Tree Physiology 21, 437–445

Effect of elevated CO_2 on monoterpene emission of young *Quercus ilex* trees and its relation to structural and ecophysiological parameters

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Received July 20, 2000

Summary We investigated growth, leaf monoterpene emission, gas exchange, leaf structure and leaf chemical composition of 1-year-old *Quercus ilex* L. seedlings grown in ambient (350 µl l^{-1}) and elevated (700 µl l^{-1}) CO_2 concentrations ($[CO_2]$). Monoterpene emission and gas exchange were determined at constant temperature and irradiance (25 °C and 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation) at an assay $[CO_2]$ of 350 or 700 µl l^{-1}. Measurements were made on intact shoots after the end of the growing season between mid-October and mid-February. On average, plants grown in elevated $[CO_2]$ had significantly increased foliage biomass (about 50%). Leaves in the elevated $[CO_2]$ treatment were significantly thicker and had significantly higher concentrations of cellulose and lignin and significantly lower concentrations of nitrogen and minerals than leaves in the ambient $[CO_2]$ treatment. Leaf dry matter density and leaf concentrations of starch, soluble sugars, lipids and hemi-cellulose were not significantly affected by growth in elevated $[CO_2]$. Monoterpene emissions of seedlings were significantly increased by elevated $[CO_2]$ but were insensitive to short-term changes in assay $[CO_2]$. On average, plants grown in elevated $[CO_2]$ had 1.8-fold higher monoterpene emissions irrespective of the assay $[CO_2]$. Conversely, assay $[CO_2]$ rapidly affected photosynthetic rate, but there was no apparent long-term acclimation of photosynthesis to growth in elevated $[CO_2]$. Regardless of growth $[CO_2]$, photosynthetic rates of all plants almost doubled when the assay $[CO_2]$ was switched from 350 to 700 µl l^{-1}. At the same assay $[CO_2]$, mean photosynthetic rates of seedlings in the two growth CO_2 treatments were similar. The percentage of assimilated carbon lost as monoterpenes was not significantly altered by CO_2 enrichment. Leaf emission rates were correlated with leaf thickness, leaf concentrations of cellulose, lignin and nitrogen, and total plant leaf area. In all plants, monoterpene emissions strongly declined during the winter independently of CO_2 treatment. The results are discussed in the context of the acquisition and allocation of resources by *Q. ilex* seedlings and evaluated in terms of emission predictions.

Keywords: carbon allocation, carbon dioxide enrichment, global change, growth, Holm oak, leaf structure, photosynthesis, volatile organic compound emission.

Introduction

A major goal in plant ecology is to assess the responses of vegetation to an increase in the concentration of atmospheric carbon dioxide concentration ($[CO_2]$), which is predicted to double within the next century (Houghton 1997). Much research has focused on primary plant metabolism and growth responses to increased $[CO_2]$, but comparatively little attention has been given to the production and emission of secondary metabolites. Among these metabolites, the volatile compounds, including isoprene and the monoterpenes, are the most common. The potential impact of increasing $[CO_2]$ on these emissions is intriguing, because it may trigger additional long-term impacts on the structure and function of ecosystems. For example, the chemical degradation of these volatiles in the atmosphere can influence climate, air quality and nutrient cycles through rainwater acidification and the formation of atmospheric photooxidants, aerosols, other greenhouse gases, and organic nitrogen compounds (Harley et al. 1999, Kesselmeier and Staudt 1999). Moreover, monoterpenes serve as carbon-based defensive compounds playing important roles in plant–parasite interactions (Langenheim 1994). An alteration of the plant's capacity to defend or to compete can be crucial in habitats where leaf growth is limited by environmental stresses such as drought. This is the case for Mediterranean vegetation, which is particularly rich in monoterpene-producing plants (Mabry and Difeo 1973, Ross and Sombrero 1991).

The pattern according to which resources in plants are allocated to the production of monoterpenes is the subject of ecological theories, namely the carbon–nutrient balance and growth–differentiation balance hypotheses (Lerdau and Gershenzon 1997, and references therein). These theories predict that when growth is more constrained than carbon assimilation, e.g., because of limited nitrogen availability, the excess photosynthates are allocated toward defense. Thus, plants developing in elevated $[CO_2]$ should increase their monoterpene production, especially when other sinks are limited. However, recent studies on conifers revealed no significant effects of elevated $[CO_2]$ on leaf monoterpene concentration or monoterpene emission (Kainulainen et al. 1998, Constable et al. 1999). This might be because monoterpenes in these species

are produced in highly specialized secretory organs, i.e., the resin ducts, whose construction and maintenance entail large metabolic costs beyond those of monoterpene synthesis alone (Lerdau and Gershenzon 1997).

In contrast, in deciduous species, isoprene is not accumulated in secretory organs, but is formed inside the chloroplasts of the leaf mesophyll. It is synthesized from recently fixed carbon in a light- and temperature-dependent process, whose potential ecological function is not well understood (Fall and Wildermuth 1998). The only study dealing with the effect of growth in elevated [CO_2] on isoprene emissions has been conducted by Sharkey et al. (1991) on two deciduous tree species. Their results showed no consistent pattern. They found that growth in elevated [CO_2] increased isoprene emission in *Quercus rubra* L. and decreased isoprene emission in *Populus tremuloides* Michx.

