

# Leaf carotenoid concentrations and monoterpene emission capacity under acclimation of the light reactions of photosynthesis

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Factors controlling the seasonal dynamics in leaf monoterpene emission capacity are not yet well understood. In particular, temperature and light algorithms alone cannot explain the pattern of volatile organic compound (VOC) emissions during spring recovery of photosynthesis in boreal forests, suggesting further physiological controls. Higher isoprenoids, such as carotenoids, and VOCs share the initial steps of their synthesis pathways. Therefore, it could be expected that the pool size of leaf carotenoids and its acclimation interact with leaf monoterpene emission capacity. We examine this interaction in evergreen foliage using the non-storing *Quercus ilex* as a model species. We modified the light environment of a number of potted trees in order to induce acclimation in leaf carotenoids, and studied the effect on monoterpene emission capacity. The results indicate that monoterpene emission capacity and photosynthetic pigment metabolism are coupled in *Q. ilex* seedlings growing at or acclimating to different light levels.

## Introduction

Biosynthesis, storage and emissions of volatile organic compounds (VOCs) by plants play several important ecological roles, such as decreasing pathogen attack and herbivory (Gershenson and Croteau 1991), attracting pollinators (Knudsen and Tollsten 1993), increasing leaf thermotolerance (Singsaas *et al.* 1997), acting as allelopathic substances (Tarayne *et al.* 1995), or even signalling means between plant individu-

als (Farmer and Ryan 1990). Globally, biogenic emissions of VOCs are important in atmospheric chemistry because they change the concentrations of O<sub>3</sub> and OH radicals promoting aerosol formation processes (Fehsenfeld *et al.* 1992, Kulmala *et al.* 2004), or affecting the residence time of radiatively active trace gases (Brasseur and Chatfield 1991). The most important VOCs in this respect are the isoprenoids, e.g. isoprene, mono- and sesquiterpenes, which are emitted by a wide range of species, including those in

boreal and temperate regions (Kesselmeier and Staudt 1999). The feedback between plant VOC emissions and atmosphere, is currently the focus of intensive research, yet mechanisms controlling seasonal patterns of VOC emissions remain controversial.

Plant VOC biosynthesis is known to take place by two different biosynthetic pathways: the mevalonic acid (MVA) pathway, and the 2-deoxyxylulose 5-phosphate/2-methylerythritol 4-phosphate pathway (MEP) (Lichtenthaler 1999). The MEP pathway for dimethylallyl diphosphate synthesis (DMADP) is responsible for most of isoprenoids synthesised in plastids (Lichtenthaler *et al.* 1997). DMADP is then converted to geranyl diphosphate GPP, which is the substrate of the last enzymatic step catalysed by monoterpene synthases in the formation of monoterpenes (Mahmoud and Croteau 2002). In addition to the numerous volatile compounds, DMADP is also a precursor to “essential isoprenoids” such as carotenoids, and thus a close link between the essential end products and other products in the pathways has been suggested (Owen and Peñuelas 2005).

At a short time-scale (minutes to hours), the temperature and light influence monoterpene emissions (Dement *et al.* 1975, Bertin and Staudt 1996). The temperature controls the monoterpene emission rate by affecting the gas vapor pressure and the resistance along the emission pathway (Staudt and Bertin 1998, Niinemets *et al.* 2004), their volatility (Peñuelas and Llusà 1999, Niinemets *et al.* 2004), and the activities of the involved enzymes (Fischbach *et al.* 2000). In contrast, light controls monoterpene emissions by regulating the carbon and energy supply through photosynthesis (Staudt and Seufert 1995, Loreto *et al.* 1996). At a diurnal time-scale, higher mid-day concentrations of DMADP in isoprene emitting, but also in some non-emitting species, suggests a general response of the pathway to light, and has been also linked to a higher turnover of essential isoprenoids (Rosentiel *et al.* 2002, Brüggemann and Schnitzler 2002, Nogués *et al.* 2006).

