Published in Plant, Cell and Environment **27**, (4) ,381–391, (2004) ©by 2004 Blackwell Publishing Ltd. All rights reserved. DOI 10.1111/j.1365-3040.2003.01151
The definitive version is available at www.blackwell-synergy.com

Isoprenoid emission in trees *of Quercus pubescens* and *Quercus ilex* with lifetime exposure to naturally high CO_2 environment.

F. Rapparini[1], R. Baraldi[1], F. Miglietta[2] & F. Loreto[3]

[1]. CNR- IBIMET - Istituto di Biometeorologia, via Gobetti 101,– Bologna, Italy.

[2]. CNR- IBIMET - Istituto di Biometeorologia, via Caproni, 8– Firenze, Italy.

[3]. CNR- IBAF - Istituto di Biologia Agroambientale e Forestale, Via Salaria Km. 29,300 – 00016 Monterotondo Scalo (Roma), Italy.

Running title: Isoprenoid emissions from *Quercus* plants at natural CO_2 springs

Correspondence: Rita Baraldi, CNR- IBIMET - Istituto di Biometeorologia, via Gobetti 101,– Bologna, Italy. Fax: +39-051-6399024; email: R.Baraldi@ibimet.cnr.it

Abstract

We investigated the long-term effect of elevated atmospheric CO_2 on isoprenoid emissions from adult trees of two Mediterranean oak species (the monoterpene-emitting *Quercus ilex* L. and the isoprene-emitting *Quercus pubescens* Willd.) native to a high-CO_2 environment. During two consecutive years, isoprenoid emission was monitored both at branch level, measuring the *actual* emissions under natural conditions, and at leaf level, measuring the *basal* emissions under the standard conditions of 30°C and at light intensity of 1000 μ mol m^{-2} s^{-1}. Long-term exposure to high atmospheric levels of CO_2 did not significantly affect the *actual* isoprenoid emissions. However, when leaves of plants grown in the control site were exposed for short-term to elevated CO_2 level by rapidly switching the CO_2 concentration in the gas-exchange cuvette, both isoprene and monoterpene *basal* emissions were clearly inhibited. These results generally confirm the inhibitory effect of elevated CO_2 on isoprenoid emission. The absence of CO_2 effect on *actual* emissions might indicate higher leaf temperature at elevated CO_2, or an interaction with multiple stresses some of which (e.g. recurrent droughts) may compensate for the CO_2 effect in Mediterranean ecosystems. Under elevated CO_2, isoprene emission by *Q. pubescens* was also uncoupled from the previous day's air temperature. In addition, pronounced daily and seasonal variations of *basal* emission were observed under elevated CO_2 underlining that correction factors may be necessary to improve the realistic estimation of isoprene emissions with empirical algorithms in the future. A positive linear correlation of isoprenoid emission with the photosynthetic electron transport and in particular with its calculated fraction used for isoprenoid synthesis was found. The slope of this relationship was different for isoprene and monoterpenes, but in either case did not change whether plants were grown in ambient or elevated CO_2. This suggests that physiological algorithms may usefully predict isoprenoid emission also under rising CO_2 levels.

Keywords: isoprene, monoterpenes, oak, elevated carbon dioxide, natural springs, algorithm.

Introduction

The increase of CO_2 concentration in the Earth's atmosphere is a well-documented and recognized part of current global changes caused by human activities (Houghton *et al*. 1995). The impact of CO_2 increase on plants, especially trees, that form an important terrestrial carbon reservoir, is receiving increasing attention. In spite of many studies carried out on the effect of elevated atmospheric CO_2 on primary physiological processes such as photosynthesis, transpiration, stomatal activity, biomass growth, and allocation, there is still limited knowledge concerning the effect of a high CO_2 world on biogenic emissions. Forests emit a wide range of VOC (volatile organic compounds), mainly isoprenoids (Isidorov, Zenkevich & Ioffe 1985). It seems that isoprenoids are emitted as protective compounds against biotic (Harrewijn, van Oosten & Piron 2001) and abiotic stresses (Kesselmeier & Staudt 1999; Sharkey & Yeh 2001; Loreto *et al*. 2001). Moreover, these natural emissions constitute a significant source of photochemically reactive carbon that affects the formation of tropospheric air pollutants and greenhouse gases, such as ozone and carbon monoxide, thus having an indirect contribution to global warming (Fehsenfeld *et al.* 1992; Fuentes *et al*. 2000). Therefore, there is a pressing need to understand the effect of plant diversity and of global changes on the forest VOC emission.

Different experimental approaches have been applied to analyze the responses of plants to elevated CO_2 in terms of VOC emission. Published data differ in species, age of plants, experimental design, CO_2 enrichment facilities, time of exposure, and water and nutrient supply, thus complicating the comparison of the results reported in the literature. Long-term effects on and adaptations of the isoprenoid emissions of natural long-lived trees exposed to increasing atmospheric levels of CO_2 are less studied than the effect of short exposure to elevated CO_2, mainly because of the inherent difficulty and the cost of the experimental set-up.

An opportunity to overcome these problems and sources of uncertainty is offered by natural CO_2 springs. All over the world there are sites where CO_2 is naturally released to the atmosphere from geological sources (Miglietta *et al*. 1995). Plants living in the vicinity of these natural CO_2 springs have experienced lifetime exposure, through many generations, to an elevated CO_2 environment. Therefore, CO_2 springs offer an opportunity to study *in situ* the long-term physiological and genetic adaptations of plants to a high CO_2 environment, and represent a promising experimental approach to gain insight for predicting larger-scale responses that have implications for the global carbon cycle or environmental quality (Norby 1996).

The main objective of the present study was to examine in a field-study, the impact of long-term exposure to a naturally high-CO_2 environment on the isoprenoid emission of two native species of mature oak trees in a Mediterranean climate in central Italy, the deciduous downy oak (*Quercus pubescens* Willd.) and the evergreen holm oak (*Quercus ilex* L.). *Q. pubescens* emits isoprene, while *Q. ilex* emits monoterpenes that are not stored in secretory tissues but are synthesized from photosynthetic carbon (Seufert *et al*. 1995; Staudt & Seufert 1995, Loreto *et al*. 1996), and likely stored only in temporary pools in chloroplasts (Ciccioli *et al*. 1997; Loreto *et al.* 1998).

Q.ilex and *Q. pubescens* represent two key plant species for the study of the effect of enhanced CO_2 levels in the Mediterranean area because of their widespread distribution (Bernetti 1998; Gratani 2000). Under the extreme conditions of the Mediterranean climate, such as high temperature and high photosynthetically active radiation (PAR) combined with soil drought, isoprenoid emissions could be very high (Loreto & Sharkey 1990, Sharkey & Loreto 1993, Guenther *et al*. 1995) and may significantly affect the chemistry of the troposphere (Fuentes *et al*. 2001). Moreover, Mediterranean-type ecosystems are expected to be particularly sensitive to any climate change (IPCC 2001).

In the past, models were used to predict isoprene emissions as influenced by meteorological parameters, namely light intensity and leaf temperature (Guenther *et al*. 1993). More recently, the physiological model developed by Niinemets *et al*. (1999, 2002a; b) provided evidence of close

relationships between isoprenoid emissions and foliar photosynthetic characteristics. Plants grown at elevated CO_2 apparently uncouple isoprenoid emissions from photosynthesis (Loreto *et al*. 2001, Rosenstiel *et al*. 2003) and perhaps also from other environmental factors. A side objective of this study was therefore to test whether the empirical and the physiological models may be able to describe isoprenoid emission under future CO_2 levels.

Materials and methods

In situ isoprenoid emission and gas-exchange measurements

The research was conducted at the Bossoleto natural CO_2 spring, situated near Rapolano Terme (Siena, central Italy) and at a nearby control site (see Scholefield *et al.* 2003,).

We measured isoprenoid emissions of two representative plant species of the Mediterranean vegetation growing in the spring and at the adjacent control site. Plants were located facing southward at 1.5-2 m above ground in both the spring and the control site.