We report on leaf emissions from the Mediterranean evergreen oak *Quercus ilex* L., which produces and releases leaf monoterpenes in a manner similar to that of isoprene (Kesselmeier et al. 1996, Loreto et al. 1996, Staudt and Bertin 1998). Our specific objectives were to: (i) determine the effect of elevated [CO_2] on the monoterpene emission capacity of *Q. ilex,* and (ii) explore the extent to which effects of [CO_2] on the emission capacity could be related to photosynthesis, growth, structure and the chemical composition of leaves.

Materials and methods

Plant material and experimental protocol

We studied 20 *Quercus ilex* seedlings of the same geographic origin (Department of Gard, southern France). In January 1998, acorns were planted in 16 × 120-cm tall PVC pipes. The pots were filled with a substrate consisting of 1 part clay and silt, 1 part sand and gravel and 1 part organic matter with a 10-cm layer of gravel above the perforated bottom to facilitate drainage. At the top, a 2-cm mulch of sand limited soil evaporation. After germination, the seedlings were randomly divided into two groups and transferred to two compartments (6.4 × 6.0 × 4.1 m) of a controlled-environment greenhouse at the CEFE-CNRS Montpellier, France. One compartment was supplied with an atmospheric [CO_2] of 350 µl l^{-1} (ambient) and the other with a [CO_2] of 700 µl l^{-1} (elevated). Temperature and vapor pressure deficit (VPD) were set to track outside ambient conditions. Plants received natural sunlight and were watered every other week with 200 ml of water. The pots were periodically repositioned to prevent shading and to minimize position effects. Differences in environmental conditions (temperature, VPD, global radiation) between the compartments were less than 5%. Growth started in early spring 1998 and continued throughout the summer. Measurements began in October 1998 after a 10-month exposure to the CO_2 treatments when leaf growth had stopped and the youngest leaves were mature. Plants remained in the greenhouse throughout the experiment except during measurements of monoterpene emission and gas exchange, which were made in a laboratory close to the greenhouse. A measurement took 1–2 h, and immediately after it, the plant was returned to the greenhouse.

Measurements were made on intact shoots in an environmentally controlled gas exchange chamber. On each plant, one lateral shoot at mid-height was randomly chosen for repeated measurements throughout the experiment (hereafter referred to as the test shoot). Test shoots were prepared by protecting the petiole at the chamber insertion point with Teflon tape. On some shoots a leaf had to be removed so that the shoot fitted into the chamber. On average, the test shoots had about 10 mature terminal leaves. Measurements were made during three consecutive measuring campaigns over a 4-month period: mid-October to mid-November (Oct–Nov), early to late December, and mid-January to mid-February (Jan–Feb).

During all campaigns, emission and gas exchange of the test shoots were repeatedly measured at 25 °C, with an irradiance of 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR), and a VPD of 1.0 ± 0.3 kPa. Shoots were acclimatized to these conditions for about 80 min before measurement. During the first and second campaigns, measurements were made only at ambient [CO_2] (367 ± 30 µl l^{-1} inside the chamber). During the third campaign (Jan–Feb) measurements were made at both the ambient and the elevated [CO_2]. Mean chamber [CO_2]s were 342 ± 36 µl l^{-1} and 717 ± 47 µl l^{-1}, respectively. Hereafter, growth [CO_2] refers to the [CO_2] in the greenhouses, and assay [CO_2] indicates the [CO_2] to which test shoots were exposed during measurement in the laboratory. Monoterpene emission rates obtained under standard light and temperature conditions (25 °C and 1000 µmol m^{-2} s^{-1} PAR) are hereafter referred to as measures of emission capacity.

During each campaign, the emission capacity of every plant was determined at the same assay [CO_2] on three different days. Every plant in both [CO_2] treatments was assayed once within 4 to 5 days in an order alternating the treatments. The order was reversed during the second and again during the third measurement period. A measurement period extended over less than 3 weeks. We also investigated within-tree variability (branch-to-branch variability on 3–5 shoots of four plants).

Monoterpene emission and gas exchange

The gas exchange chamber comprised a Teflon-coated cylinder, 100 mm in diameter and 30 mm high, with the lower part inserted in a temperature-controlled metal housing. Chamber air was thoroughly mixed by a fan. The chamber was flushed with pressurized air at controlled flow rates, usually 500 ml min^{-1} (Mass Flow Controller 5850, Brooks Instrument, Hatfield, PA). Water and CO_2 were removed by filters and re-introduced by means of a humidifier and by injection of pure gaseous CO_2. A light source with a water-cooled glass filter provided a range of PAR values up to 2000 µmol m^{-2} s^{-1} at chamber level. Leaf temperature was measured with two thermocouples and irradiance was measured with a modified photodiode calibrated against a quantum sensor (PAR-SB 190, Li-Cor Inc., Lincoln, NE). The CO_2 mixing ratio and relative humidity of the air entering and leaving the chamber were

measured by an infrared gas analyzer and a thermo-hygrometer, respectively (ADC Bioscientific Ltd., Hoddesdon, England). Transpiration, photosynthesis (net CO_2 assimilation) and stomatal conductance for water vapor were calculated according to von Caemmerer and Farquhar (1981).