At a longer time-scale (days to months) the capacity of VOC emissions (rate of emissions under standard conditions) is by no means con-

stant, but varies with several orders of magnitude, especially in boreal and temperate regions where the seasonality in environmental factors and plant activity is well-defined (Peñuelas and Llusà 1999, Llusà and Peñuelas 2000, Staudt *et al.* 2002, 2003, Bäck *et al.* 2005). Similarly, light environment during leaf development (Sharkey *et al.* 1991, Bertin *et al.* 1997), leaf ontogeny (Staudt and Bertin 1998, Staudt *et al.* 2003, Hakola *et al.* 2001), circadian rhythms (Wilkinson *et al.* 2006, Loivamäki *et al.* 2007), and ecotype (Peñuelas and Llusà 1999, Staudt *et al.* 2001, Tarvainen *et al.* 2005) also influence plant VOC emission capacity. In particular, we have observed very high monoterpene emission rates during spring recovery of photosynthesis in boreal Scots pine, which could not be fully explained by temperature (Tarvainen *et al.* 2005). Interestingly, strong changes in needle carotenoid contents also take place during spring recovery of photosynthesis in boreal Scots pine (Porcar-Castell *et al.* 2008).

We hypothesize that the seasonal acclimation in the pool size of leaf carotenoids interacts with the seasonality in emission capacity of VOCs, i.e. in the long term (weeks), an increase in the total pool of a specific essential isoprenoid, such as carotenoids, would require upregulation of the MEP pathway, increasing the available DMADP and enhancing VOC synthesis capacity (Owen and Peñuelas 2005). This mechanism could be of significance particularly in evergreen boreal forests, where large seasonal adjustments in leaf carotenoid contents take place in the needle to cope with excess light during the unfavorable growth conditions (Ensminger *et al.* 2006, Porcar-Castell *et al.* 2008). However, the interaction between volatile isoprenoid and carotenoid biosynthesis is difficult to study in a species possessing massive storage pools, such as Scots pine. Therefore, in this study we selected a model species without storage capacity to look for field evidence for this interaction.

*Quercus ilex* is a vigorous monoterpene emitter which has been extensively studied due to its light-dependent emissions and lack of storage pools, facilitating the study of the emission responses to many environmental factors, and the correlation with simultaneous rates of synthesis (Bertin *et al.* 1997, Llusà and Peñuelas

2000, Loreto *et al.* 2001). In this study, we modified the light environment of potted *Q. ilex* seedlings in order to induce acclimation in leaf carotenoids. Our aim was to examine the interaction between leaf carotenoid concentration, its acclimation, and monoterpene emission capacity in evergreen foliage. We analysed the relationship between leaf total carotenoid concentration, photosynthetic capacity and total monoterpene emission capacity in *Q. ilex* seedlings both during a period when carotenoid contents were adjusting, and at the steady-state.

## Material and methods

### Species and experimental setting

Thirty potted four-year-old *Quercus ilex* trees (Holm oak) were purchased from the Forestal Catalana S.A. (Barcelona) and transferred to the experimental site on 20 June 2006. On 21 June, the seedlings were separated into two different light environments (15 in full sunlight, and 15 in shade: ca. 50% of full sunlight) for their long-term acclimation. All seedlings were watered weekly. We assumed that the acclimation of the pigment composition to the new light environments had reached a steady-state after three months under relatively constant summer conditions. On 26 September, manipulations of the light environments were done. Eight *Q. ilex* seedlings from the shaded conditions were transferred to the full sunlight (SHADESUN), and eight seedlings from full sunlight transferred to shade (SUNSHADE). The rest of the seedlings in each treatment were left as controls (SHADE: shade control; and SUN: sun control).

### Environmental data

Two light sensors (LI-190, LI-COR, NE), and two self-manufactured and calibrated Thermistors were used to monitor photosynthetic photon flux density (PPFD) and the air temperature in each of the light environments. A single measurement was recorded every 30 seconds with a data logger (Micrologger 21X, Campbell Scientific Ltd., UK).