During the first year of the project (2000), leaf level measurements were made during June, July, August, and September, while branch level measurements were made during June and September. During the second year (2001), both leaf and branch measurements were made in June and late September. Emissions were estimated approximately every hour during the sampling period (9 am to 4 pm), over 2 or 3 days. All the measurements were made simultaneously at elevated and ambient CO_2 concentration.

At the leaf level, basal emission, E_B, defined as the emission that occurs when a leaf is exposed to 30°C and 1000 μ mol m^{-2} s^{-1} PAR, was measured with a leaf cuvette. *Basal* emission, photosynthesis (A), and stomatal conductance (g_s) were measured by clamping a portion of fully expanded leaves of the canopy of adult trees in the cuvette of the portable gas-exchange system Li-Cor 6400 (Li-Cor, Lincoln, Nebraska, USA), as described by Scholefield *et al*. (2003). This system also allowed immediate switching from elevated to ambient CO_2 and *vice versa*, as explained by

120Scholefield *et al*. (2003). E_B was measured 30 min after switching CO_2, when A and g_s had reached
121a new steady-state.
122At the branch level, the emission measured under actual environmental conditions, E_A, was
123measured by enclosing sun-exposed branches in translucent Teflon bags mounted on an aluminum
124frame with a volume of 12 L. Briefly, the bags, flushed with charcoal-filtered air at a rate of about
12512 L min^{-1}, were equipped with a fan and a sensor for bag air temperature. The difference between
126temperature inside the bag and the outside air temperature was around 2-3°C. A quantum sensor
127mounted outside close to the top of the chamber measured PAR. Air samples were withdrawn from
128the Teflon lines by using an aspirating pump at a flow rate of 100-200 mL min^{-1}. Both inlet and
129outlet air flows were measured with mass-flow meters, and the data were stored using a data logger.
130Both in the leaf and branch enclosures, compounds entering and leaving the bag were collected on
131two-stage traps consisting of glass tubes filled with 0.034 g of Carbograph 1 and 0.17 g of
132Carbograph 2 (Lara, Rome, Italy). Isoprenoids retained on carbon traps were thermodesorbed at
133250°C and cryofocused at –150°C on a fused silica liner connected to a 5890 gas chromatograph
134(Hewlett Packard, Palo Alto, CA, USA) and using a 5970 quadrupole mass spectrometer (Hewlett
135Packard) as detection system (Baraldi *et al*. 1999; Rapparini *et al*. 2001). Isoprenoids were
136transferred to a capillary column (60 m x 0.25 mm I.D.; 0.25 μ m film of polymethylsiloxane; HP1,
137Hewlett Packard). The column was maintained at 40°C for 10 min and then heated to 220°C at 5°C
138min^{-1}. The identity of the compounds of interest was determined by comparison of their retention
139time and mass spectra to that of authentic standards. Quantification of isoprenoids was performed
140after calculation of standard curves and response factors for each compound, and using d_{14}-cymene
141as internal standards. The biogenic VOC emission rates were calculated by multiplying the
142concentration difference between the chamber's inlet and outlet air with the air flow through the
143chamber divided by leaf area.
144PAR and cuvette air temperature were measured while emission samples were taken. During each
145measuring campaign, the branches enclosed in the bag were cut to determine the leaf area and dry

weight. Leaf area was measured by Video Image. For the image acquisition, a CCD camera (JVC model TK-880) was used, interfaced with a computer by an ELVIS board and Chameleon software (Sky Instr. Ltd., UK). Leaf dry weight was determined after drying at 70°C in a ventilated oven until the weight stabilized.

In the July and August 2000 field campaigns, measurements of predawn leaf water potentials were used as stress indices. They were measured on 16 fully expanded leaves of different plants with a pressure chamber (Model 3005; Soil Moisture Equipment Corp., Santa Barbara, CA, USA).

Emission modelling

Branch-level isoprenoid emission measured under experimental conditions were normalized to standard conditions, based on algorithm of Guenther *et al*. (1993). This algorithm usefully described isoprene emission as dependent to PAR and leaf temperature. As *Q. ilex* monoterpene emissions has been found to have temperature and light dependencies very similar to those of isoprene emissions (Staudt & Seufert 1995), the model of Guenther *et al*. (1993) has also been applied to monoterpene emissions of *Q. ilex* in the field (Ciccioli *et al*. 1997; Kesselmeier *et al*. 1996, 1997; Bertin *et al*. 1997).

In the model of Guenther *et al*. (1993) isoprene emission (E) is given as the product of the *basal* emission factor (E_B) at temperature of 30°C and a PAR of 1000 μ mol m^{-2} s^{-1} and two correction factors to account for temperature (C_T) and light (C_L) dependencies:

$$E = E_B C_T C_L$$

To check the possible influence of CO_2 air concentration on the temperature dependence of isoprene emission, we correlated averaged E_A for branches of plants growing at ambient and elevated CO_2 with temperatures of single sampling day and with temperature averaged over a growing number of days before measurements.

To model isoprenoid emission at different CO_2 levels we also applied the algorithm developed by Niinemets *et al*. (1999; 2002a) using the E_B, and the corresponding photosynthetic parameters. The rate of photosynthetic electron transport (J) is calculated from leaf gas exchange measurements as (Brooks and Farquhar 1985):

$$J = \frac{(A + R_d)(4C_i + 8\Gamma^*)}{(C_i - \Gamma^*)} \quad \text{Eqn 1}$$

Where R_d is the rate of mitochondrial respiration continuing in the light (mmol m^{-2} s^{-1}), Γ^* is the CO_2 compensation point in the absence of R_d (μ mol mol^{-1}, Laisk 1977), and C_i is the intercellular CO_2 concentration (μ mol mol^{-1}).

Taking into account the different coenzyme (NADPH and ATP) cost of isoprenoids per mole CO_2 assimilated as compared to that for sugar synthesis, and according to the methyl erythritol 4-phosphate pathway, the rate of electron transport necessary to support the measured isoprenoid emissions (J_E) and the extra photosynthetic electron transport required to increase the reduction state from the level of sugar to isoprenoids (J_e) are given by:

for isoprene (Niinemets *et al*. 1999)

$$J_E = \frac{6E(4.67C_i + }{(C_i - \Gamma^*)} \quad \text{Eqn 2}$$

$$J_e = \frac{6E(0.67C_i + }{(C_i - \Gamma^*)} \quad \text{Eqn 3}$$

for monoterpenes (Niinemets *et al*. 2002a)

$$J_E = \frac{12E(4C_i + 8\Gamma^*)}{(C_i - \Gamma^*)} \quad \text{Eqn 4}$$

Eqn 5

16
198
199
200
201
202
203 $J_e = 2E(\vartheta - 24)$
204
205 Where ϑ is the NADPH cost of monoterpenes (mol m^{-1})
206 From Eqns 1-5, the fraction of photosynthetic electron transport used for isoprenoid emission, ε, is
207 determined as the ratio between the rate of electron transport necessary to support the measured
208 isoprenoid emission (J_E) and the total photosynthetic electron transport (J_T). J_T is calculated from
209 foliar photosynthesis and isoprenoid emission as J (Eqn 1) + J_e (Eqn 3 or 5).
210
211 *Statistical analysis*
212 One-way analysis of variance (ANOVA) was performed for all the parameters to test the effect of
213 growth under different CO_2 level on physiological characteristics of *Q. ilex* and *Q. pubescens* plants
214 for individual field campaign in 2000 and 2001. Regression analysis was used to detect the
215 influence of temperature of the sampling day or averaged over a growing number of days before
216 measurements, and of photosynthetic characteristic parameters calculated applying the Niinemets
217 algorithm (1999, 2002a) on isoprenoid emission, determining if the parameters of the regression are
218 significantly different from zero. All statistical analyses were performed in SAS (SAS System 8.1,
219 SAS Institute Inc., Cary, NC, USA).
220
221 **Results**
222 *In situ isoprenoid emission and physiological measurements*
223 The profile of the emitted monoterpenes by *Q. ilex* was similar at both branch and leaf level. The
224 major emitted compounds were α-pinene, β-pinene, sabinene and β-myrcene, which accounted

for more than 60-70% of the total emission, as commonly found for this species (Bertin *et al*. 1997; Staudt & Seufert 1995; Csiky & Seufert 1999; Sabillon & Cremades 2001). The proportions of the main compounds were constant and independent of daytime, season, environmental conditions and CO_2 level (data not shown).