Monoterpenes were sampled by passing 500 ml of chamber air at 100 ml min^{-1} through glass tubes filled with about 200 mg of Tenax TA (20–35 mesh, Chrompack, Varian Inc., Palo Alto, CA). Samples were analyzed in a Chrompack gas chromatograph (CP9003) fitted with a fused silica capillary column (CP-Sil 8 CB, 25 m × 0.32 mm, 1.2 mm d.f., Chrompack), a flame ionization detector (FID) and a thermal desorption and cold trap injection unit. The unit consisted of an oven for thermal desorption of the Tenax tubes, a glass liner with an injection port for introduction and evaporation of liquid samples, and a capillary sample loop placed in a stainless steel tube that was cooled by liquid nitrogen and flash heated by the hot-wire method. A custom-built, programmable, 4-channel temperature controller included one channel for flash-heating control, and a 6-2 Valco valve and transfer line into the GC oven to maintain the temperature of 150 °C. Measurements were calibrated by injecting an aliquot (usually 1 µl) of freshly prepared monoterpene solution (pure monoterpene standards (Fluka, Sigma-Aldrich Corp., St. Louis, MO) dissolved in MeOH) in the glass liner and evaporating it on a clean Tenax tube with 300 ml of N_2 (quality C, flow rate 30 ml min^{-1}). The sample was then analyzed as usual. The accuracy of the emission measurement was tested by replicating the standard analysis (coefficient of variation = 4.1%, $n = 7$). There was no significant difference in the FID-responses among the individual monoterpenes.

Emission rate was calculated as the difference between the air concentration in the chamber enclosing a shoot and the concentration measured in the empty chamber multiplied by airflow. Emissions and CO_2 and H_2O gas exchange rates were expressed on the basis of projected leaf area (ng m^{-2} s^{-1}) and leaf dry mass (µg g^{-1} h^{-1}). Emission rates were related to photosynthetic rates measured simultaneously to calculate the percentage of recently fixed carbon that was lost by monoterpene production.

Leaf structure and chemistry and plant biomass

Total leaf area of each plant was assessed by regression between leaf length and leaf width measured on every leaf, assuming an ellipsoidal shape ($r^2 = 0.99$, $n = 46$ leaves, leaf area ranging from 2 to 10 cm^2). The total volume of stem and branches (axial organs) of each plant was calculated from lengths and mean diameters, assuming a cylindrical shape. Mean diameter of a branch (or stem) was averaged from three measurements made close to the base, close to the tip and in the middle of the branch. At the end of the third campaign, the test shoots of each plant were collected to determine leaf dry mass (LDM), leaf thickness (LT) and projected leaf area (LA). The results were used to calculate mean leaf mass per area (LMA, g m^{-2}), leaf volume (LV, cm^3) and leaf dry matter density (DMD, kg m^{-3}). Leaf area was determined with a video leaf area meter (Delta-T Area Meter MK2, Delta-T Devices, Ltd., Cambridge, U.K.). Leaf dry mass was determined after drying for 48 h in a ventilated oven at 60 °C. Leaf thickness was measured at five points per leaf lamina with a linear displacement transducer (LVDT model L5R, Ifélec, Paris, France). The instrument was calibrated daily with metal plates of known thickness. The signal was strongly linear ($r^2 > 0.99$) between 0.1 and 0.5 mm.

After drying, leaves of the test shoots were milled (Cyclotec 1093 Sample Mill, Tecator, Höganäs, Sweden). The samples were scanned with a near-infrared reflectance spectrophotometer (NIRSystems 6500, Foss NIRSystems, Inc., Silver Spring, MD) following the procedure described by Joffre et al. (1992). Leaf concentrations of nitrogen (N), total ash (TA), phosphorus (P), cellulose (Cellu), hemi-cellulose (Hemi), lignin, starch and soluble sugars were determined based on calibration equations built on the spectral and wet chemical database of *Quercus* spp. collected throughout the French Mediterranean area (Meuret et al. 1993, Damesin et al. 1997). The leaf chemical data were used to calculate: total non-structural carbohydrates (TNCs = starch + sugars), leaf sclerophyll index (Sclero = (Hemi + Cellu + Lignin)/N ratio), and starch/N ratio. The starch/N ratio is considered a key parameter in predicting carbon allocation in plants: a high ratio indicates an accumulation of carbon as a result of growth limitations, and consequently favors the production of carbon-based defensive compounds.

Statistical analysis

The influences of season (campaign) and growth [CO_2] on emission capacity and gas exchange were evaluated by two-way ANOVA (SigmaStat 2.0, SPSS Inc., Chicago, IL). To avoid pseudo-replication, we used the means of the three replicate measurements made on the same leaves during each campaign. The influence of growth [CO_2] and assay [CO_2] (campaign January–February) on emission capacity and gas exchange was evaluated by two-way ANOVA. When ANOVA was significant ($P < 0.05$), the Tukey test was applied to determine differences among treatments. The ANOVA was applied on ranked data when tests for normality and equal variances failed. A two-tailed student *t*-test was used to examine effects of growth [CO_2] on plant growth parameters, leaf structure and chemical composition. When tests for normality and equal variances failed, the Mann-Whitney Rank Sum Test was applied.

