased from September onwards (DOY 242). The seasonal variation of E on this plot followed mainly the seasonal temperature and light patterns. The maximal E in summer were considerably higher on the irrigated plot than those observed on the dry and control plot at the same time, although climate conditions (see Fig. 1b and Fig. 2a) as well as leaf temperatures and incident PPFD were similar (data not shown).

3.3 Seasonal trends of standard monoterpene emission factors

Standard monoterpene emission factors (≡ basal emission capacity; E_S) on the dry and control plots were higher in 2005 than in 2006 (Fig. 3a and 3b) and presented a similar intra-annual variability than E. The periods of decreasing E_S corresponded approximately to the periods of decreasing E and were even more pronounced in summer 2006. In

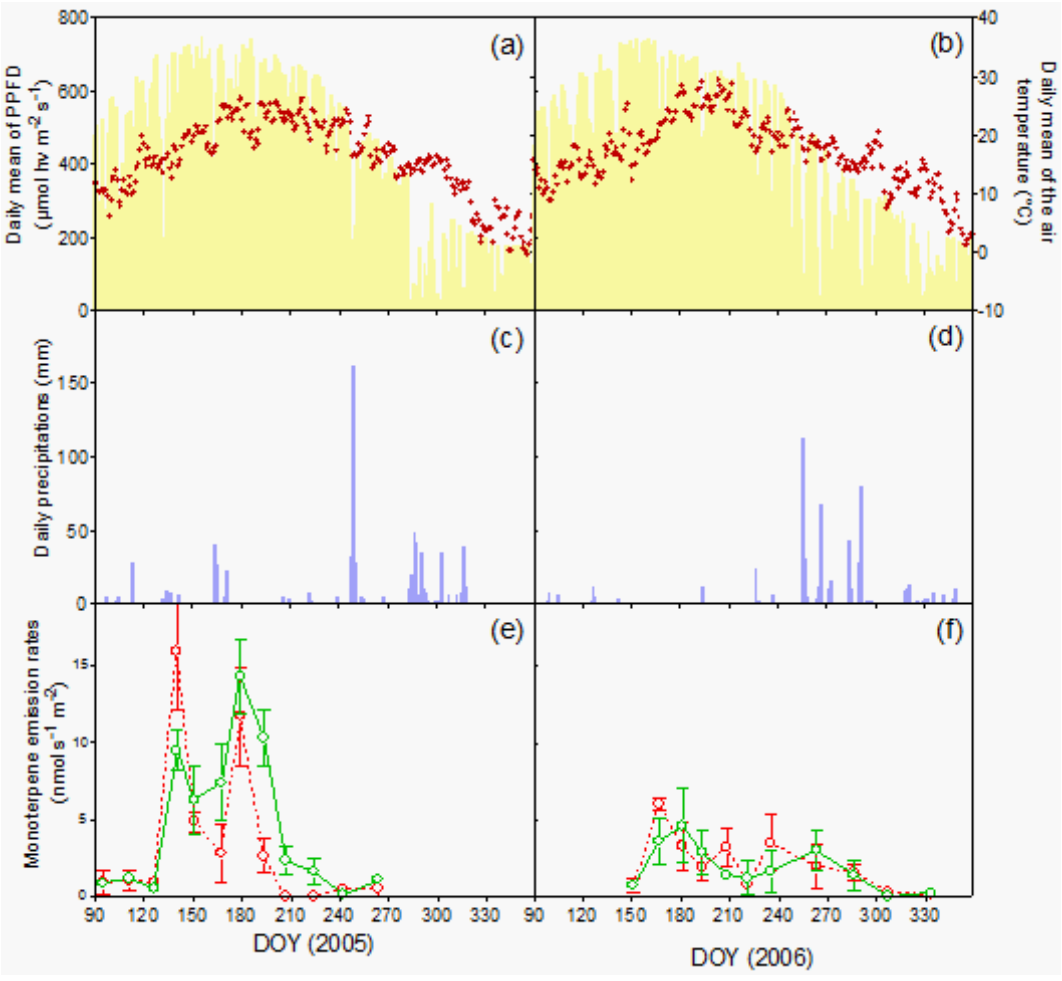

Fig. 1. Seasonal variations of the daily mean of photosynthetic Photon Flux Density (PPFD, yellow bars, μmol m^{-2} s^{-1}) and the daily mean of air temperature (dots, °C) **(a, b)**, daily sum of precipitation (mm) **(c, d)** and foliar monoterpene emissions rates **(e, f)** measured over two years (DOY=day of the year) in the forest of Puechabon. Monoterpene emissions rates (nmol s^{-1} m^{-2}) are means ±SE of n=3 measurements made on different individual trees in the control (green symbols) and dry plot (red symbols).

