Isoprenoid emission in trees of Quercus pubescens and Quercus ilex with lifetime exposure to naturally high CO₂ environment.

F. Rapparini¹, R. Baraldi¹, F. Miglietta² & F. Loreto³

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 – 1100016 Monterotondo Scalo (Roma), Italy.

Running title: Isoprenoid emissions from Quercus plants at natural CO₂ springs

Correspondence: Rita Baraldi, CNR- IBIMET - Istituto di Biometeorologia, via Gobetti 20101,– Bologna, Italy. Fax: +39-051-6399024; email: R.Baraldi@ibimet.cnr.it
Abstract

We investigated the long-term effect of elevated atmospheric CO₂ 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-CO₂ 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 μmol m⁻² s⁻¹. Long-term exposure to high atmospheric levels of CO₂ 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 CO₂ level by rapidly switching the CO₂ concentration in the gas-exchange cuvette, both isoprene and monoterpane basal emissions were clearly inhibited. These results generally confirm the inhibitory effect of elevated CO₂ on isoprenoid emission. The absence of CO₂ effect on actual emissions might indicate higher leaf temperature at elevated CO₂, or an interaction with multiple stresses some of which (e.g. recurrent droughts) may compensate for the CO₂ effect in Mediterranean ecosystems. Under elevated CO₂, 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 CO₂ 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 CO₂. This suggests that physiological algorithms may usefully predict isoprenoid emission also under rising CO₂ 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 µmol 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
Scholefield et al. (2003). $E_B$ was measured 30 min after switching $CO_2$, when $A$ and $g_s$ had reached a new steady-state.

At the branch level, the emission measured under actual environmental conditions, $E_A$, was measured by enclosing sun-exposed branches in translucent Teflon bags mounted on an aluminum frame 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 temperature inside the bag and the outside air temperature was around 2-3$^\circ$C. A quantum sensor mounted outside close to the top of the chamber measured PAR. Air samples were withdrawn from the Teflon lines by using an aspirating pump at a flow rate of 100-200 mL min$^{-1}$. Both inlet and outlet air flows were measured with mass-flow meters, and the data were stored using a data logger.

Both in the leaf and branch enclosures, compounds entering and leaving the bag were collected on two-stage traps consisting of glass tubes filled with 0.034 g of Carbograph 1 and 0.17 g of Carbograph 2 (Lara, Rome, Italy). Isoprenoids retained on carbon traps were thermodesorbed at 325°C and cryofocused at –150°C on a fused silica liner connected to a 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) and using a 5970 quadrupole mass spectrometer (Hewlett Packard) as detection system (Baraldi et al. 1999; Rapparini et al. 2001). Isoprenoids were transferred to a capillary column (60 m x 0.25 mm I.D.; 0.25 µm film of polymethylsiloxane; HP1, Hewlett Packard). The column was maintained at 40°C for 10 min and then heated to 220°C at 5°C min$^{-1}$. The identity of the compounds of interest was determined by comparison of their retention time and mass spectra to that of authentic standards. Quantification of isoprenoids was performed after calculation of standard curves and response factors for each compound, and using d$_{14}$-cymene as internal standards. The biogenic VOC emission rates were calculated by multiplying the concentration difference between the chamber’s inlet and outlet air with the air flow through the chamber divided by leaf area.

PAR and cuvette air temperature were measured while emission samples were taken. During each measuring 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):

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

Where $R_d$ is the rate of mitochondrial respiration continuing in the light (mmol m$^{-2}$ s$^{-1}$), $\Gamma^*$ is the CO$_2$ compensation point in the absence of $R_d$ ($\mu$ mol mol$^{-1}$, Laisk 1977), and $C_i$ is the intercellular CO$_2$ concentration ($\mu$ 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)

$$J_E = \frac{6E(4.67C_i + 9.33\Gamma^*)}{(C_i - \Gamma^*)}$$

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

for monoterpenes (Niinemets et al. 2002a)

$$J_E = \frac{12E(4C_i + 8\Gamma^*)}{(C_i - \Gamma^*)}$$
Where $\vartheta$ is the NADPH cost of monoterpenes (mol m$^{-1}$)

From Eqns 1-5, the fraction of photosynthetic electron transport used for isoprenoid emission, $\varepsilon$, is determined as the ratio between the rate of electron transport necessary to support the measured isoprenoid emission ($J_E$) and the total photosynthetic electron transport ($J_T$). $J_T$ is calculated from foliar photosynthesis and isoprenoid emission as $J$ (Eqn 1) + $J_e$ (Eqn 3 or 5).