### Monitoring of photosynthetic capacity

The maximum yield of photochemistry ( $F_v/F_m$ ) was monitored with a fluorometer (PAM-2000, Walz GmbH, Germany). Minimum chlorophyll fluorescence intensity ( $F_o$ ) was measured after a minimum of one hour of dark-acclimation, and maximum chlorophyll fluorescence intensity ( $F_m$ ) was obtained next by supplying a saturating light pulse to the leaf. The maximum quantum yield of photochemistry was calculated as  $F_v/F_m$ , (Kitajima and Butler 1975), where  $F_v = F_m - F_o$ . Fluorescence measurements were performed around noon (11:00–12:00, solar time). Three leaves per seedling and three seedlings from each treatment were fluorometrically measured on each sampling day. A healthy, current-year and fully-developed leaf was marked on the same three seedlings per treatment to repeatedly measure photosynthetic capacity during the experiment. The photosynthetic capacity ( $\text{CO}_2$  uptake) under standard conditions ( $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $30^\circ\text{C}$ , and  $360\text{--}380 \text{ ppm CO}_2$ ) was monitored in the leaf with a gas exchange system (CIRAS-2, PP-Systems, USA). Once the steady-state was reached, typically after ten minutes, five recordings were made and stored. A digital photo was taken perpendicular to the CIRAS-2 cuvette plane for later estimation of the leaf area. Gas exchange measurements took several hours and were usually performed between 07:30 and 16:30 (solar time). The treatments were sampled following a different sequence on each date to avoid possible differences induced by the diurnal emission pattern.

### Water content and leaf mass per area

Measurements of relative water content (RWC), and leaf mass per area (LMA) were carried out towards the middle of the experiment (5 October 2006), in order to register any potential changes induced by the treatments. Two current-year and fully developed leaves per each seedling and treatment were collected. After measuring the fresh weight (FW), and the leaf area (LA) with a leaf area meter (LI-COR 3100, NE), each sample was placed in a separate tube and distilled water was added to cover the leaf petioles. Leaves

were left in the tubes overnight at 4 °C, and the next morning the turgor weight was measured (TW). Finally, leaf samples were dried at 60 °C until no further reduction in weight was observed, and the dry weight (DW) was measured. The following equations were applied to estimate RWC and LMA:

$$\text{RWC}(\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} 100 \quad (1)$$

$$\text{LMA} = \frac{\text{DW}}{\text{LA}} \quad (2)$$

### Monitoring of pigment contents

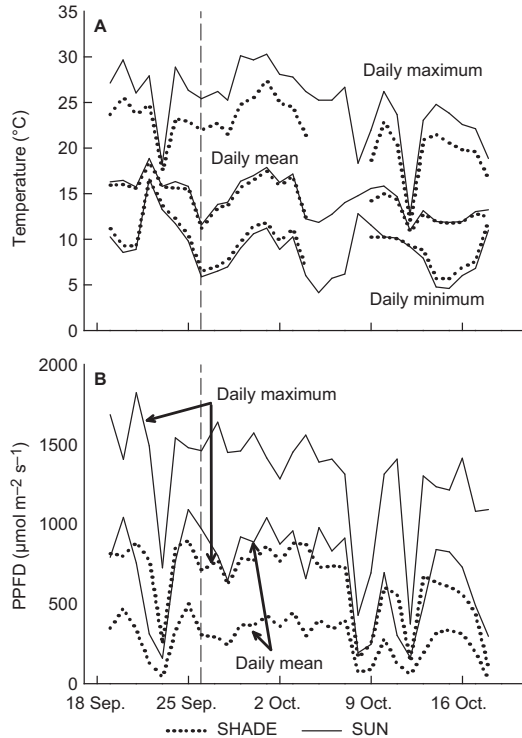
On each sampling day, two to three current-year and fully developed leaves were collected per seedling and immediately frozen in liquid nitrogen. Three seedlings per treatment were sampled. Pigment sampling was carried out around 10:00–12:00 (solar time). Samples were subsequently stored at –80 °C until extraction (a few weeks later). Samples were ground in liquid nitrogen with mortar and pestle, and 50 mg of a sample (fresh weight) was put into an Eppendorf® tube, 1.5 ml of 85% acetone was added into the tube and the sample was suspended with a vortex. Next, samples were placed in an ultrasound bath for 15 minutes at 4 °C, resuspended with a vortex, and centrifuged for 5 minutes at 16 000 g. The supernatant was stored and the pellet re-extracted with 1 ml of 100% acetone following the same procedure. The resulting extract was adjusted with distilled water to attain a final acetone concentration of 82%–83% and filtered prior to HPLC analysis, as described by Munné-Bosch and Alegre (2000). In short, pigments were separated on a Dupont non-encapped Zorbax ODS-5  $\mu\text{m}$  column (250 mm  $\times$  4.6 mm, 20% C, Scharlau, Barcelona, Spain) at 30 °C at a flow rate of 1 ml  $\text{min}^{-1}$ . Detection was carried out at 445 nm (Diode array detector, HP1100 Series, Agilent Technologies, Santa Clara, CA).