Days were cloudless and dry over most of the experiments. During the sampling period (9 am-4 pm solar time), PAR showed a typical daily cycle with maximum values at midday (around 1700 μ mol^{-2} m^{-2} s^{-1} and 1300 μ mol^{-2} m^{-2} s^{-1} in June and September field campaigns, respectively), and minimum values in the late afternoon (approximately 100-250 μ mol^{-2} m^{-2} s^{-1}). Bag air temperature varied between 25°C and 37°C.

E_A were in the range previously reported in literature for the two different oak species (Biogenic Emissions in the Mediterranean Area - BEMA project 1997). Only *Q. ilex* monoterpene emissions in September 2000 under ambient CO_2 were significantly higher than in the other months (Tab 1). During the June 2000 field campaign, monoterpene E_A of *Q. ilex* was not affected by long-term exposure to high CO_2, with a daily average emission rate of 8.2 nmol m^{-2}s^{-1} under elevated CO_2 and of 8.6 nmol m^{-2}s^{-1} under ambient CO_2. In September 2000, the monoterpene emissions of plants grown under elevated CO_2 were comparable to those found in June, but E_A was significantly higher in plants grown under ambient CO_2 than under elevated CO_2 (Tab 1).

During June 2000, E_A measured at branch level in *Q. pubescens* plants under elevated CO_2 were significantly lower (3.5 nmol^{-2} m^{-2} s^{-1}) than those determined under ambient CO_2 (7.8 nmol^{-2} m^{-2} s^{-1}), while in September 2000 and in both 2001 field campaigns no significant differences between the two sites were observed, except in some hours of the day (Tab 1).

Leaf weight per leaf area was 152 and 72 g m^{-2} for *Quercus ilex* and *Quercus pubescens* in the control site respectively, and 172 and 81 g m^{-2} for the same species under elevated CO_2. Consequently when isoprene and monoterpene emission rates were expressed on leaf mass basis, differences between ambient and elevated CO_2 were maintained or, in some cases, even more evident.

The photosynthetic rates (A) of *Q. ilex* plants were always higher under elevated CO_2 with respect to ambient CO_2 (Tab 1). The percentage of carbon emitted as monoterpenes relative to carbon fixed by photosynthesis was always higher under ambient CO_2 compared to elevated CO_2, reaching 4% in September. In *Q. pubescens* plants the stimulation of photosynthesis at elevated CO_2 was observed only in September 2000 and in June 2001. As in *Q. ilex*, the percentage of carbon lost as isoprene was higher under ambient CO_2 compared to elevated CO_2.

The diurnal trends of E_A and E_B of isoprene emitting *Q. pubescens* plants grown at elevated CO_2 showed in September 2001 a clear daily change with lower values early in the morning and maximum values in the afternoon (Fig. 1a, b). The same trend was observed in plants grown under ambient CO_2 (data not shown). The diurnal trend of E_B and E_A was associated to a similar trend of photosynthesis, at both CO_2 levels (Fig. 1a). In *Q. pubescens* there was also evidence of seasonality-related variability in isoprene E_B measured along 2000 at both sites, with peaks of emission during July and September (Fig. 2a, b). Photosynthesis maximum was also observed in July 2000 (Fig. 2a, b).

At the control site, rapid switches of the CO_2 concentration from 350 to 1000 ppm had a strong and very rapid negative effect on E_B of both species (Fig. 3a, b). Photosynthesis increased (Fig. 3c, d) and g_s decreased (Fig. 3e, f) in response to CO_2 rise. At the Bossoleto site, E_B slightly increased or remained unchanged in response to CO_2 switches from 1000 to 350 ppm (Fig. 3a, b). Photosynthesis was also unaffected (Fig. 3c, d), but g_s increased in response to CO_2 reduction (Fig. 3e, f)

Predawn leaf water potentials measured in July and August 2000 were particularly low in both oak species compared to values reported for the same species in the same Mediterranean area (Schwanz & Polle 1998; Loreto *et al*. 2001), indicating a high drought stress (Fig. 4a, b). The predawn leaf water potential was slightly lower in *Q. ilex* than in *Q. pubescens*, reaching mean values of about 10.3 MPa and 7.2 MPa, respectively, independently of CO_2 growth conditions (Fig. 4a, b).

Emission modelling

Normalization of E_A according to Guenther (1993) on the basis of meteorological factors (light and temperature) (i.e. Tab 1) would not be correct if the relationship between these factors and emission is affected by growth at elevated CO_2. Day time changes of light intensity should cause only limited changes in isoprenoid emission (Guenther *et al*. 1993). Temperature represents a more important factor in controlling isoprenoid emission and recent works showed the influence of past temperature conditions on the temperature dependence of isoprene emission (Guenther *et al*. 1999; Sharkey *et al*. 1999; Geron *et al*. 2000; Hanson & Sharkey 2001; Pétron *et al*. 2001). To test the influence of temperature on the E_A of *Q. pubescens* plants, we correlated average isoprene E_A (Tab 1) with the temperature of the sampling day and the calculated average temperature of 1 to 15 days before measurements. At ambient CO_2 there was a clear increase of the correlation between isoprene emission and air temperature by increasing the number of days over which the air temperature was averaged, and the strongest relationship was observed when the temperature was averaged over 14 days (Tab 2). However, no strong relationship was found under elevated CO_2.

In alternative to the empirical model based on environmental factors, isoprenoid emission can also be estimated with a physiological model based on photosynthesis (Niinemets *et al*. 1999; 2002a,b). For both *Quercus* species and both field campaigns, E_B tended to scale positively with A and the photosynthetic electron flux (J) under ambient CO_2 concentrations ($r^2 = 0.43$ $P < 0.02$ and $r^2 = 0.60$ $P < 0.005$ for photosynthesis and electron transport, respectively), while no correlation was found in plants grown at the Bossoleto site ($r^2 = 0.04$ $P > 0.50$ and $r^2 = 0.13$ $P > 0.20$ for photosynthesis and electron transport, respectively) (data not shown).

Isoprenoid E_B of both species correlated positively with the photosynthetic electron transport rate required to sustain the observed isoprenoid emission (J_E), independently of the year and of the CO_2-growth conditions ($r^2 = 0.73$ $P < 0.0005$ and $r^2 = 0.92$ $P < 0.0001$ for *Q. pubescens* and *Q. ilex*, respectively) (Fig. 5a). Isoprenoid E_B also correlated positively with the fraction of electrons going into the isoprenoid synthesis pathway, ε ($r^2 = 0.77$ $P < 0.0002$ and $r^2 = 0.43$ $P < 0.05$ for *Q.*

303*pubescens* and *Q. ilex*, respectively) (Fig. 5b). In both species, the intercepts were not statistically
304different from zero, but the slope was higher in *Q. pubescens* than in *Q. ilex* plant. We did not
305observe any significant effect of growth under different CO_2 conditions on this positive correlation
306(data not shown).