Statistical analysis

One-way analysis of variance (ANOVA) was performed for all the parameters to test the effect of growth under different CO$_2$ level on physiological characteristics of $Q. ilex$ and $Q. pubescens$ plants for individual field campaign in 2000 and 2001. Regression analysis was used to detect the influence of temperature of the sampling day or averaged over a growing number of days before measurements, and of photosynthetic characteristic parameters calculated applying the Niinemets algorithm (1999, 2002a) on isoprenoid emission, determining if the parameters of the regression are significantly different from zero. All statistical analyses were performed in SAS (SAS System 8.1, SAS Institute Inc., Cary, NC, USA).

Results

In situ isoprenoid emission and physiological measurements

The profile of the emitted monoterpenes by $Q. ilex$ was similar at both branch and leaf level. The major emitted compounds were $\alpha$-pinene, $\beta$-pinene, sabinene and $\beta$-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 µmol m$^{-2}$ s$^{-1}$ and 1300 µmol m$^{-2}$ s$^{-1}$ in June and September field campaigns, respectively), and minimum values in the late afternoon (approximately 100-250 µmol 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 $Q. ilex$ monoterpenes emissions in September 2000 under ambient CO$_2$ were significantly higher than in the other months (Tab 1).

During the June 2000 field campaign, monoterpane $E_A$ 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 8.6 nmol m$^{-2}$ s$^{-1}$ under ambient CO$_2$. In September 2000, the monoterpane 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 m$^{-2}$ s$^{-1}$) than those determined under ambient CO$_2$ (7.8 nmol 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 monoterpane 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, 2b).

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, 3d) and g, 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, 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; Gerón 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, $\varepsilon$ ($r^2 = 0.77$ P < 0.0002 and $r^2 = 0.43$ P < 0.05 for *Q.*
pubescens and Q. ilex, respectively) (Fig. 5b). In both species, the intercepts were not statistically different from zero, but the slope was higher in Q. pubescens than in Q. ilex plant. We did not observe any significant effect of growth under different CO₂ conditions on this positive correlation (data not shown).
Exposure to or growth at elevated CO₂ 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 CO₂ 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 CO₂ induced in both Quercus species a rapid and clear inhibition of leaf-level isoprenoid emission, confirming the results obtained in Phragmites in the same CO₂ 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 CO₂, even after normalization, which indicates that both actual and basal emissions were not reduced by growth at elevated CO₂. 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 CO₂. Certainly the night temperature of the spring was higher than in the surrounding since CO₂ 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 CO₂
conditions, did not observe the inhibitory effect of high CO\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2} in our experiments.

We have shown that isoprene E\textsubscript{B} by \textit{Q. pubescens} is subjected to daily and seasonal variations.
Pronounced daily and seasonal variations of E\textsubscript{B} were observed by other authors (Fuentes \textit{et al.}
1995; Ciccioli \textit{et al.} 1997; Street \textit{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 \textit{et al.} 1999; Lehning \textit{et al.} 2001; Ciccioli \textit{et al.} 2003). It is clear that similar
correction factors should be used also when monitoring emission under elevated CO\textsubscript{2}. However, at
least seasonal variations of E\textsubscript{B} seems to be similar under ambient and elevated CO\textsubscript{2}. Rapid
temperature fluctuations (Singsaas \textit{et al.} 1999; Singsaas & Sharkey 2000), usually occurring in our
experimental conditions, and past temperatures experienced by the plants prior to measurements
(Guenther \textit{et al.} 1999; Sharkey \textit{et al.} 1999; Geron \textit{et al.} 2000; Hanson & Sharkey 2001; Pétron \textit{et
al.} 2001) could have influenced isoprenoid emissions, particularly under elevated CO\textsubscript{2}. Our
experiments have shown that isoprene E\textsubscript{A} in ambient CO\textsubscript{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 \textit{et
al.} 1999; Geron \textit{et al.} 2000; Hanson & Sharkey 2001) or similarly (Guenther \textit{et al.} 1999; Petron \textit{et
al.} 2001) long-lasting than previously reported. However, under elevated CO\textsubscript{2} E\textsubscript{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 $\epsilon$ (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 $\epsilon$ 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).