both years, E_S temporary decreased between June and middle of August (DOY 180–240 in 2005 and DOY 170–220 in 2006) by about 90%. There were no significant differences between the two treatments in Puechabon (P=0.09). However in 2006, E_S measured on the irrigated plot (Fig. 4a) was significantly higher (P=0.02) compared to the other plots in Puechabon. To characterise this difference, treatment effects were tested with linear models assuming three different periods of water effects on E_S (Table 1). *AICc* of the reference model (1) was 468, above which models have to be considered as less suitable to fit the data. Among the tested models only the model 3 incorporating a treatment effect exclusively during the "drought" period yielded a lower value of *AICc* (454).

3.4 Plant and soil water status

At the Puechabon site, soil water status (*SWS*) values decreased during summer until a minimum of 80 mm in both years (Fig. 3c and 3d) and reached the field capacity (210 mm) after the autumnal rainfalls. Some rainfalls in June 2005 (DOY 164) caused a small temporary increase of *SWS*. In 2005, *SWS* was lower in the dry plot than in the control plot during spring and summer (DOY 90–248). Differences between plots were less pronounced in 2006.

The predawn water potential g(ψ_{PD}) decreased notably twice in 2005 (Fig. 3c): first in June (DOY 158) to −2.5 MPa on the dry plot and to −1.5 MPa on the control plot, and secondly in July and August (DOY 179–242) to −5 MPa and to −4 MPa respectively on the dry and control plot. In 2006, ψ_{PD} reached −4.5 MPa in both plots in mid August (DOY 221; Fig. 3d). The difference between dry and control plots

was significant in 2005 and 2006 (for both year $P<0.01$), with smaller values in the dry plot.

Thus, the data suggest the occurrence of three distinguished drought events in the 2-years measurement period at Puechabon: a first moderate drought event in May–June 2005 (DOY 140–163), a stronger one lasting from July to September 2005 (DOY 180–247) and another stronger one lasting from May to September 2006 (DOY 140–254).

3.5 Photosynthesis and PSII status

During the first drought period in early summer 2005 (DOY 140–163) net assimilation rates (A; Fig. 3e) decreased more in the dry plot than in the control plot. In both plots, A recovered after the June rainfalls but decreased again and more drastically during the second drought period (DOY 180-242), reaching negative values (respiration) in leaves from the dry plot. In 2006, A was strongly reduced in leaves of both plots at Puechabon throughout the summer period (DOY 151–235; Fig. 3f), recovered after the mid-August rainfalls (DOY 237) and reached its annual maximum under mild temperatures in fall (DOY 286-333). Oppositely in the irrigated plot, A increased in spring (DOY 144–185) and remained high during the whole experimental period (DOY 185–326; Fig. 4b). We observed no significant differences between dry and control plot leaves in 2005 (P=0.14). However, a significant treatment effect was seen in 2006 (P=0.01) with higher net assimilation rates of leaves from the irrigated plot compared to the others during the drought period.

Fv/Fm values remained stable throughout 2005 and 2006 except at the end of the most severe drought periods (DOY 242 in 2005, DOY 221 in 2006) when they decreased (Fig. 3e and 3f). In 2005 the *Fv/Fm* values were significantly lower in leaves from the dry plot than in control leaves ($P<0.01$) and remained lowered even after a rainfall event in September. During the drought in 2006, *Fv/Fm* values decreased again more in leaves subjected to additional drought compared to control leaves (P=0.04).

3.6 Seasonal variation of monoterpene synthase activities

In leaves from both treatments at Puechabon and from the irrigated site at Montpellier, in vitro mono-TPS activities followed a similar seasonal cycle in 2006 (P=0.23; Fig. 3g and 4c): mono-TPS activities increased after leaf development to a summer plateau and progressively declined during the fall and winter periods. No clear inhibition of enzyme activities was detected during the drought period. Instead, remarkable between-tree variability was observed as reported in a previous study on mono-TPS activity in *Q. ilex* leaves (Fischbach et al., 2002).