Discussion

Exposure to or *growth* at elevated CO_2 often reduces isoprenoid emission by vegetation (Monson & Fall. 1989; Loreto & Sharkey 1990; Sharkey *et al*. 1991; Loreto *et al*. 2001; Scholefield et al. 2003; Rosenstiel *et al.* 2003), with few exceptions (Tognetti *et al*. 1998; Constable *et al*. 1999; Sharkey *et al*. 1991; Buckley 2001; Staudt *et al*., 2001). A clear enzymological and biochemical regulation has been recently demonstrated to occur under elevated CO_2 and is likely to account for this effect (Loreto *et al*. 2001; Scholefield *et al*. 2003; Rosenstiel *et al*. 2003). We showed that short term *exposure* to elevated CO_2 induced in both *Quercus* species a rapid and clear inhibition of leaf-level isoprenoid emission, confirming the results obtained in *Phragmites* in the same CO_2 spring site by Scholefield *et al*. (2003). However, branch-level isoprenoid emissions from trees of *Q. ilex* and *Q. pubescens* grown in the Bossoleto spring were not significantly different than at ambient CO_2, even after normalization, which indicates that both *actual* and *basal* emissions were not reduced by *growth* at elevated CO_2. In the Mediterranean region, multiple stresses, such as high temperature and irradiance combined with limited water availability (Scarascia-Mugnozza *et al*. 2000) could have strong effects on isoprenoid emission, especially in summer. Elevated temperatures stimulate isoprenoid emission (Loreto & Sharkey 1990), probably because of activation of isoprene synthase (Monson *et al*. 1992). It may be that temperatures in the Bossoleto spring were slightly higher than in the control site, therefore compensating for the inhibitory effect of elevated CO_2. Certainly the night temperature of the spring was higher than in the surrounding since CO_2 formed overnight a dense layer covering the bottom of the spring (see cover picture).

Isoprene emission has been found to be scarcely inhibited by environmental stresses, and recovery from stresses, particularly drought, can even temporarily stimulate the emission of isoprene (Loreto & Delfine, 2000; Sharkey & Loreto 1993; Llusià & Penuelas 1998; Bruggemann & Schnitzler 2002).

In a Mediterranean area, limited water availability represents a major environmental stress. Loreto et al. (2001), in a long-term study on field-grown *Q. ilex* plants growing under elevated CO_2

conditions, did not observe the inhibitory effect of high CO_2 on monoterpene emission when plants experienced a very severe summer drought, suggesting that the effect of these environmental stresses can counteract and overcome the negative effect of elevated CO_2. Similar results have been reported by M.J. Potosnak, K.L. Griffin, R.K. Monson, R. Murthy, J. van Haren, A. Wright, B. Farnsworth, C.A. Klimas, T. N. Rosenstiel and V.C. Engel (2002, personal communication) for isoprene emission by poplar plants grown under elevated CO_2 in the mesocosms of Biosphere-2 Center.

In our experimental conditions, very low predawn water potentials were measured, especially during summer 2000. Thus, it may be speculated that severe drought stress conditions stimulated the isoprenoid emission and counteracted the negative effect of elevated CO_2 in our experiments.

We have shown that isoprene E_B by *Q. pubescens* is subjected to daily and seasonal variations. Pronounced daily and seasonal variations of E_B were observed by other authors (Fuentes *et al*. 1995; Ciccioli *et al*. 1997; Street *et al.* 1997) and in few recent models an additional factor to account for seasonal influences on emission has been introduced (Guenther 1997; Pier & McDuffie 1997; Guenther *et al*. 1999; Lehning *et al.* 2001; Ciccioli *et al*. 2003). It is clear that similar correction factors should be used also when monitoring emission under elevated CO_2. However, at least seasonal variations of E_B seems to be similar under ambient and elevated CO_2. Rapid temperature fluctuations (Singsaas *et al*. 1999; Singsaas & Sharkey 2000), usually occurring in our experimental conditions, and past temperatures experienced by the plants prior to measurements (Guenther *et al*. 1999; Sharkey *et al*. 1999; Geron *et al*. 2000; Hanson & Sharkey 2001; Pétron *et al*. 2001) could have influenced isoprenoid emissions, particularly under elevated CO_2. Our experiments have shown that isoprene E_A in ambient CO_2 is positively correlated with previous day temperature. The best correlation was found by averaging two week temperature before measurements, which suggests that weather regulation on isoprene may be even more (Sharkey *et al*. 1999; Geron *et al*. 2000; Hanson & Sharkey 2001) or similarly (Guenther *et al*. 1999; Petron *et al*. 2001) long-lasting than previously reported. However, under elevated CO_2 E_A did not well correlate with air temperature of the sampling day as well as with long-term variations of this

parameter. One explanation for this might be related to stomatal closure under elevated CO_2 leading to raising leaf temperature with respect to air temperature. Irrespective of the physiological reason, this may reduce the model performance under elevated CO_2.

Under elevated CO_2 modelling based on physiological traits may be more successful than that on meteorological parameters. In general, when we compared the calculated photosynthetic parameters (A, J) with E_B of isoprenoids, we found a positive relationship, in ambient but not at elevated CO_2. In contrast, a positive relationship was always found when E_B was compared with the photosynthetic electron transport necessary to support the measured isoprenoid emissions or with the fraction of photosynthetic electron transport used for isoprenoid synthesis. These positive correlations between were, to a certain extent, expected, as the basal emission is a factor to calculate J_E and ε (Niinemets *et al*. 1999; 2002a, b). But our results indicate that these relationships are not influenced by CO_2 level and suggest that the physiological algorithm may usefully predict isoprenoid emission also under rising CO_2, and even under multiple stress conditions such as those probably experienced by plants during our experiments. On the other hand, differences between the *Quercus* species in the slope of ε *versus* isoprenoid emissions reflect a higher electron cost for monoterpenes than for isoprene, which is consistent with the higher metabolic energy of monoterpene compared to that of isoprene synthesis (Niinemets *et al*. 1999; 2002a).

Aknowledgements

We thank Dr. A. E. Sztein for revising and editing the manuscript. Researches were supported by the EC-Environment project EVK2-CT-1999-00042 (Future-VOC).

This work is dedicated to the memory of Wolfgang Zimmer.

References

386Baraldi R., Rapparini F., Rossi F., Latella A. & Ciccioli P. (1999). Volatile organic compound
387 emissions from flowers of the most occurring and economically important fruit tree species.
388 *Physics and Chemistry of the Earth* **6**, 729-732.

389Bernetti G. (1998) II. Selvicoltura speciale. Scienze Forestali e Ambientali (eds G. Giordano),
390 UTET, Torino, Italy.

391Bertin N., Staudt M., Hansen U., Seufert G., Ciccioli P., Foster P., Fugit J.L. & Torres L. (1997)
392 Diurnal and seasonal course of monoterpene emissions from *Q. ilex* (L.) under natural conditions.
393 Applications of light and temperature algorithms. *Atmospheric Environment*. **31**, 135-144.

394Brooks A. & Farquhar G.D. (1985) Effects of temperature on the CO_2/O_2 specificity of ribulose-1,5-
395 bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Estimates from gas-
396 exchange measurements on spinach. *Planta* **165**, 397-406.

397Bruggemann N. & Schnitzler J.-P. (2002) Comparison of isoprene emission, intercellular isoprene
398 concentration and photosynthetic performance in water-limited oak (*Quercus pubescens* Willd.
399 and *Quercus robur* L.) saplings. *Plant Biology* **4**, 456-463.

400Buckley P.T. (2001) Isoprene emissions from a Florida scrub oak species grown in ambient and
401 elevated carbon dioxide. *Atmospheric Environment* **35**, 631-634.

402Ciccioli P., Brancaleoni E., Frattoni M., Marta S., Brachetti A., Vitello M., Tirone G. & Valentini
403 R. (2003) Relaxed eddy accumulation, a new technique for measuring emission and deposition
404 fluxes of volatile organic compounds by capillary gas chromatography and mass spectrometry.
405 *Journal of Chromatography A* **985**, 283-296.

406Ciccioli P., Fabozzi, C., Brancaleoni, E., Cecinato A., Frattoni, M., Loreto, F., Kesselmeier, J.,
407 Schaefer, L., Bode, K., Torres, L. & Fugit, J.L. (1997) Use of the isoprene algorithm for
408 predicting the monoterpene emission from the Mediterranean holm oak *Quercus ilex* L. :
409 performance and limits of this approach. *Journal of Geophysical Research* **102**, 23319-23328.