Correlations between emission capacity and photosynthesis, plant growth parameters, leaf structure and chemical composition of all plants ($n = 20$) were made with Pearson Product Moment Correlation test (SigmaStat 2.0). In addition, scatter charts were made to check the data distribution for nonlinear relationships and for differences between the CO_2 treatments.

Results

Leaf structure and chemistry of test shoots

Table 1 summarizes structural properties and chemical composition of the leaves of the test shoots. Elevated [CO_2] signif-

icantly increased leaf thickness by about 10%. Mean LMA and mean leaf size were increased about 5%, but differences between CO_2 treatments were not significant. Mean DMD of seedlings was similar in both CO_2 treatments. Among the nine chemical constituents analyzed, five were significantly affected by CO_2 treatment. Growth at elevated [CO_2] significantly increased cellulose and lignin concentration and significantly decreased nitrogen, total ash and phosphorus concentration. Among the derived parameters, mean sclerophyll index and starch/N ratio were significantly increased in response to elevated [CO_2].

Leaf chemical composition was also expressed on a Cellu + Lignin-free basis to check whether the concentrations of other compounds changed independently of the quantitatively most important change in leaf chemical composition. When concentrations were calculated on a Cellu + Lignin-free basis, the difference in phosphorus became insignificant ($P = 0.106$, $n = 10$), whereas total ash and nitrogen concentrations were still significantly lower in seedlings grown in elevated [CO_2] compared with ambient [CO_2] ($P = 0.024$ and 0.017, $n = 10$). Because LMA increased in seedlings grown in elevated [CO_2], differences in nitrogen, total ash and phosphorus concentrations were not significant when expressed on a leaf area basis. There were no correlations among leaf structural and leaf chemical parameters except for a positive correlation between leaf thickness and LMA ($r^2 = 0.36$, $P = 0.005$, $n = 20$), and between leaf thickness and cellulose concentration ($r^2 = 0.33$, $P = 0.010$, $n = 20$).

Table 1. Structural and chemical properties of leaves of test shoots from *Q. ilex* seedlings grown in ambient (350 µl l^{-1}) and elevated (700 µl l^{-1}) [CO_2] regimes. Values are means ± standard deviation of 10 plants per treatment. Abbreviations: LT = leaf thickness, LMA = leaf mass per area, DMD = leaf dry matter density. Leaf chemical composition determined by NIRS is expressed as percentage of leaf dry weight. Abbreviations: N = nitrogen, TA = total ash, Hemi = hemicellulose, Cellu = cellulose, P = phosphorus, TNCs = total nonstructural carbohydrates (i.e., starch + sugars), Sclero = leaf sclerophyll index (i.e., (Hemi + Cellu + Lignin)/N ratio), starch/N = starch/nitrogen ratio. Means were tested for effects of [CO_2] treatment and differences were considered significant when $P < 0.05$.

Parameter	[CO_2] (µl l^{-1})		*P*
	350	700	
Leaf size (cm^2)	3.8 ± 1.4	4.5 ± 1.7	0.361
LT (mm)	0.30 ± 0.02	0.33 ± 0.02	0.018
LMA (g m^{-2})	188 ± 19	198 ± 17	0.215
DMD (kg m^{-3})	626 ± 33	601 ± 59	0.257
N	1.74 ± 0.21	1.51 ± 0.08	0.013
TA	4.7± 0.87	3.7 ± 0.61	0.012
Hemi	19.9 ± 0.75	19.5 ± 0.48	0.158
Cellu	21.9 ± 1.16	23.3 ± 0.73	0.009
Lignin	15.6 ± 1.21	17.1 ± 0.75	0.011
Starch	8.5 ± 0.65	8.8 ± 0.78	0.325
Sugars	11.8 ± 0.88	11.4 ± 1.09	0.421
Lipids	13.4 ± 1.39	13.7 ± 1.07	0.582
P	0.16 ± 0.01	0.15 ± 0.01	0.022
TNCs	20.2 ± 1.0	20.2 ± 1.2	0.939
Sclero	33.6 ± 4.8	39.8 ± 3.0	0.004
Starch/N	5.0 ± 0.61	5.8 ± 0.57	0.004

Plant growth and morphology

Although individual plants in both treatments varied considerably in morphological properties such as height, ramification pattern, color of bark and size and form of leaf lamina, mean tree growth was significantly enhanced in elevated [CO_2]. In response to CO_2 enrichment, leaf area was increased 40%, and leaf dry mass and leaf volume were increased about 50% (Table 2). Total leaf number per plant was also significantly higher in the elevated [CO_2] treatment than in the ambient [CO_2] treatment, whereas mean leaf size was similar in both CO_2 treatments. In response to elevated [CO_2], branches were significantly longer and thicker, but there were no more branches per plant in the elevated [CO_2] treatment than in the ambient [CO_2] treatment. Total volume of axial organs, estimated from length and mean diameter of stem and shoots, indicated that biomass of axial organs increased 90% in response to CO_2 enrichment.