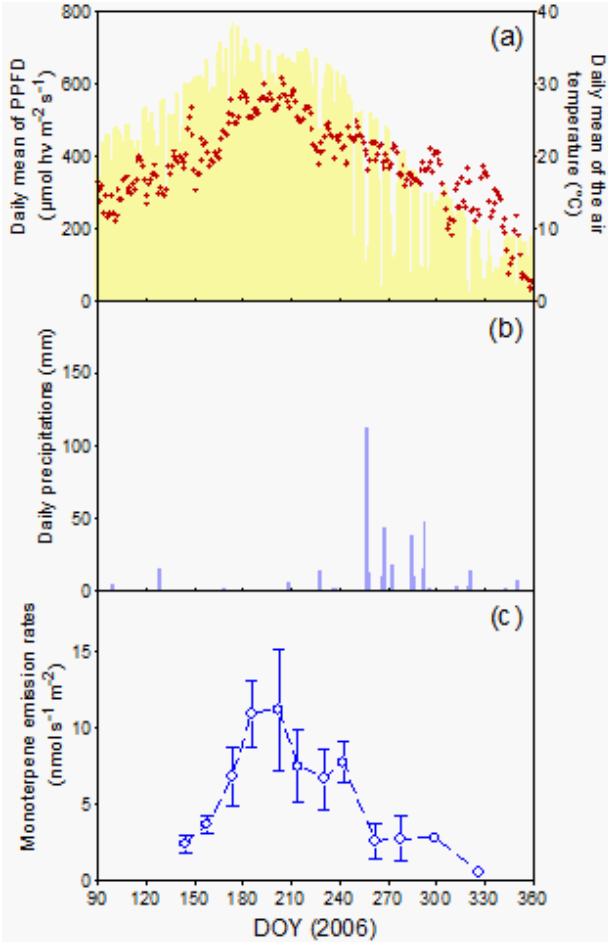

Fig. 2. Seasonal variations of daily means of photosynthetic Photon Flux Density (yellow bars, μmol m^{-2} s^{-1}) and daily means of air temperature (dots, °C) **(a)**, daily sum of precipitation (mm) **(b)** and foliar monoterpene emissions rates **(c)** measured in 2006 on the irrigated plot of the experimental field site at CEFE-CNRS in Montpellier. Monoterpene emission rates (nmol s^{-1} m^{-2}) are means ±SE of n=3 measurements made on different trees.

3.7 Correlation analysis between monoterpene emission and plant water status

When data from both treatments and years were pooled, E_S increased exponentially with ψ_{PD} (i.e. could be modelled as E_S=a. exp(b.gψ_{pd}) + error term, see Fig. 5; procedure "gnls", library "nlme", R©, Vienna, Austria). To exclude the seasonal effect (emission start in spring and emission inhibition in fall), only the measurements made in summer were taken into account. We computed the 95% confidence prediction interval using an ordinary bootstrap method (500 replicates of the dataset were generated and modelled using exponential models; procedure "gnls", library "boot", R©, Vienna, Austria). The delimited interval of prediction was larger at high ψ_{pd} (−1/0 MPa) compared to lower values (−5/−2 MPa).