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This work is dedicated to the memory of Wolfgang Zimmer.

References


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


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 ($\varepsilon$) in leaves of *Q. pubescens* (closed diamond) and *Q. ilex* (open squares). Each point corresponds to a separate field campaign of measurements.
Tab. 1. Daily average actual emission ($E_A$) measured at branch level, photosynthesis ($A$) measured at leaf level from *Q. ilex* and *Q. pubescens* plants, and percent of photosynthetic carbon lost as isoprenoid during 2000 and 2001 field campaigns. The values shown in the parenthesis represent $E_B$ estimated using the equations of Guenther *et al.* (1993). Values are means ± standard error (S.E.), $n = 3-4$. * $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$.

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<tr>
<th></th>
<th><strong>Q. ilex</strong></th>
<th></th>
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<th><strong>Q. pubescens</strong></th>
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<td>$A$</td>
<td>% C</td>
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<td>% C</td>
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<td>(µmol m$^{-2}$ s$^{-1}$)</td>
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<td>(nmol m$^{-2}$ s$^{-1}$)</td>
<td>(µmol m$^{-2}$ s$^{-1}$)</td>
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<td>8.6 ± 1.7 (5.7 ± 1.4)</td>
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<td>8.2 ± 1.2 (4.9 ± 0.7)</td>
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<td></td>
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<td>(18.9 ± 2.1)</td>
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<td></td>
<td>16.9 ± 2.1 (3.3 ± 0.8)**</td>
<td>4.7 ± 1.3</td>
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<td>6.1 ± 1.0*** (3.3 ± 0.8)**</td>
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<td>5.9 ± 0.9 (5.6 ± 1.1)</td>
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<td>8.2 ± 1.7 (6.4 ± 0.9)</td>
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Tab. 2. Parameters estimated by linear regression analysis between the mean daily (24-hr) temperature of the sampling day (0) and that averaged over 1 to 15 days prior to midnight before the day of measurement and actual isoprene emissions of *Q. pubescens* plants grown under ambient CO$_2$ concentration at the control site and those grown under elevated CO$_2$ conditions at the Bossoleto site.

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<th>Slope</th>
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<th>$P$</th>
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| **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   |
Figure 1

(a) 

Isoprene emission (nmol m\(^{-2}\) s\(^{-1}\))

Photosynthesis (\(\mu\)mol m\(^{-2}\) s\(^{-1}\))

(b) 

Isoprene emission (nmol m\(^{-2}\) s\(^{-1}\))

Photosynthesis (\(\mu\)mol m\(^{-2}\) s\(^{-1}\))

Time of the day (h)

0 5 10 15

0 5 10 15 20

9 10 11 12 13 14 15 16

- Isoprene
- Photosynthesis
Figure 2

(a) Isoprene emission (nmol m$^{-2}$ s$^{-1}$)
(b) Photosynthesis (µmol m$^{-2}$ s$^{-1}$)

(b)
Figure 3
Figure 4

(a) Predawn water potential (MPa)

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(b) Predawn water potential (MPa)

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Figure 5

(a) Graph showing the relationship between photosynthetic electron rate for terpene synthesis ($\mu$ mol m$^{-2}$ s$^{-1}$) and terpene emission rate (nmol m$^{-2}$ s$^{-1}$), with the following statistics:

- $r^2 = 0.73$
- $P < 0.0005$

(b) Graph showing the relationship between the fraction of electrons for terpene synthesis ($\mu$ mol m$^{-2}$ s$^{-1}$) and terpene emission rate (nmol m$^{-2}$ s$^{-1}$), with the following statistics:

- $r^2 = 0.77$
- $P < 0.0002$

- $r^2 = 0.43$
- $P < 0.05$

- $r^2 = 0.92$
- $P < 0.0001$