Regression analyses between plant growth parameters (Table 2) and structural or chemical properties of test shoots (Table 1) indicated positive relationships between total leaf area and cellulose ($r^2 = 0.32$, $P = 0.01$, $n = 20$), lignin ($r^2 = 0.36$, $P = 0.005$, $n = 20$), sclerophyll index ($r^2 = 0.39$, $P = 0.003$, $n = 20$) and leaf thickness ($r^2 = 0.29$, $P = 0.02$, $n = 20$); and negative relationships with nitrogen ($r^2 = 0.33$, $P = 0.009$, $n = 20$) and phosphorus ($r^2 = 0.24$, $P = 0.03$, $n = 20$). Thus, plants with more foliage tended to have thicker and more sclerophyllous leaves than plants with less foliage.

Monoterpene emission and gas exchange

All test shoots emitted the same monoterpenes including α-pinene, sabinene, β-pinene, myrcene and limonene, which

Table 2. Growth parameters of *Q. ilex* seedlings after 10 months in ambient (350 µl l^{-1}) and elevated (700 µl l^{-1}) [CO_2] regimes. Values are means ± standard deviations of 10 plants per treatment. *P*-Values indicate significance of [CO_2] treatment effect ($P < 0.05$).

Parameter	[CO_2] (µl l^{-1})		*P*
	350	700	
Plant height (cm)	46 ± 7.6	62 ± 16.6	0.026
Stem diameter (mm)	3.5 ± 0.3	4.4 ± 0.7	0.005
No. of branches	6.1 ± 3	8.9 ± 4	0.100
Total length of axis (cm)	99 ± 16	151 ± 46	0.005
Total volume of axis[1] (cm^3)	5.7 ± 1.0	12.1 ± 5.5	<0.001
No. of leaves	118 ± 27	155 ± 44	0.035
Leaf size (cm^2)	5.3 ± 1.4	6.0 ± 2.2	0.405
Total leaf area (cm^2)	620 ± 129	855 ± 161	0.002
Total leaf dry mass[2](g)	11.1 ± 1.7	16.9 ± 3.5	<0.001
Total leaf volume[2] (cm^3)	17.8 ± 2.8	28.2 ± 6.3	<0.001

[1] Calculated from lengths and diameters of stem and branches.
[2] Estimated from total leaf areas and LMAs and leaf thickness of test shoots (Table 1).

accounted for about 90% of the total terpene release. Trace emissions were detected for α-thujene, camphene, μ-terpinene, *p*-cymene, cis- and trans- β-ocimene, and one compound tentatively identified as 1,8-cineol. Here, only the major compounds are considered further.

Between-branch variability in leaf emission capacity determined during the December campaign was slightly higher than the mean variability of the three replicate measurements made on the same shoot of the same plants (coefficient of variation of 34 versus 30%, $n = 4$). The coefficient of variation for leaf emission capacity during the December campaign was 67 and 36% for the ambient and elevated [CO_2] treatments, respectively ($n = 10$). Between-tree variability was always higher in the ambient [CO_2] treatment than in the elevated [CO_2] treatment (see also Table 3 and Figure 1).

Table 3 summarizes mean emission capacities, photosynthesis, carbon loss and total emissions of plants in the two CO_2 treatments measured at ambient [CO_2] during the first two campaigns (Oct–Nov and December), and measured at both ambient and elevated [CO_2] during the Jan–Feb campaign. Table 4 summarizes the results of two-way ANOVAs of the effects of growth [CO_2] and campaign, and on the effects of growth [CO_2] and assay [CO_2] for the Jan–Feb campaign. Mean emission capacities of seedlings in both treatments significantly declined during the season; decreasing by a factor of about 5 from Oct–Nov to December and by a factor of 2 from December to Jan–Feb. Photosynthetic rates decreased by 30% between the first and second campaigns and then remained rather stable. As a result, carbon losses were highest in the first campaign and lowest in the third campaign. Transpiration rates and stomatal conductance were not significantly different between campaigns (data not shown). There was no significant correlation between emission capacity and photosynthetic rate in any of the campaigns.

Mean emission capacities and total emission per plant significantly increased in response to elevated [CO_2]. On average, plants grown in elevated [CO_2] had 1.8-fold higher emission capacities and released 2.8-fold more monoterpenes per plant than plants grown in ambient [CO_2]. The differences between CO_2 treatments tended to increase with campaign, although the interaction between CO_2 treatment and campaign was not significant (Table 4).

In both CO_2 treatments, changing the assay [CO_2] between 350 and 700 μl l^{-1} had no short-term effect on emission capacity, but it rapidly influenced photosynthesis. When assay [CO_2] was switched from 350 to 700 μl l^{-1}, photosynthesis increased 1.9-fold within 1 h, whereas emission capacity remained unchanged (Table 3, Jan–Feb campaign). When measured at the same assay [CO_2], mean photosynthesis was not significantly different between the two growth [CO_2] treatments (Tables 3 and 4). Differences in photosynthesis between treatments were significant only when the seedlings were assayed at their respective growth [CO_2]. Mean carbon losses assayed at the respective growth [CO_2] were not significantly different between the growth CO_2 treatments. Thus, exposure to elevated [CO_2] exerted a short-term effect but no long-term effect on photosynthesis. Conversely, elevated [CO_2] had no short-term effect on monoterpene emission capacity, but a long-term effect was observed.