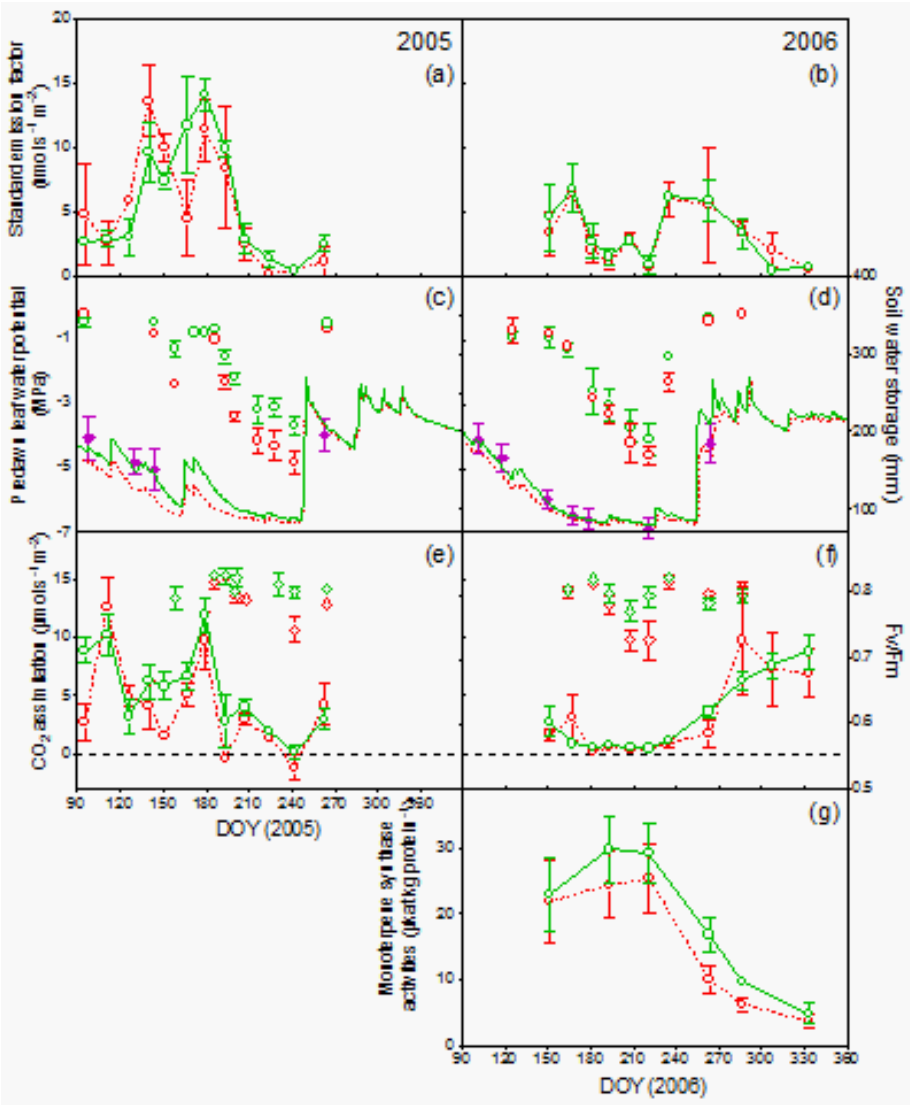

Fig. 3. Seasonal variation of physiological parameters and soil water storage during 2005 **(a, c, e)** and 2006 **(b, d, f, g)** in the control plot (green symbols) and the dry plot (red symbols) of the *Q. ilex* forest site at Puechabon: (a, b) standard emission factor (nmol s^{-1} m^{-2}, mean ±SE of n=3); (c, d) predawn leaf water potential (circles, MPa, mean ±SE of n=3) and soil water storage measured (violet diamonds, mm, mean ±SD of n=35) and simulated (green line for the control plot and red-dotted line for the dry plot); (e, f) CO_2 assimilation (circles, μmol s^{-1} m^{-2}, mean ±SE of n=3) and F_V/F_M (diamonds, mean ±SE of n=3); (g) monoterpene synthase activity (μkat kg protein^{-1}, means ±SE of n=3).

4 Discussion

We observed a strong inter-annual variation of MT emission rates (E) as well as of MT emission factors (E_S) at the Puechabon forest. In 2005 maximum E_S of about 15 nmol s^{-1} m^{-2} (30 μg h^{-1} g^{-1} leaf dry weight) were observed at the end of May and again at the end of June. These values agree with those reported in previous studies for the same species (respectively 40, 32 and 21 μg h^{-1} g^{-1} in Owen et al., 2001; Sabillon and Cremades, 2001; Staudt et al., 2002). However, in 2006 comparable values were found only on the irrigated plot in Montpellier but not in the Puechabon site, where maximum E_S were about 7 nmol s^{-1} m^{-2} (16 μg h^{-1} g^{-1}). This year-to-year difference at Puechabon might be due to variations in climate conditions, to the caterpillar attack in 2005 or to the resulting difference in the leaf age between the two measurement years. The latter possibility can however be ruled out given that in 2006 similar large differences in E_S appeared between the same leaf age class of the irrigated plot within a plantation and the forest site, and in 2007, no significant differences could be observed between 1-year and current-year leaves. Correspondingly,