### Monoterpene emissions

Immediately after measuring the photosynthetic capacity, and while keeping the same light and

temperature conditions, monoterpene emissions were measured from the same leaf. Air coming out of the CIRAS-2 cuvette, in which the leaf was placed, flowed through a T-system to a glass tube (8-cm long and 0.3-cm internal diameter) manually filled with terpene adsorbents Carbopack B, Carboxen 1003, and Carbopack Y (Supelco, Bellefonte, PA) separated by plugs of quartz wool. Terpenes did not suffer any chemical transformations in the tubes as checked with standards ( $\alpha$ -pinene,  $\beta$ -pinene, camphene, myrcene, *p*-cymene, limonene, sabinene, camphor, and dodecane) (Llusà *et al.* 2006). Prior to use, the tubes were conditioned for 10 minutes at 350 °C with a stream of purified helium. Air flow through the tube was approximately 0.5 l  $\text{min}^{-1}$  (Escort Elf, MSA, PA), and the exact flow was registered for later calculations. The sampling time was 10 minutes, and it started once photosynthesis had reached the steady state under the standard measuring conditions (1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 30 °C and 360–380 ppm  $\text{CO}_2$ ) for at least 10 minutes. Based on previous tests with PTR-MS, 10 minutes is sufficient to attain stabilization of monoterpene emissions in *Q. ilex* (data not shown). CIRAS-2 was left running without any leaves for 5–10 minutes between measurements in order to clean the system. In addition, one blank sample was taken every three measurements to register the system background. The sample tubes were stored in a freezer at –30 °C until analysis (7 to 20 days). Emission rate calculations were made on a mass balance basis. The obtained system background of the blank samples was subtracted.

Terpene analyses were conducted in a GC-MS (Hewlett Packard HP59822B, CA). Trapped emitted monoterpenes were injected automatically by a robotic sample processor (FOCUS) (ATAS GL International BV 5500 AA Veldhoven, The Netherlands) in an OPTIC3 injector (ATAS GL International BV 5500 AA Veldhoven, The Netherlands) for 5 minutes and passed into a 30 m  $\times$  0.25 mm  $\times$  0.25 mm film thickness capillary column (Supelco HP-5, Crosslinked 5% Me Silicone, Supelco Inc., Bellefonte, PE, USA). After sample injection, the initial temperature (60 °C) was increased at the rate of 20 °C  $\text{min}^{-1}$  up to 150 °C, and thereafter at the rate of 30 °C  $\text{min}^{-1}$  up to 300 °C, and maintained for 5 minutes. The helium flow was 0.7 ml  $\text{min}^{-1}$ .



**Fig. 1.** (A) Temperature and (B) photosynthetic photon flux density (PPFD) at the experimental site. Solid lines correspond to the full sunlight environment (SUN), and dots to the shaded environment (SHADE). Vertical dashed lines mark the date when the treatments SHADESUN and SUNSHADE were transferred to the new growing conditions.

The identification of monoterpenes was carried out with GC-MS and comparison with standards from Fluka (Buchs, Switzerland), literature spectra, and GCD Chemstation G1074A HP (Hewlett Packard). Frequent calibration with  $\alpha$ -pinene, limonene and  $\alpha$ -humulene standards was done once every 4–6 analyses and was used for quantification. Terpene calibration curves (four concentration levels) always had  $r^2 > 0.91$ .

### Statistical analysis

Differences between two treatment groups were analysed using a *t*-test, and differences among treatment groups were analysed using one-way ANOVA with a Bonferroni post-hoc test. Correlations between variables were studied by estimating coefficient of determinations ( $r^2$ ). Confi-

dence intervals (CI) of the mean ( $\mu$ ) were estimated according to Student's *t* distribution, as  $\mu \pm tsn^{1/2}$ , where *t* is the statistic, *s* is the standard deviation of the mean, and *n* the sample size.