Correlation of emission capacity with growth, leaf structural and chemical parameters

Regression analyses of pooled data of growth [CO_2] treatments were based on the mean emission capacity of each plant

Table 3. Monoterpene emissions and photosynthesis of *Q. ilex* seedlings grown in ambient (350 μl l^{-1}) and elevated (700 μl l^{-1}) [CO_2]s. Measurements were made in three consecutive measuring campaigns over 4 months: Oct–Nov, December and Jan–Feb. During each campaign, emission and photosynthesis were repeatedly measured at the same temperature and light conditions of 25 °C and 1000 μmol m^{-2} s^{-1} PAR. In the first and second campaigns, all measurements were made at the same assay [CO_2] of 350 μl l^{-1} (= ambient growth [CO_2]). In the third campaign, measurements were made at an assay [CO_2] of 350 μl l^{-1} (= ambient growth [CO_2]) and 700 μl l^{-1} (= elevated growth [CO_2]). Values are means ± standard deviation of $n = 10$ plants per treatment with three replications per plant and campaign. Parameter carbon loss is the percentage of assimilated carbon used for monoterpene emission. Emission per plant was calculated by multiplying emission capacity of each plant by its total plant leaf area.

	Growth [CO_2] 350 μ l^{-1}				Growth [CO_2] 700 μ l^{-1}			
	Campaign/Assay [CO_2]				Campaign/Assay [CO_2]			
	Oct–Nov/ 350	Dec/ 350	Jan–Feb/ 350	Jan–Feb/ 700	Oct–Nov/ 350	Dec/ 350	Jan–Feb/ 350	Jan–Feb/ 700
Monoterpene emission								
ng m^{-2} LA s^{-1}	288 ± 142	56 ± 38	22 ± 27	22 ± 26	402 ± 117	94 ± 34	53 ± 31	49 ± 29
μg g^{-1} LDM h^{-1}	5.5 ± 2.4	1.0 ± 0.6	0.4 ± 0.4	0.4 ± 0.4	7.3 ± 1.9	1.7 ± 0.5	1.0 ± 0.6	0.9 ± 0.5
Emission per plant								
ng $plant^{-1}$ s^{-1}	18.0 ± 10.8	3.2 ± 2.1	1.3 ± 1.5	1.2 ± 1.5	34.7 ± 13.1	8.1 ± 3.5	4.9 ± 3.7	4.6 ± 3.5
Photosynthesis								
μmol m^{-2} LA s^{-1}	7.8 ± 2.1	5.4 ± 2.4	5.5 ± 2.6	9.9 ± 3.5	7.2 ± 1.9	5.1 ± 2.0	5.2 ± 2.1	10.0 ± 3.0
Carbon loss								
%	0.28 ± 0.11	0.07 ± 0.04	0.03 ± 0.03	–	–	–	–	0.04 ± 0.02