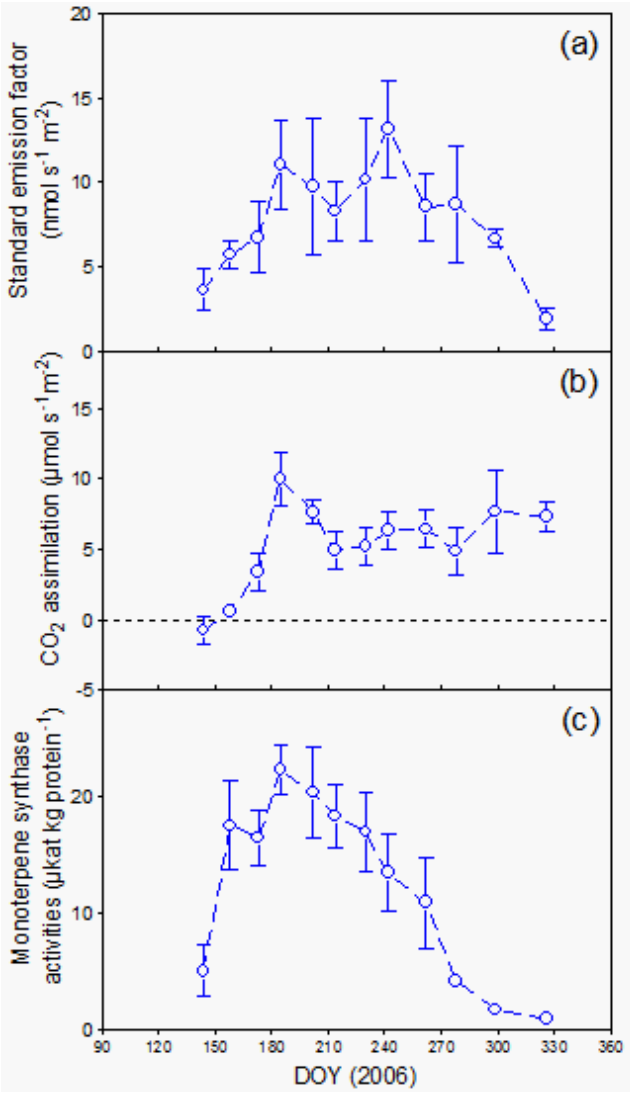

Fig. 4. Seasonal variation of physiological parameters in 2006 measured on three irrigated *Q. ilex* trees at the experimental field site of the CEFE-CNRS: **(a)** standard emission factor (mean ±SE of n=3, nmol s^{-1} m^{-2}); **(b)** CO_2 assimilation (mean ±SE of n=3, μmol s^{-1} m^{-2}); **(c)** monoterpene synthase activity (mean ±SE of n=3, μkat kg $protein^{-1}$).

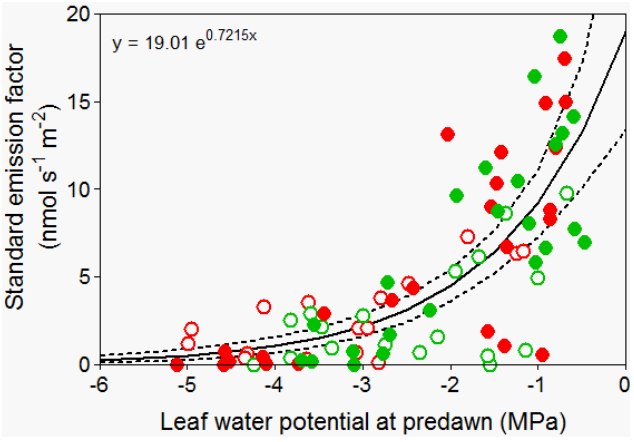

Fig. 5. Plot of the standard emission factor (nmol s^{-1} m^{-2}) against the predawn leaf water potential (MPa) at the Puechabon site. The black curve represents the fitted exponential regression (see equation curve) and the dotted curves the prediction intervals (95%). All data (2005 (full points) and 2006 (empty points), control (green) and dry plots (red)) except fall and spring measurements are shown. Then, plotted data corresponded to both periods defined as drought periods with the plant and soil water status measurements (DOY 140–242 in 2005 and DOY 150–235 in 2006).