410Constable J.V.H., Litvak M.E., Greenberg J.P. & Monson R.K. (1999) Monoterpene emission from
411 coniferous trees in response to elevated CO_2 concentration and climate warming. *Global Change*
412 *Biology* **5**, 255-267.
413Csiky O. & Seufert G. (1999) Terpenoid emissions of Mediterranean oaks and their relation to
414 taxonomy. *Ecological Applications* **9**, 1138-1146.
415Fehsenfeld F., Calvert J., Fall R., Goldan P., Guenther A.B., Hewitt N., Lamb B., Liu S., Trainer
416 M., Westberg H. & Zimmerman P. (1992). Emissions of volatile organic compounds from
417 vegetation and the implications for atmospheric chemistry. *Global Biogeochemical Cycles* **6**,
418 390-430.
419Fuentes J.D., Hayden, B.P., Garstang, M., Lerdau, M., Fitzjarrald, D., Baldocchi, D.D. & Monson,
420 R. (2001) New Directions: VOCs and biosphere-atmosphere feedbacks. *Atmospheric*
421 *Environment* **35**, 189-191.
422Fuentes J.D., Lerdau M., Atkinson R., Baldocchi D., Bottenheim J.W., Ciccioli P., Lamb B., Geron
423 C., Guenther A., Sharkey T.D. & Stockwell W. (2000) Biogenic hydrocarbons in the atmospheric
424 boundary layer: a review. *Bulletin of the American Meteorological Society* **81**, 1537-1575.
425Fuentes J.D., Wang D., den Hartog G., Neumann H.H., Dann T.F. & Puckett K.J. (1995) Modelled
426 and field measurements of biogenic hydrocarbon emissions from a Canadian deciduous forest.
427 *Atmospheric Environment* **29**, 3003-3017.
428Geron C., Guenther A., Sharkey T. & Arnts R.R. (2000) Temporal variability in basal isoprene
429 emission factor. *Tree Physiology* **20**, 799-805.
430Gratani L. (2000) Leaf temperature effects on gas-exchange in *Quercus ilex* L. growing under field
431 conditions. *Plant Biosystems* **134**, 19-24.
432Guenther A. (1997) Seasonal and spatial variations in natural volatile organic compound emissions.
433 *Ecological Applications* **7**, 34-45.

434Guenther A., Baugh B., Brasseur G., Greenberg J., Harley P., Klinger L., Serça D. & Vierling L.
435 (1999) Isoprene emission estimates and uncertainties for the Central African EXPRESSO study
436 domain. *Journal of Geophysical Research*, **104**, 30,625-30,639.

437Guenther A., Hewitt C.N., Erickson D., Fall R., Geron C., Graedel T., Harley P., Klinger L., Lerdau
438 M., McKay W.A., Pierce T., Scholes B., Steinbrecher R., Tallamraju R., Taylor J. & Zimmerman
439 P. (1995) A global model of natural volatile organic compound emissions. *Journal of*
440 *Geophysical Research* **100**, 8873-8892.

441Guenther A., Zimmerman P., Harley P., Monson R. K. & Fall R. (1993) Isoprene and monoterpene
442 emission rate variability: model evaluation and sensitivity analysis. *Journal of Geophysical*
443 *Research* **98**, 12609-12617.

444Hanson D.T. & Sharkey T.D. (2001) Rate of acclimation of the capacity for isoprene emission in
445 response to light and temperature. *Plant, Cell & Environment* **24**, 937-946.

446Harrewijn P., van Oosten M.A. & Piron G.M. (2001) Natural Terpenoids as Messengers: a
447 multidisciplinary study of their production, biological functions and practical application. Kluwer
448 Academic Publishers, Netherlands.

449Houghton J.T., Meiro-Filho L.G., Callander B.A., Harris N., Kattenburg A. & Maskell K. (1995)
450 Climate Change 1995 – the Science of Climate Change. Second Assessment Report of the
451 Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

452IPCC. Intergovernmental Panel on Climate Change (IPCC). Third Assessment Report - Climate
453 Change 2001.

454Isidorov V.A., Zenkevich I.G. & Ioffe B.V. (1985) Volatile organic compounds in the atmosphere
455 of forests. *Atmospheric Environment* **19**, 1-8.

456Kesselmeier J. & Staudt M. (1999) Biogenic Volatile Organic Compound (VOC): An overview on
457 emission, physiology and ecology. *Journal of Atmospheric Chemistry* **33**, 23-88.

458Kesselmeier J., Schaefer L., Ciccioli P., Brancaleoni E., Cecinato A., Frattoni M., Foster P., Jacob
459 V., Denis J., Fugit J.L., Dutaur L. & Torres L. (1996) Emission of monoterpenes and isoprene

from a Mediterranean oak species *Quercus ilex* L. measured within the BEMA (Biogenic Emissions in the Mediterranean Area) project. *Atmospheric Environment* **30**, 1841-1850.

Laisk A. (1977). Kinetika fotosinteza I fotodyhaniya C3-rastenii (Kinetics of photosynthesis and photorespiration in C_3-plants) (in Russian). Moscow, Russia: Nauka.

Lehning A., Zimmer W., Zimmer I. & Schnitzler J.-P. (2001) Modeling of annual variations of oak (*Quercus robur* L.) isoprene synthase activity to predict isoprene emission rates. *Journal of Geophysical Research* **106**, 3157-3166.

Llusià J. & Penuelas J. (1998) Changes in terpene content and emission in potted Mediterranean woody plants under severe drought. *Canadian Journal of Botany* **76**, 1366-1373.

Loreto F. & Delfine S. (2000). Emission of isoprene from salt-stressed *Eucalyptus globulus* leaves. *Plant Physiology* **123**, 1605-1610.

Loreto F. & Sharkey T.D. (1990) A gas exchange study of photosynthesis and isoprene emission in red oak (*Quercus rubra* L.). *Planta* **182**, 523-531.

Loreto F., Ciccioli P., Brancaleoni E., Cucinato E. & Frattoni M. (1998) Measurement of isoprenoid content in leaves of Mediterranean *Quercus* spp. by a novel and sensitive method and estimation of the isoprenoid partition between liquid and gas phase inside the leaves. *Plant Science* **136**, 25-30.

Loreto F., Ciccioli P., Cecinato A., Brancaleoni E., Frattoni M. & Tricoli D. (1996) Influence of environmental factors and air composition on the emission of α -pinene from *Quercus ilex* leaves. *Plant Physiology* **110**, 267-275.

Loreto F., Fischbach R.J., Schnitzler J.-P., Ciccioli P., Brancaleoni E., Calfapietra C. & Seufert G. (2001) Monoterpene emission and monoterpene synthase activities in the Mediterranean evergreen oak *Quercus ilex* L. grown at elevated CO_2 concentrations. *Global Change Biology* **7**, 709-717.

Miglietta F., Badiani M., Bettarini I., van Gardingen P., Selvi F. & Raschi F. (1995) Preliminary studies of the long-term CO_2 response of Mediterranean vegetation around natural CO_2 vents. In:

Global Change and Mediterranean-Type Ecosystems (eds. J. M. Moreno & W. C. Oechel), pp 102-120. Springer-Verlag , New York.

Monson R.K. & Fall R. (1989) Isoprene emission from aspen leaves. *Plant Physiology* **90**, 267-274.

Monson R.K., Jaeger C.H., Adams III W.W., Driggers E.M., Silver G.M. & Fall R. (1992) Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. *Plant Physiology* **98**, 1175-1180.

Niinemets Ü., Hauff K, Bertin N., Tenhunen J.D., Steinbrecher R. & Seufert G. (2002a) Monoterpene emissions in relation to foliar photosynthetic and structural variables in Mediterranean evergreen *Quercus* species. *New Phytologist* **153**, 243-256.

Niinemets Ü. Seufert G., Steinbrecher R. & Tenhunen J.D. (2002b). A model coupling foliar monoterpene emissions to leaf photosynthetic characteristics in Mediterranean evergreen *Quercus* species. *New Phytologist* **153**, 257-275.