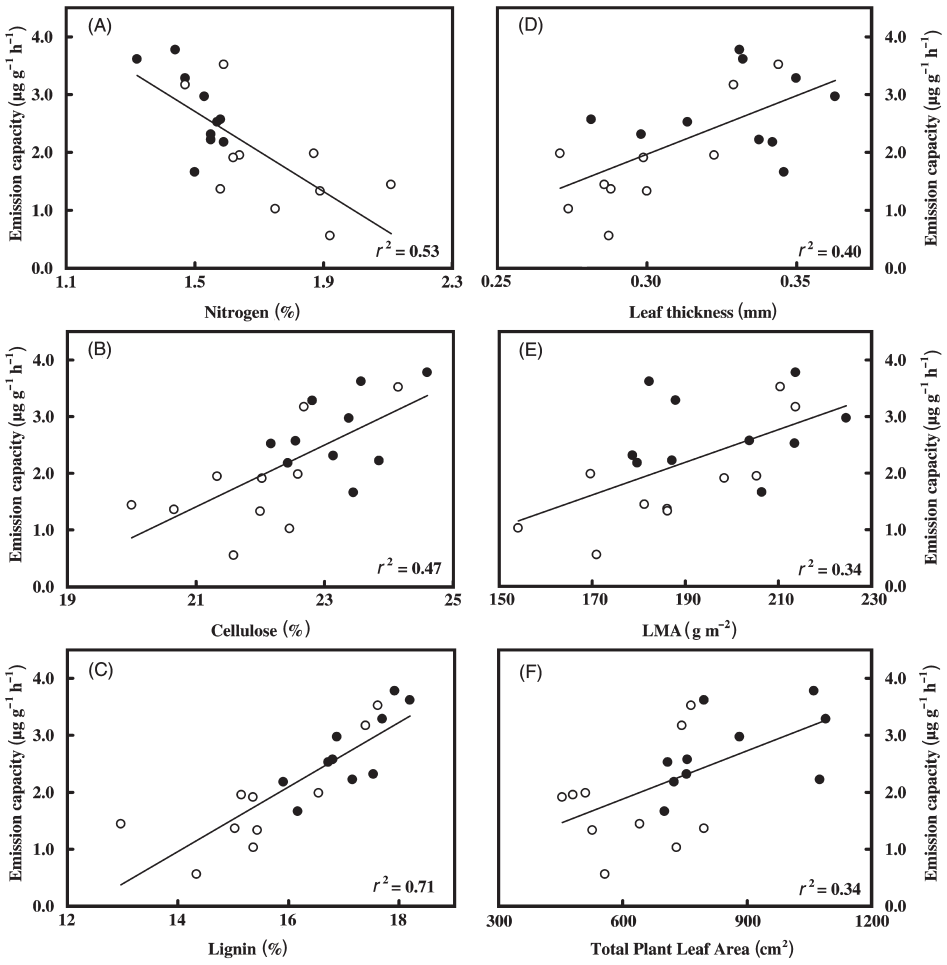

Figure 1. Monoterpene emission capacity versus leaf concentrations of nitrogen (A), cellulose (B) and lignin (C), leaf thickness (D), leaf dry mass per area (E) and total plant leaf area (F) of *Q. ilex* seedlings grown in ambient (○) and elevated (●) [CO_2]. Emission capacities are mean values measured in three campaigns (see Table 3). All correlations are statistically significant ($P < 0.05$) assuming a linear relationship.

($n = 20$), on a leaf dry weight basis for all campaigns (Figure 1). Additional analyses were run on emission capacities expressed on a leaf area basis as well as on carbon losses (i.e., emission capacity related to photosynthesis). Changing the unit of expression slightly changed the correlation coefficients, but rarely changed their significance levels.

Among the investigated plant morphological and leaf structural properties, there were significant positive correlations between emission capacity and leaf thickness, LMA and total plant leaf area (or total plant leaf dry mass) (Figure 1). The latter relationship was more significant for carbon losses than for emission capacities ($r^2 = 0.58$ versus $r^2 = 0.34$, $n = 20$). There was no significant correlation with DMD, leaf size, leaf density or other morphological properties.

Regression analyses between emission capacity and leaf chemical constituents yielded a negative correlation in the case of nitrogen and positive correlations in the case of cellulose, lignin (Figure 1) and the derivative sclerophyll index ($r^2 = 0.666$, $P < 0.001$, $n = 20$). Emission capacity was not correlated with leaf concentrations of total ash, hemi-cellulose,

Table 4. The *P*-values of two 2-way ANOVAs on the effects of growth [CO_2] and season (campaign), and on the effects of growth [CO_2] and assay [CO_2] during the Jan–Feb campaign (see Table 3) on monoterpene emission, photosynthesis and net carbon loss in *Q. ilex*.

	Emission capacity[1]	Emission per plant	Photosynthesis	Carbon loss
Growth [CO_2]	0.010	< 0.001	0.637	
Campaign	< 0.001	< 0.001	0.003	< 0.001
Growth [CO_2] × Campaign	0.500	0.551	0.976	
Growth [CO_2]	0.004	< 0.001	0.948	0.460
Assay [CO_2]	0.814	0.877	< 0.001	
Growth [CO_2] × Assay [CO_2]	0.830	0.837	0.853	

[1] Significance levels for emission were the same when expressed on a leaf dry weight or a leaf area basis.

lipids, phosphorus, starch, soluble sugars, TNCs or starch/N ratio. Use of carbon loss instead of emission capacity did not improve correlations with any of the chemical constituents or derivatives. The data distribution suggested that some relationships were nonlinear, and correlation coefficients were improved when an exponential instead of a linear function was applied.

Discussion

The observed changes in leaf structure and plant morphology in response to elevated [CO_2] are consistent with earlier results summarized by Poorter (1993), Gunderson and Wullschleger (1994) and Pritchard et al. (1999), and especially for oak species by Norby (1996). Increased foliage biomass, elongation and thickening of stems and shoots, and increased leaf thickness and high LMA (Tables 1 and 2) are common morphological responses to elevated atmospheric [CO_2]. Plants grown in elevated [CO_2] often exhibit an accumulation of foliar TNCs (i.e., starch and sugars), resulting in increased LMA independently of changes in leaf thickness. Frequently, this accumulation of TNCs is accompanied by a decrease in leaf nitrogen (hence an increase in starch/N ratio) and a reduction in photosynthetic capacity, commonly referred to as down-regulation (e.g., Harley 1995, Poorter et al. 1997, Tjoelker et al. 1998, Wolfe et al. 1998, Pritchard et al. 1999). Photosynthetic down-regulation in leaves grown in elevated [CO_2] is often discussed in terms of limitations in the utilization of leaf carbohydrates by various sinks. In general, down-regulation occurs faster in fast-growing species than in slow-growing species. It is promoted by experimental conditions that limit the sink capacity of plants during growth in elevated [CO_2], such as the use of small pots (e.g., Gunderson and Wullschleger 1994, Norby 1996, Hättenschwiler et al. 1997, Kainulainen et al. 1998, Tjoelker et al. 1998, Pritchard et al. 1999). In our study, sink limitation was minimized by the use of large pots and the gas exchange measurements made over a 4-month period did not display photosynthetic down-regulation in response to growth in elevated [CO_2]. The increase in LMA of seedlings grown in elevated [CO_2] was mainly a result of an increase in leaf thickness, because mean DMDs of seedlings in the two CO_2 treatments were similar, and there was no significant accumulation of TNCs in leaves of seedlings in the elevated [CO_2] treatment (Table 1). Thus, it seems that *Q. ilex* leaves grown in elevated [CO_2] were able to maintain their carbon balance. The extra carbon gained during the exposure period must have been utilized in sink functions, such as leaf and root growth, secondary growth of stems, accumulation of reserve or production of secondary metabolites such as monoterpenes.