## Results

### Micrometeorology

There was no precipitation registered during the experiment (UAB Meteorological Station). The daily-mean air temperature was practically equal in shaded (SHADE) and exposed treatments (SUN) (Fig. 1A). However, the diurnal temperature fluctuation was slightly higher in case of sun-exposed seedlings: 3–4 °C higher maximum temperature and approximately 1 °C lower minimum temperatures. Both maximum and daily mean photosynthetic photon flux densities (PPFD) were approximately half in the SHADE as compared with those in the SUN treatments (Fig. 1B). Note also the cloudy days with lower maximum and minimum PPFD levels.

### Water content and leaf mass per area

Mean relative water content (RWC) measured on 5 October, after a sunny and rainless two-week period, was above 90% in all four treatments:  $93.0\% \pm 9.4\%$  (SUN),  $97.2\% \pm 7.1\%$  (SHADE),  $95.5\% \pm 2.9\%$  (SUNSHADE) and  $90.2\% \pm 24.0\%$  (SHADESUN) (mean  $\pm$  95% CI). Similarly, leaf mass per area (LMA) measured on 5 October was  $248.2 \pm 39.5$  g m<sup>-2</sup> (SUN),  $265.9 \pm 45.1$  g m<sup>-2</sup> (SHADE),  $261.6 \pm 118.3$  g m<sup>-2</sup> (SUNSHADE) and  $254.8 \pm 29.9$  g m<sup>-2</sup> (SHADESUN) (mean  $\pm$  95% CI). No significant differences in RWC nor in LMA were found among treatments (one-way ANOVA: RWC,  $F_{3,8} = 0.93$ ,  $p = 0.4701$ ; LMA,  $F_{3,8} = 0.24$ ,  $p = 0.8646$ ).

### Photosynthetic performance

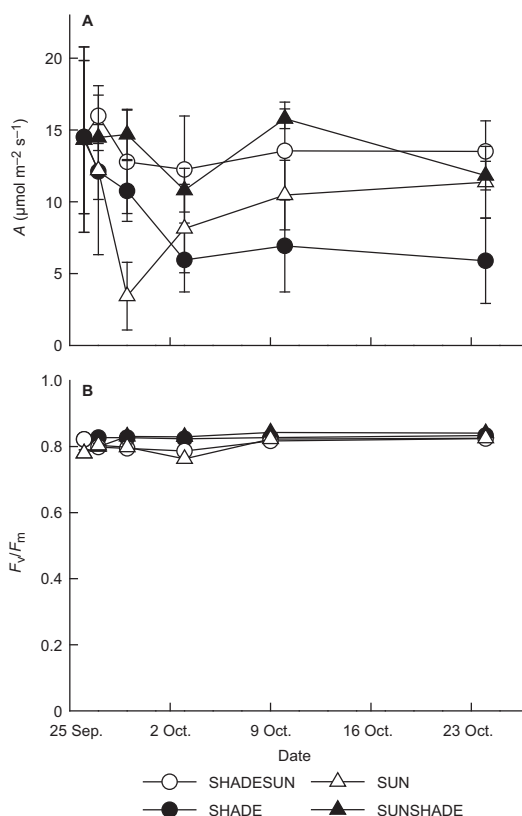
Photosynthetic CO<sub>2</sub> assimilation capacity measured under standard conditions (PPFD = 1000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, 30 °C and 360–380 ppm CO<sub>2</sub>) was similar in SUN and SHADE seedlings prior

to the transfer between light environments on 26 September. After transfer to full sunlight, the SHADESUN seedlings did not significantly differ in photosynthetic capacity from either the SHADE or the SUN treatments for the whole duration of the experiment (Fig. 2A) (One way ANOVA:  $p > 0.05$  for all dates). The seedlings transferred to shade conditions (SUNSHADE) had slightly higher photosynthetic capacity as compared with those in the SUN treatment three days after the transfer (one-way ANOVA:  $F_{3,8} = 3.87$ ,  $p = 0.056$ ), but did not differ from the control treatments for the rest of the experiment (one-way ANOVA:  $p > 0.05$  for all dates). Unexpectedly, photosynthetic capacity had decreased transiently on the third day in the SUN treatment, and tended to gradually decrease in all treatments during the first week.