Niinemets Ü., Tenhunen J.D., Harley P.C. & Steinbrecher R. (1999) A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. *Plant Cell & Environment* **22**, 1319-1335.

Norby R.J. (1996). Oaks in a high-CO_2 world. *Annales des Sciences Forestieres* **53**, 413-429.

Petron G., Harley P., Greenberg J. & Guenther A. (2001). Seasonal temperature variations influence isoprene emission. *Atmospheric Environment* **28**, 1707-1710.

Pier P.A. & McDuffie C. (1997) Seasonal isoprene emission rates and model comparisons using whole-tree emissions from white oak. *Journal of Geophysical Research* **102**, 23963-23971.

Rapparini F., Baraldi R. and & Facini O. (2001) Seasonal variation of monoterpene emission from *Malus domestica* and *Prunus avium*. *Phytochemistry* **57**, 681-687.

Rosenstiel, T.N., Potosnak M.J., Griffin K.L., Fall R. & Monson R.K. (2003) Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem. *Nature* **421**, 256-259.

Sabillón D. & Cremades L.V. (2001) Diurnal and seasonal variation of monoterpene emission rates for two typical Mediterranean species (*Pinus pinea* and *Quercus ilex*) from field measurements-relationship with temperature and PAR. *Atmospheric Environment* **35**, 4419-4431.

Scarascia-Mugnozza G., Oswald H., Piussi P. & Radoglou K. (2000) Forests of the Mediterranean region: gaps in knowledge and research needs. *Forest Ecology and Management* **132**, 97-109.

Scholefield P.A., Doick K.J., Herbert B., Hewitt C. N., Schnitzler J.-P., Pinelli P. & Loreto F. (2003) Impact of rising CO_2 on VOC emissions: isoprene emission from *Phragmites australis* growing at elevated CO_2 on a natural carbon dioxide spring. *Plant Cell & Environment* (XXX).

Schwanz P. & Polle A. (1998). Effects of lifetime exposure to elevated CO_2 on antioxidative enzymes in mature oak trees. *New Phytologist* **140**, 411-423.

Seufert G., Kotzias D., Spartá C. & Versino B. (1995) Volatile organics in Mediterranean shurbs and their potential role in a changing environment. In: Global Change ad Mediterranean-type Ecosystems (eds. J. M. Moreno & W. C. Oechel), pp. 343-370, Springer-Verlag, New York.

Sharkey T.D. & Loreto F. (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. *Oecologia* **95**, 328-333.

Sharkey T.D. & Yeh S. (2001) Isoprene emission from plants. *Annual Review of Plant Molecular Biology* **52**, 407-436.

Sharkey T.D., F. Loreto & C.F. Delwiche. (1991) High carbon dioxide and sun/shade effect on isoprene emissions from oak and aspen tree leaves. *Plant Cell & Environment* **14**, 333-338.

Sharkey T.D., Singsaas E.L., Lerdau M.T. & Geron C.D. (1999) Weather effects on isoprene emission capacity and applications in emissions algorithms. *Ecological Applications* **9**, 1132-1137.

Singsaas E.L. & T.D. Sharkey. (2000) The effects of high temperature on isoprene synthesis in oak leaves. *Plant, Cell & Environment* **23**, 751-757.

535Singsaas E.L., LaPorte M.M., Shi J-Z., Monson R.K., Bowling D.R., Johnson K., Lerdau M.,
536 Jasentuliytana A. & Sharkey T.D. (1999) Kinetics of leaf temperature fluctuation affect isoprene
537 emission from red oak (*Quercus rubra*) leaves. *Tree Physiology* **19**, 917-924.

538Staudt M. & Seufert G. (1995) Light dependent emission of monoterpenes by holm oak (*Quercus*
539 *ilex* L.). *Naturwissenschaften* **82**, 89-92.

540Staudt M., Joffre R., Rambal S. & Kesselmeier J. (2001) Effect of elevated CO_2 on monoterpene
541 emission of young *Quercus ilex* trees and its relation to structural and ecophysiological
542 parameters. *Tree Physiology* **21**, 437-445.

543Street R.A., Owen S., Ducham S. C., Boissard C. & Hewitt C.N. (1997) Effect of habitat and age on
544 variations in volatile organic compound (VOC) emissions from *Quercus ilex* and *Pinus pinea*.
545 *Atmospheric Environment* **31**, 89-100.

546Tognetti R, Johnson J.D., Michelozzi M. & Raschi A. (1998). Response of foliar metabolism in
547 mature trees of *Quercus pubescens* and *Quercus ilex* to long.term elevated CO_2. *Environmental*
548 *and Experimental Botany* **39**, 233-245.

Figure legends

Fig. 1. Diurnal trend of isoprene *basal* (a) and *actual* (b) emission (closed diamond) and of photosynthesis (open squares) from *Q. pubescens* leaves. Measurements were taken under elevated CO_2 at the Bossoleto site during September 2001 field campaign. Means ± S.E., *n*=3-4, are shown.

Fig. 2. Seasonal trend of isoprene *basal* emission and of photosynthesis from *Q. pubescens* leaves under ambient (a) and elevated (b) CO_2 level. Measurements taken along 2000. Means ± S.E., *n*=3-4, are shown.

Fig. 3. Response of isoprenoid *basal* emission, photosynthesis and stomatal conductance of *Q. pubescens* (a, c, e) and *Q. ilex* (b, d, f) plants to CO_2 switches. CO_2 was suddenly decreased from 1000 to 350 ppm for plants growing at the Bossoleto site, while it was suddenly increased from 350 to 1000 ppm for plants growing in the control site. Measurements taken at both sites during September 2001 are relative to emission before switching (shown as 1; dashed line). Means (n = 5) ± S.E.

Fig. 4. Predawn water potential of (a) *Q. pubescens* and (b) *Q. ilex* leaves of plants grown under high-CO_2 air concentrations at the Bossoleto site or under ambient CO_2 conditions in the control site. Means (n = 16) ± S.E. Differences between control and Bossoleto site were not statistically significant at $P < 0.1$.

Fig. 5. Isoprenoid *basal* emissions in relation with (a) photosynthetic electron transport rate required to sustain the observed rates of isoprenoid emission (J_E) and with (b) the fraction of electrons for the isoprenoid synthesis (ε) in leaves of *Q. pubescens* (closed diamond) and *Q. ilex* (open squares). Each point corresponds to a separate field campaign of measurements.

574 Tab.1. Daily average a*ctual* emission (E_A) measured at branch level, photosynthesis (A) measured
575 at leaf level from *Q. ilex* and *Q. pubescens* plants, and percent of photosynthetic carbon lost as
576 isoprenoid during 2000 and 2001 field campaigns. The values shown in the parenthesis represent
577 E_B estimated using the equations of Guenther *et al*. (1993). Values are means ± standard error
578 (S.E.), n = 3-4. * P< 0.1, ** P< 0.05, *** P< 0.01.

		Q. ilex			***Q. pubescens***		
		E_A (nmol m^{-2} s^{-1})	A (μ mol m^{-2} s^{-1})	% C	E_A (nmol m^{-2} s^{-1})	A (μ mol m^{-2} s^{-1})	% C
June 2000	Ambient CO_2	8.6 ± 1.7 *(5.7 ± 1.4)*	4.8 ± 0.7	1.2	7.8 ± 1.5 *(4.4 ± 0.7)*	12.9 ± 0.4	0.2
	Elevated CO_2	8.2 ± 1.2 *(4.9 ± 0.7)*	8.5 ± 2.0*	0.6	3.5 ± 0.7** *(2.6± 0.6)***	11.9 ± 2.1	0.1
September 2000	Ambient CO_2	16.9 ± 2.1 *(18.9 ± 2.1)*	4.7± 1.3	4.0	7.7 ± 0.5 *(8.7± 0.6)*	6.3 ± 0.3	0.7
	Elevated CO_2	6.1 ± 1.0*** *(3.3 ± 0.8)****	10.8 ± 0.3**	0.3	8.4 ± 2.1 *(7.9 ± 1.9)*	10.2 ± 2.0*	0.4
June 2001	Ambient CO_2				17.6 ± 2.2 *(24.1 ± 2.8)*	7.0 ± 0.8	5.6
	Elevated CO_2				20.2 ± 2.7 *(17.6 ± 2.4)*	17.4 ± 5.2**	1.3
September 2001	Ambient CO_2				5.9 ± 0.9 *(5.6 ± 1.1)*	10.1 ± 1.3	4.6
	Elevated CO_2				8.2 ± 1.7 *(6.4 ± 0.9)*	8.8 ± 1.6	2.7

579

50

580Tab. 2. Parameters estimated by linear regression analysis between the mean daily (24-hr) 581temperature of the sampling day (0) and that averaged over 1 to 15 days prior to midnight before the 582day of measurement and *actual* isoprene emissions of *Q. pubescens* plants grown under ambient 583CO_2 concentration at the control site and those grown under elevated CO_2 conditions at the 584Bossoleto site.