Staudt et al. (2003) found no increase of E_S with increasing leaf age but rather a decrease or no change in E_S of *Q. ilex* leaves. Similarly, Fischbach et al. (2002) observed lower enzyme activities in 1-year-old than in current-year *Q. ilex* leaves. Another possibility is that caterpillar feeding in 2005 induced increased emissions in the non-attacked 1-year leaves. Staudt and Lhoutellier (2007) observed a slight temporary increase in the E_S of *Q. ilex* saplings infested by Gypsy moth caterpillars. This increase lasted however only a few days and therefore neither can explain the year-to-year difference in E_S after the caterpillar attack nor the site difference observed in 2006. Hence the year and site specific differences in E_S must be mainly related to variation in the prevailing climate conditions. Generally, year-to-year variations in temperature and light are rather small in Mediterranean climates, whereas the annual precipitation pattern can be highly variable (Fig. 2c and 2d), causing more or less pronounced drought periods throughout the year. In the present study annual PPFD and temperature patterns were indeed similar in both years and both locations but not the water limitation. Loreto et al. (2001) reported inter-annual variation of *Q. ilex* emissions up to a factor of ten, which they attributed in part to variable water stress conditions. According to Granier et al. (2007) inter-annual variation in drought can be characterized by three indices describing the beginning of drought, the drought duration and the drought intensity (daily drought duration weighted by water stress in equivalent days EqD). In 2005 and 2006 the drought period in the Puechabon forest began approximately at the same period (DOY 148 and 140, respectively), but the rainfall event occurring in June 2005 limited drought duration to 84 days and drought intensity to 28 EqD. No comparable rainfall events happened in spring 2006 and therefore the drought period was longer (127 days) and more intense (48 EqD). Consequently E_S did not reach an annual maximum during early summer 2006 as observed during 2005 or at the irrigated plot in the same year.

The treatment effect characterized by MANOVA depended on the seasonal period. E_S was significantly higher on the irrigated plot during the drought period (9.8± (SEM) 3.6 nmol s^{-1} m^{-2} for the irrigated plot versus 1.8±1.1 nmol s^{-1} m^{-2} for the Puechabon plots in average), but it was similar at the three plots during the rest of the year.

In Puechabon, all trees suffered from water limitation but the stress was more intensive on the dry plot than on the untreated control plot. MANOVA results showed a significant effect of the throughfall displacement treatment on plant water status and photosynthetic apparatus as reflected by lower ψ_{pd} and F_v/F_m on the dry plot in summers 2005 and 2006. Despite the significant treatment effect on these physiological parameters, E_S was seemingly unaffected by the additional drought treatment, even though emissions were undoubtedly inhibited by water stress. One major reason for this apparent discrepancy is that E_S did not linearly respond to the plant water status but rather followed an exponential way (Fig. 5). Around the -2 MPa value, the curve showed an inflexion: below this value E_S seemed to be limited by the plant water status. Above, the increase of the interval of prediction suggested that E_S is controlled by other factors. Other studies also recorded the existence of a threshold of water stress for the reduction of BVOC emissions (Pegoraro et al., 2004; Brilli et al., 2007). Given that the exclusion displacement resulted only in a 25% *SWS* difference and that ψ_{pd} decreased rapidly from -1 to -4 MPa within one month in both plots, the periods during which ψ_{pd} of the dry trees has passed the threshold while ψ_{pd} of the control trees has not, were brief and rare and hence were hardly ever detected in our measurements made twice per month.

The mechanisms responsible for this threshold response of emission to drought remain to be elucidated. Water stress could affect emissions by altering the activity of key-enzymes within the specific MT biosynthesis pathway or by altering the availability of basic substrates entering this pathway. Our results showed seasonal patterns of mono-TPS activity similar in all plots without evidence for drought effects. These results agree with previous studies on *Q. ilex* and *Q. robur* (Schnitzler et al., 1997; Fischbach et al., 2002) but not with recent greenhouse studies by Brilli et al. (2007) and Fortunati et al. (2008) reporting an inhibition of isoprene synthase activity in water-stressed poplar saplings. However, it cannot be ruled out that other key-enzymes of terpenoid biosynthesis upstream in the MEP pathway could be affected in *Q. ilex* leaves during water stress such as the deoxyxylulose-5-phosphate synthase (Wolfertz et al., 2004). Furthermore, the enzymatic activity determined in vitro does not necessarily reflect the activity in vivo. Especially under stress conditions the concentrations of substrates and cofactors or the cellular pH may differ from in vitro conditions, which may potentially affect the activity status since mono-TPS activities display a very sharp pH optimum in vitro at 6.7 (Fischbach et al., 2000). On the other hand, in the present study both in vitro and field emission measurements showed similar product patterns with consistent between-tree differences suggesting that the in vitro assays indeed reproduced the foliar in vivo enzyme activities.