The maximum quantum yield of photochemistry in photosystem II (PSII), estimated fluorometrically as  $F_v/F_m$ , remained rather stable, around 0.8, in all the treatments for the entire duration of the experiment (Fig. 2B). Yet, it was significantly lower in the seedlings exposed to full sunlight (SUN) ( $F_v/F_m = 0.779 \pm 0.026$ ) as compared with that in the seedlings in the shaded environment (SHADE) ( $F_v/F_m = 0.822 \pm 0.011$ ) prior to the transfer ( $t$ -test:  $t_{14} = 3.40$ ,  $p = 0.004$ ). After transfer to full sunlight,  $F_v/F_m$  slightly decreased in SHADESUN as compared with that in the seedlings remaining in the shade (SHADE), and increased in SUNSHADE compared to the seedlings remaining under full sunlight (SUN).

## Pigment contents

As anticipated, during the study period the total xanthophyll-cycle pool pigments and total carotenoids (based on chlorophyll) increased significantly in the seedlings transferred from shade to sun (SHADESUN) (Fig. 3), from  $0.259 \pm 0.142$  mol mol<sup>-1</sup> (Car Chl<sup>-1</sup>) on 26 September to  $0.375 \pm 0.042$  mol mol<sup>-1</sup> (Car Chl<sup>-1</sup>) on 24 October ( $t$ -test:  $t_4 = 3.38$ ,  $p = 0.0277$ ). Both VAZ/Chl and Car/Chl remained consistently higher in SUN than in SHADE for the entire period. Furthermore, the change in light environment affected the SHADESUN and SUNSHADE treatments

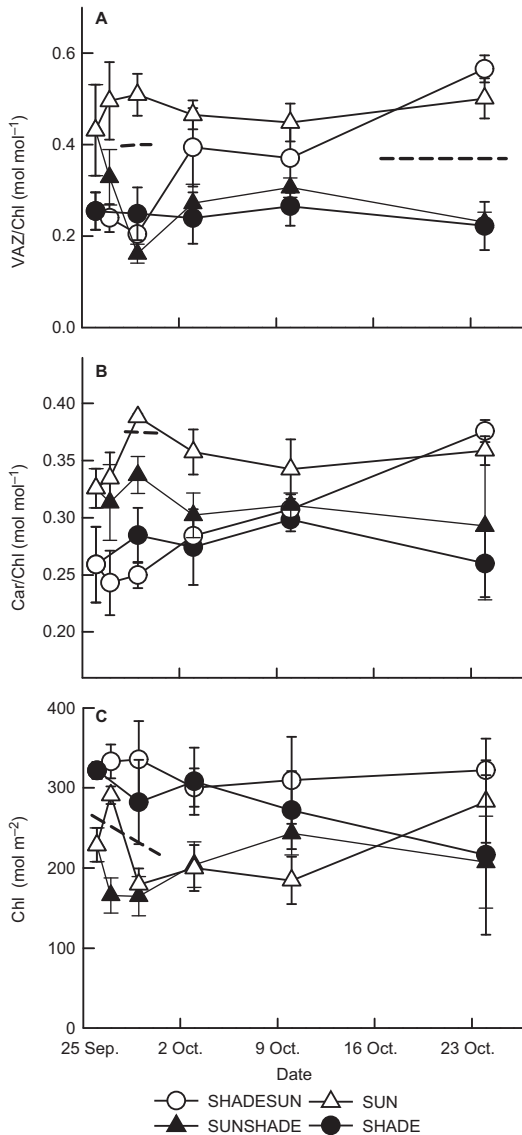


**Fig. 2.** (A) Evolution of the photosynthetic capacity in *Quercus ilex* in each of the treatments (measured at  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD,  $30^\circ\text{C}$  and  $360\text{--}380$  ppm  $\text{CO}_2$ ). (B) Evolution of the maximum quantum yield of photochemistry ( $F_v/F_m$ ) in *Q. ilex* in each of the light treatments. Points correspond to mean values  $\pm$  SEs ( $n = 3$ ).

as expected: increasing VAZ/Chl and Car/Chl in SHADESUN and decreasing them in SUNSHADE.