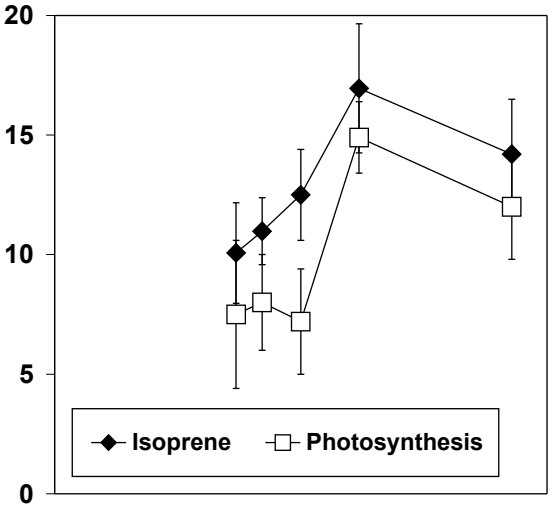
585

586

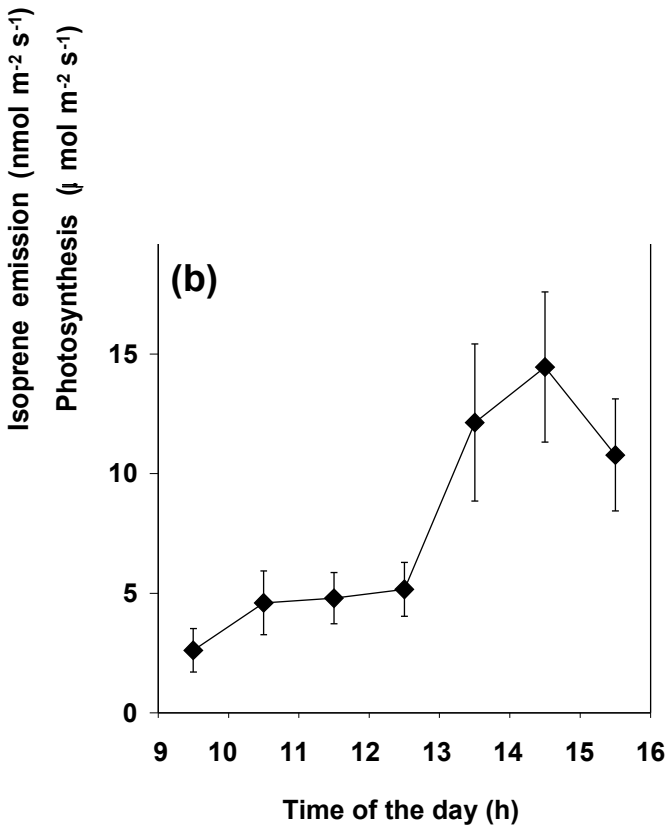
	N° of days	Intercept	*P*	Slope	r^2	*P*	*n*
Ambient CO_2	**0**	-20.66	0.294	1.53	0.33	0.135	8
	1	-36.35	0.113	2.37	0.49	0.055	8
	2	-34.53	0.090	2.32	0.53	0.041	8
	3	-38.64	0.054	2.57	0.61	0.023	8
	4	-37.84	0.062	2.56	0.59	0.027	8
	5	-38.72	0.061	2.63	0.59	0.027	8
	6	-38.61	0.073	2.64	0.56	0.030	8
	7	-39.55	0.064	2.70	0.58	0.029	8
	8	-43.24	0.033	2.89	0.66	0.014	8
	9	-45.50	0.018	3.02	0.73	0.007	8
	10	-47.03	0.011	3.11	0.76	0.005	8
	11	-49.54	0.009	3.27	0.78	0.004	8
	12	-50.49	0.006	3.33	0.81	0.002	8
	13	-45.33	0.005	3.06	0.82	0.002	8
	14	-40.95	0.005	2.82	0.83	0.002	8
	15	-36.85	0.011	2.59	0.79	0.003	8
Elevated CO_2	**0**	-13.69	0.716	1.15	0.07	0.070	8
	1	-38.22	0.397	2.43	0.18	0.296	8
	2	-39.94	0.327	2.57	0.23	0.233	8
	3	-50.45	0.209	2.16	0.32	0.144	8
	4	-51.95	0.192	3.27	0.34	0.132	8
	5	-51.84	0.204	3.30	0.32	0.141	8
	6	-47.71	0.260	3.10	0.27	0.188	8
	7	-50.98	0.228	3.28	0.30	0.161	8
	8	-56.06	0.178	3.55	0.35	0.124	8
	9	-60.52	0.135	3.79	0.40	0.092	8
	10	-61.52	0.127	3.86	0.41	0.086	8
	11	-65.77	0.110	4.12	0.44	0.075	8
	12	-71.96	0.070	4.48	0.52	0.046	8
	13	-68.27	0.051	4.28	0.56	0.032	8
	14	-62.45	0.048	3.97	0.58	0.029	8
	15	-56.44	0.065	3.63	0.54	0.038	8

587

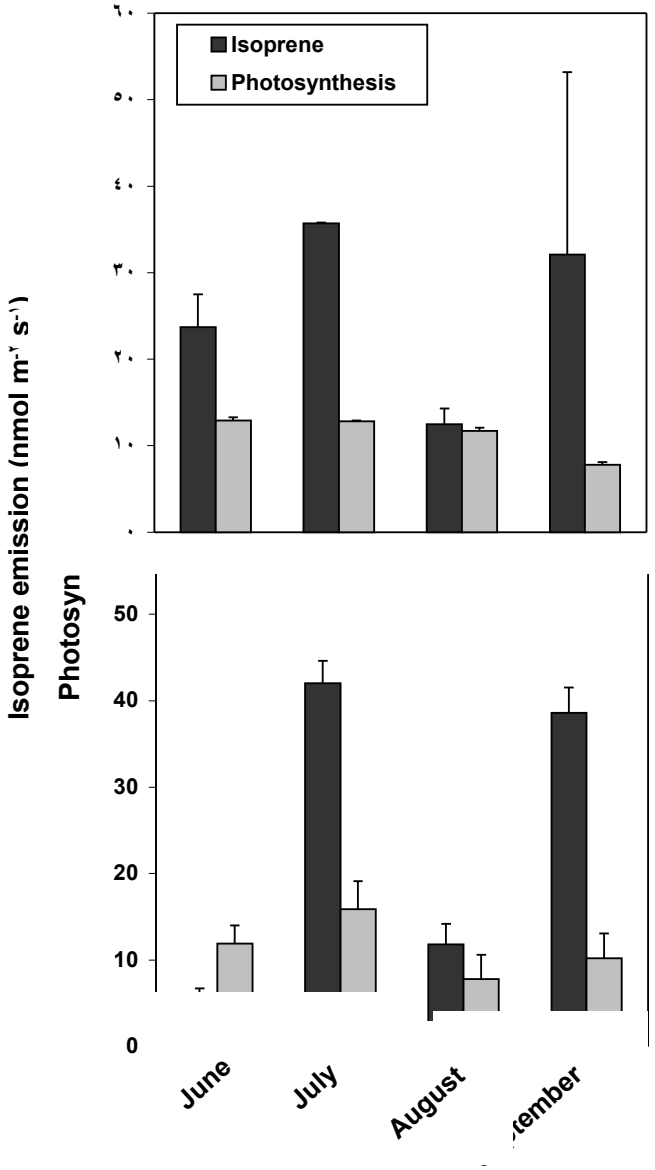
588



(a)