Alternatively the drop in emissions in response to severe drought can mainly be caused by a lack of primary substrates coming from photosynthetic processes. Usually the fraction of photosynthates (reduced carbon and reduction power) consumed in chloroplast terpenoid synthesis is rather small (Niinemets et al., 2004; Schnitzler et al., 2004) but may increase substantially under summer drought. As biosynthesis of volatile terpenoids competes with other, possibly more persistent metabolic sinks such as carotenoid/xanthophyll biosynthesis, starch formation, or amino-acid biosynthesis, the availability of photosynthates may decrease under severe and extended drought as shown by Funk et al. (2004) and by Brilli et al. (2007) with $^{13}CO_2$ labelling experiments. In the present study the mean percentage of assimilated carbon emitted as MT was about 2.5% when $A > 2.5\,\mu\text{mol}\,\text{s}^{-1}\,\text{m}^{-2}$ and was on average doubled during the drought periods with top values reaching more than 30%. Hence carbon assimilation was more severely affected by water stress than MT emissions, as it has been observed in previous studies (Llusia and Penuelas, 1998; Brüggemann and Schnitzler, 2002; Pegoraro et al., 2004). During summer drought, assimilation was predominantly limited by increased diffusion resistance through stomatal closure and by high temperatures favoring photorespiration. In addition, metabolic limitations had likely impaired photosynthesis under the most severe drought conditions when a decrease in the maximum photochemical efficiency (predawn F_V/F_M) was observed indicating a persistent, irreversible inhibition of PSII (Methy et al., 1997). Nevertheless, MT emissions were still detectable even when the carbon gain approached zero. This indicates that alternative carbon sources contribute to MT biosynthesis under stress as reported for isoprene biosynthesis (Affek and Yakir, 2003; Schnitzler et al., 2004).

In summary the present field study demonstrates that water limitation strongly reduce MT fluxes from Mediterranean Holm oak forests. This negative impact was visible over two consecutive years with lower annual emission rates during the second year and over the seasons with a sharp emission decrease during summer when emissions would otherwise reach their annual maximum. A bell-shaped seasonal evolution of MT emissions, as reported in previous studies (e.g. Schnitzler et al., 1997; Staudt et al., 2002) was seen only at the irrigated plot, while the time course of emission at the non-irrigated plots showed remarkable drops in emission coinciding with the periods of summer drought characterized by low ψ_{pd}, *SWS* and A. *Q. ilex* is known to be a very widespread, highly poly-morphological and drought tolerant tree species that is considered as the most important single BVOC source of the Mediterranean vegetation (Kesselmeier et al., 1997; Sabillon and Cremades, 2001). Strikingly, substantial drought-related diminutions in the foliar emissions of this species were observed not only on natural trees being subjected to an additional, partial exclusion of precipitation but also on untreated trees to a similar extent and this during two successive years that were quite different with respect to occurrence and intensity of drought. We conclude that drought-related reductions of MT emissions are highly relevant already under present climatic conditions and therefore