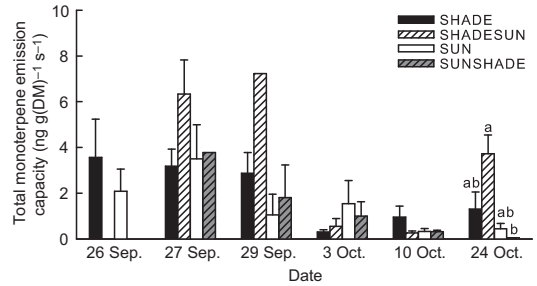
## Monoterpene emissions

No significant differences could be detected in total monoterpene emission capacity between seedlings exposed to full sunlight (SUN) and shaded conditions (SHADE) at the start of the experiment ( $2.09 \pm 4.17$  and  $3.56 \pm 7.21$  ng g(DM)<sup>-1</sup> s<sup>-1</sup> (mean  $\pm$  95% CI); see Fig. 4). Monoterpene emission capacity tended to decrease in the SHADE, SUN, and SUNSHADE treatments with time, but the tendency was only significant in the treatments SHADE



**Fig. 3.** The ratios of (A) xanthophyll-cycle pool pigments (VAZ) to total chlorophyll and (B) total carotenoid to total chlorophyll, as well as (C) total chlorophyll contents (Chl *a* + *b*) per leaf area. Points correspond to mean values  $\pm$  SEs ( $n = 3$ ). Dashed lines separate treatment groups that are significantly different ( $p < 0.05$ ).

(one-way ANOVA:  $F_{1,3} = 8.02$ ,  $p = 0.0472$ ) and SUNSHADE (one-way ANOVA:  $F_{1,3} = 24.76$ ,  $p = 0.0156$ ). In contrast, in the treatment SHADESUN, the observed pattern was different, with a transient, short-term increase in emissions registered immediately (1–3 days) after the transfer to full sunlight, followed by a period



**Fig. 4.** Mean ( $\pm$  SE) total monoterpene emission capacity of *Q. ilex* leaves under standard conditions (1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD, 30 °C and 360–380 ppm CO<sub>2</sub>). Standard error bars are not presented when  $n < 3$  (missing data due to problems during the analysis). Significant differences between groups are represented with different letters when appropriate (ANOVA and post-hoc test:  $p < 0.05$ ).

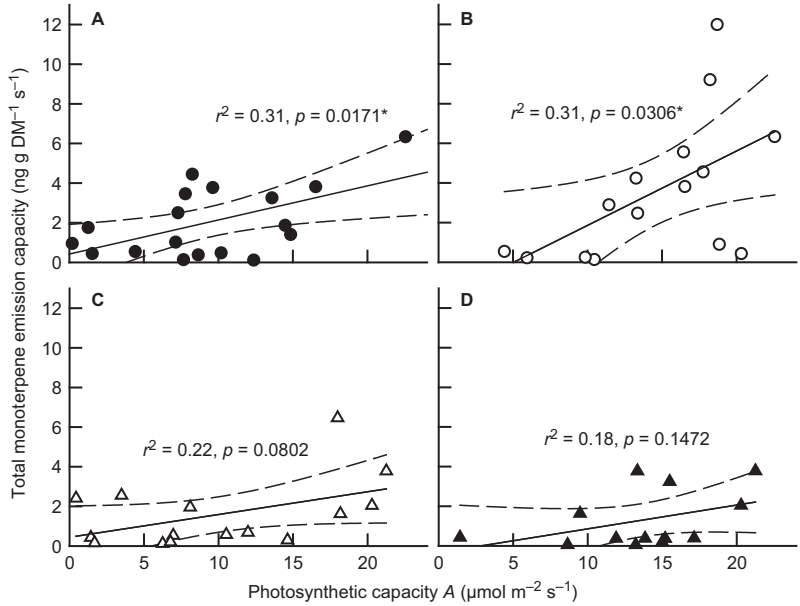
of very low emissions and a later increase four weeks after the transfer. Emission capacity in SHADESUN four weeks after the transfer tended to be higher than in other treatments, although the differences were found to be significant only between SHADESUN and SUNSHADE treatments (one-way ANOVA and post-hoc test:  $F_{3,8} = 7.235$ ,  $p = 0.029$ ;  $p_{(\text{SHADESUN}:\text{SUNSHADE})} = 0.049$ ].

### Controls over monoterpene emission capacity

The total monoterpene emission capacity and the photosynthetic capacity correlated significantly in the treatment SHADESUN, SHADE, to some extent but not significantly in the treatment SUN, and did not correlate in the treatment SUNSHADE (Fig. 5). Generally, monoterpene emissions and photosynthetic capacities increased concomitantly.