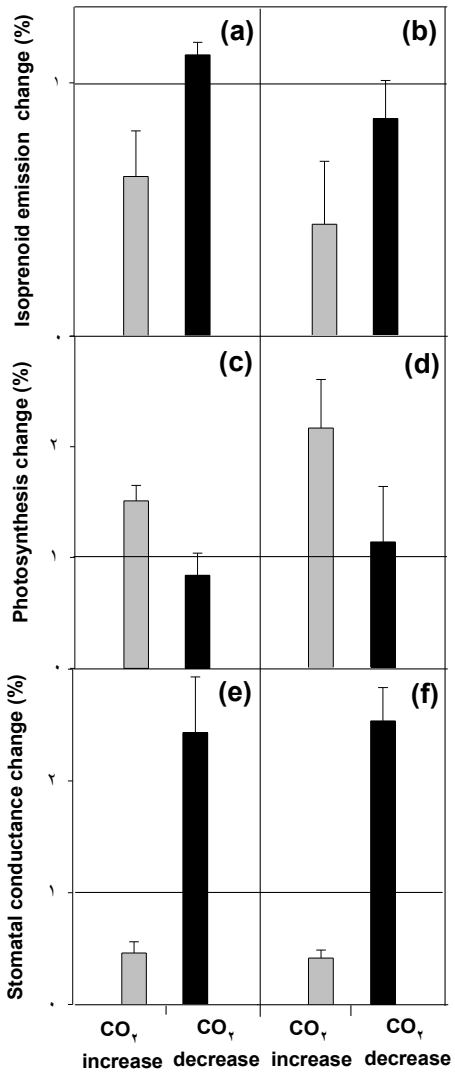
592Figure 1


Isoprene
Photosynthesis
Isoprene emission (nmol m-2 s-1)
Photosyn
50
40
30
20
10
0
June
July
August

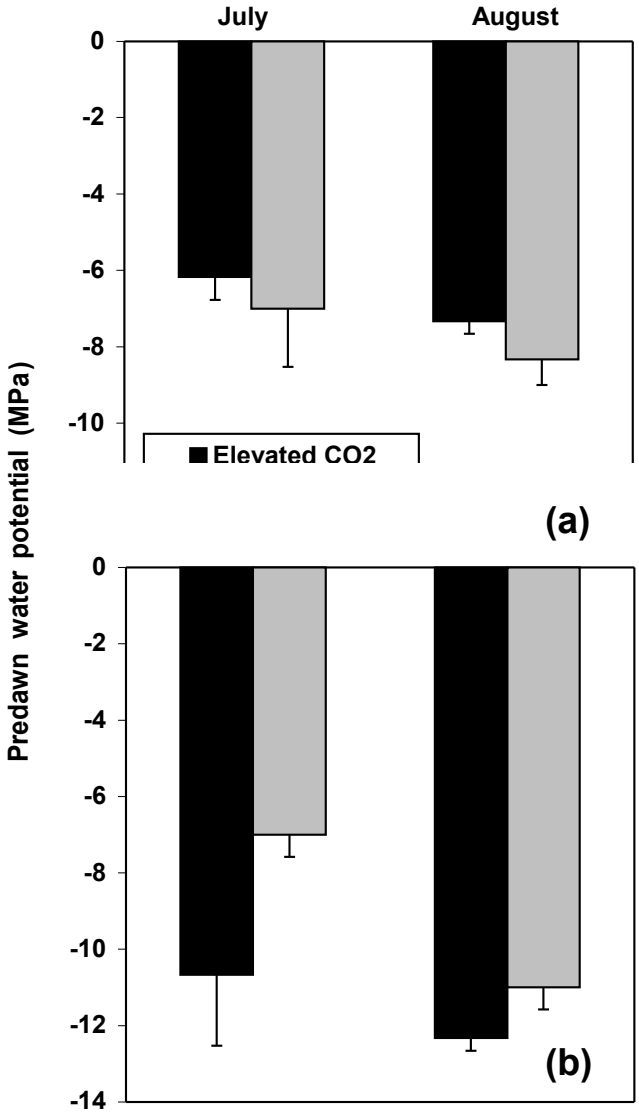
(b)

Figure 2

a

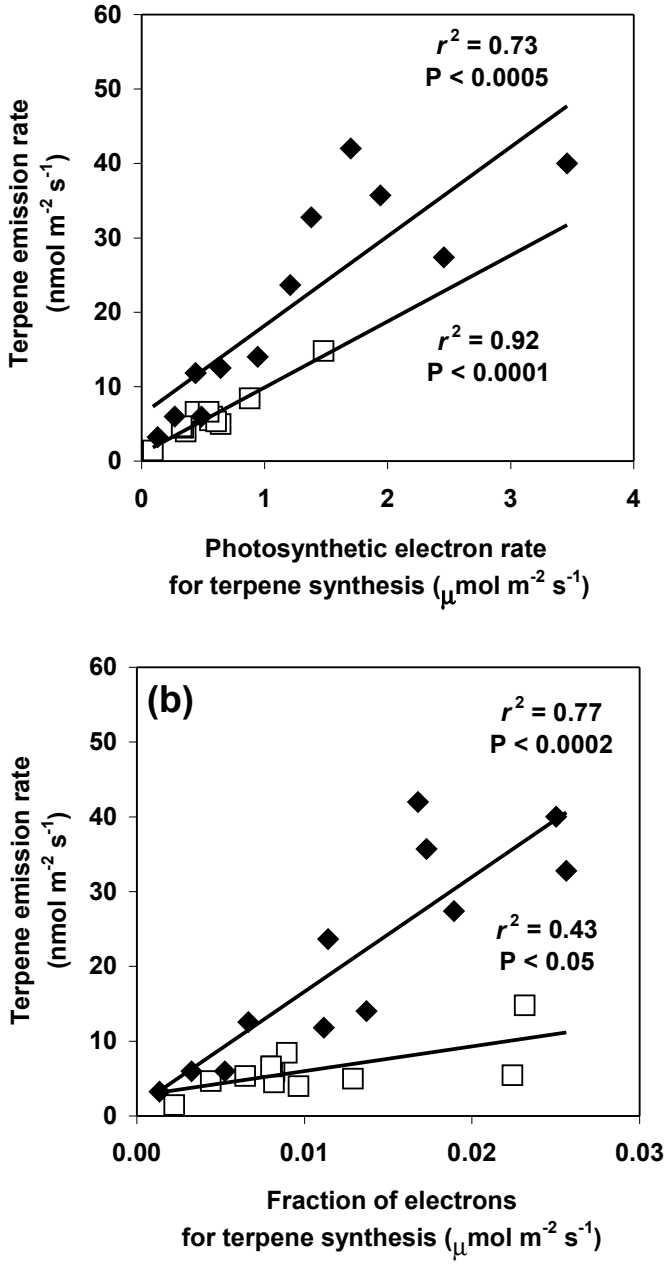


601
602Figure 3
603



608
609
610Figure 4

(a) (b)


r^2 = 0.73
P < 0.0005
r^2 = 0.92
P < 0.0001
Terpene emission rate
(nmol m^{-2} s^{-1})
Photosynthetic electron rate
for terpene synthesis (μmol m^{-2} s^{-1})
(b)
r^2 = 0.77
P < 0.0002
r^2 = 0.43
P < 0.05
Terpene emission rate
(nmol m^{-2} s^{-1})
Fraction of electrons
for terpene synthesis (μmol m^{-2} s^{-1})