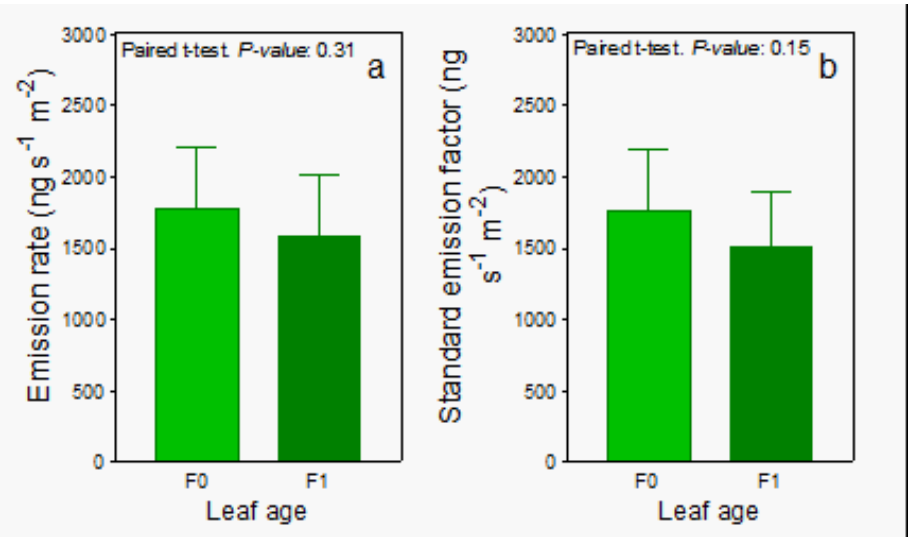

Fig. A1. Monoterpene emission rate (**a**, in $\mathrm{ng\,s^{-1}\,m^{-2}}$) or monoterpene standard emission factor (**b**, in $\mathrm{ng\,s^{-1}\,m^{-2}}$) from two leaf age classes: F0 (current leaves), F1 (one-year-old leaves). Measurements were made on adult *Quercus ilex* trees in the forest of Puechabon, 13 July 2007. Leaf age classes were compared by means of a paired t-test (n=3 trees).

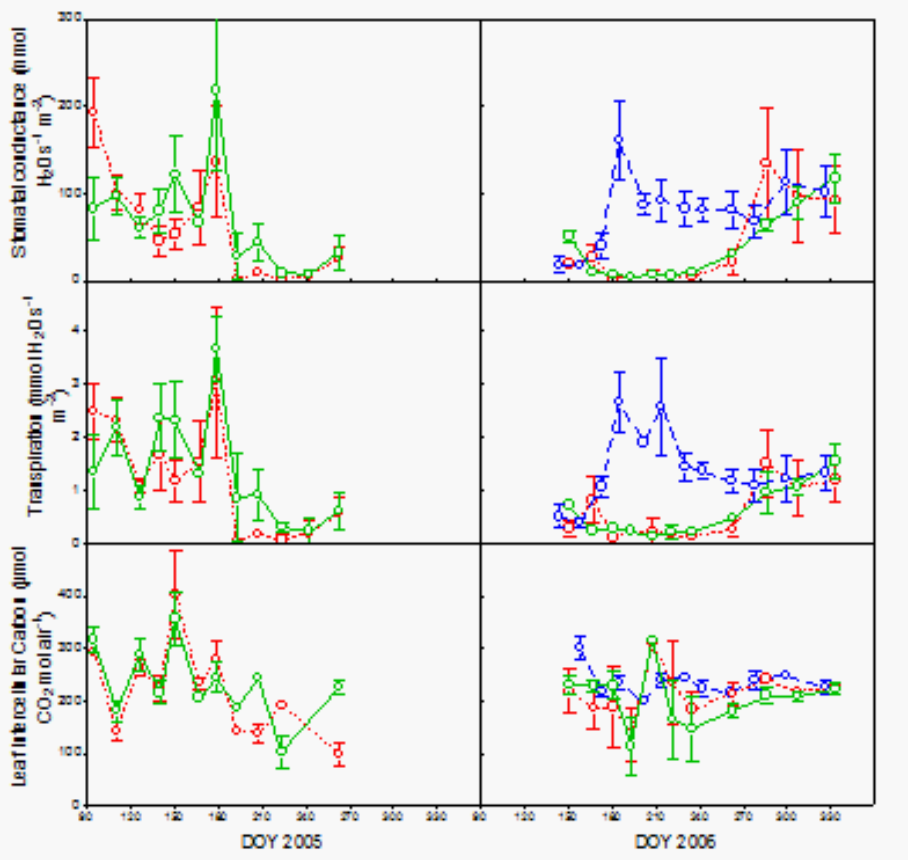

Fig. A2. Seasonal variation of physiological parameters during 2005 (left graphs) and 2006 (right graphs) in the control plot (green symbols), the dry plot (red symbols) and the irrigated plot (blue symbols). First graphs are stomatal conductance ($\mathrm{mmol\,s^{-1}\,m^{-2}}$, mean ± SE of n=3), seconds are transpiration ($\mathrm{mmol\,s^{-1}\,m^{-2}}$, mean ±SE of n=3) and thirds are Leaf Intercellular CO_2 concentration ($\mu\mathrm{mol\,CO_2\,mol\,air^{-1}}$, mean ±SE of n=3).

should be considered in current large scale emission estimations. To date, only a very few studies incorporate water stress effects in BVOC emission estimations basically by applying an empirical decrease of E_s to simulate drought (Parra et al., 2004; Guenther et al., 2006). In an accompanying follow-up study we use the present data set to develop and validate a process-based modelling approach that potentially allows predicting drought effects on emission at leaf level by means of a biochemical model of BVOC biosynthesis that is linked to photosynthesis and soil water model (Grote et al., 2009).

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