Based on common resource allocation theories, we predicted that *Q. ilex* plants grown in elevated [CO_2] would have increased production of carbon-based defensive compounds (Kainulainen et al. 1998, Constable et al. 1999). We found that *Q. ilex* leaves grown in elevated [CO_2] had increased emission capacities and decreased starch/N ratios. They also had increased proportions of lignin and cellulose, and were thick and sclerophyllous, a characteristic that is positively related with leaf longevity (Poorter et al. 1997, Damesin et al. 1998) and leaf resistance to low water potential (Cunnigham et al. 1999, Niinemets et al. 1999). The growth of axial organs was more favored than that of leaves, indicating that plants increased the allocation of resources to woody tissues, which would favor the capture of belowground resources. On the other hand, leaves in the elevated [CO_2] treatment did not allocate more carbon to monoterpene production in relation to their carbon gain, because carbon losses measured at the respective growth [CO_2] were similar. Furthermore, the between-tree variability in carbon loss as well as in emission capacities were not correlated with leaf TNC concentration or with starch/N ratio, but were correlated with whole-plant leaf biomass. These results suggest that the emission capacity of plants grown in elevated [CO_2] was not enhanced by accumulation of reduced carbon or by limited sink activity. On the contrary, the emission capacity of leaves increased together with the capacity to process and export photosynthates, i.e., the plants exhibited increased sink strength and growth rates.

The observed correlations between leaf emission capacity and morphological or chemical attributes do not necessarily reflect causal relationships, but may simply result from covariations. Between-tree variation in leaf nitrogen can be explained at least partly by a dilution effect at the leaf level as well as at the whole-plant level, because leaf nitrogen negatively scaled with leaf thickness and whole-plant leaf area. However, correlations between emission capacity and leaf attributes such as LMA or photosynthetic capacity may be useful for predicting variation in emission capacity, as has been demonstrated for some isoprene-emitting tree species (Harley et al. 1996, 1997, Lerdau and Throop 1999). Our results suggest that the leaf chemical components describing leaf sclerophylly (i.e., ratio between fibers and nitrogen), rather than LMA, are the best indicators of between-tree variation in emission capacity but are difficult to measure. Future work should evaluate whether other easy-to-measure attributes can be used to improve the assessment of leaf emission capacity, for instance a combination of LMA with leaf lamina narrowness (i.e., ratio of lamina length and width) or canopy leaf area index (Cunnigham et al. 1999, Rambal 2001). We found that a linear combination of LMA and whole-plant leaf area significantly improved the correlation with emission capacity (r^2 = 0.56 against r^2 = 0.34; Figure 1). Photosynthesis, on the other hand, was unsuitable for predicting between-tree variability in emission capacity. However, photosynthesis might be a useful indicator of seasonal variation in emission capacity, because it decreased from fall to winter in parallel with the decline in emission capacity. Temporary decreases in photosynthesis and emission capacity during the winter have been observed previously on the same and other evergreen Mediterranean tree species (Damesin 1996, Llusià and Peñuelas 2000, Staudt et al. 2000; Staudt et al., unpublished results).

Based on our finding that *Q. ilex* seedlings grown in elevated [CO_2] have an increased emission capacity and in-

creased foliage biomass, we conclude that, in a future high [CO_2] world, the atmospheric load of monoterpenes from Mediterranean forests will rise. However, mature trees of populations native to environments with elevated [CO_2] may respond differently than our seedlings. Mature trees may down-regulate growth and emission capacity after many generations of exposure to elevated [CO_2]. Comparative tree ring analysis made in *Q. ilex* stands in natural CO_2 springs revealed that growth acceleration occurs mainly during the juvenile phase, and diminishes as trees become older (Hättenschwiler et al. 1997). Another uncertainty concerns the impacts of global warming and increasing summer drought on *Q. ilex* emissions. Hättenschwiler et al. (1997) provided evidence that trees grown near CO_2 springs benefit from increased resistance to water stress, probably because of a reduction in leaf area per unit tree biomass, a feature consistent with our findings. An improved capacity to cope with water limitations in response to growth in elevated [CO_2] could indirectly promote emissions, because drought stress reduces the emission capacity as well as limits the growth of *Q. ilex* canopies (Rambal and Debussche 1995, Bertin and Staudt 1996).

Acknowledgments

This work was undertaken as part of the EU project ECOVOC (ENV4-CT97-0412). We thank N. Mandl for help with leaf chemical analysis, and F. Jardon for his technical assistance and in particular for building the environmentally controlled gas exchange chamber. We are grateful to Drs. F. Helleis and J. Fabreguettes for their valuable support in setting up the GC thermo-desorption and injection control system, Dr. J. Roy for the CO_2 facilities, and Dr. E. Garnier for help with various materials and instruments.

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