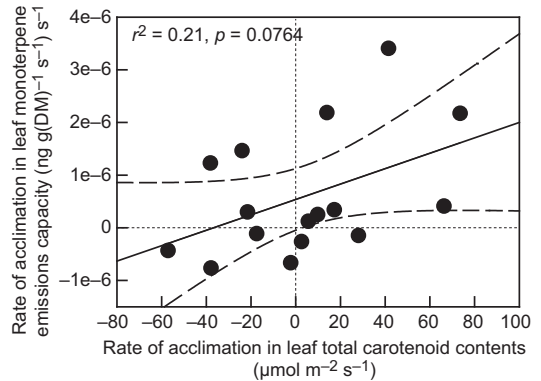
In order to study the relationship between emissions and pigment contents after the transfer to different light environments, we examined the correlation between the rate of acclimation in leaf total carotenoid contents ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and the rate of acclimation in leaf monoterpene emission capacities ( $[\text{ng g DM}^{-1} \text{s}^{-1}] \text{s}^{-1}$ ) of individual seedlings (Fig. 6). Rates of acclimation and individual seedlings were used, instead of absolute amounts and treatment means, in order to exclude the effect of individual variability in

**Fig. 5.** Correlations between the leaf total monoterpene emission rates, obtained at a steady state under standard conditions ( $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD,  $30^\circ\text{C}$  and  $360\text{--}380 \text{ppm CO}_2$ ), and the steady state photosynthetic carbon assimilation rates obtained under the same conditions. Data points correspond to measurements of monoterpene emissions and carbon assimilation of a single leaf obtained during the course of the experiment. Solid lines = regressions, dashed lines = 95% CIs.



monoterpene emission capacity. Only the data from the last three sampling dates were used, when combined emissions and pigment samples were obtained from the same seedlings. Combined pigment and emission sampling was not carried out during the first week in order to avoid short-term interactions between the collection of leaves and monoterpene emissions. The results showed a relationship between these variables (Fig. 6). In the trees in which the carotenoid contents had increased between measurements, the emission capacities also tended to increase during the same period, and *vice versa*. Only in a very few seedlings the emissions had increased with the slightly decreasing carotenoid content.

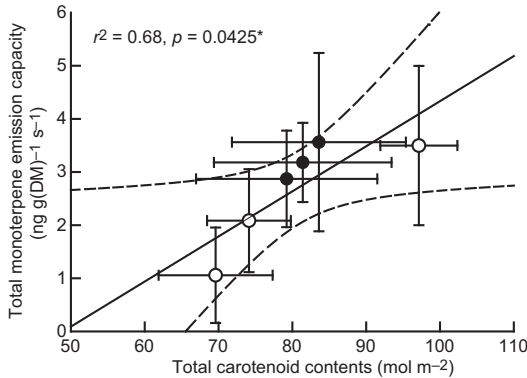
Finally, we studied the steady-state relationship between pigment contents and emission capacity, using the data from the control treatments SUN and SHADE obtained during the first three days of the study period, assuming that the seedlings would have attained the steady-state after spending three months under relatively constant summer conditions. We found a significant and positive correlation between levels of pigment contents and monoterpene emission capacity (Fig. 7). The higher the treatment mean carotenoid content was, the higher was the treatment mean monoterpene emission capacity, and *vice versa*. When the data from all sampling



**Fig. 6.** Correlation between the rate of acclimation in total monoterpene emission capacity measured under standard conditions ( $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD,  $30^\circ\text{C}$ , and  $360\text{--}380 \text{ppm CO}_2$ ) and the rate of acclimation in leaf total carotenoid contents. Data are from the last three sampling dates, when carotenoid contents and emissions were concurrently measured from the same trees. Data from all treatments were used. Rates of change were calculated as  $([x]_{t_1} - [x]_{t_0})/t$ , where  $[x]$  is the monoterpene emission capacity or carotenoid concentration and  $t$  is the time in seconds between sampling points. Solid line = regression, dashed lines = 95% CIs.

dates were included, ranging from 26 September to 24 October, the correlation was no longer significant (data not shown).





**Fig. 7.** Correlation between the total leaf monoterpene emissions under standard conditions ( $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD,  $30^\circ\text{C}$ , and  $360\text{--}380 \text{ppm CO}_2$ ) and the total leaf carotenoid contents. Points correspond to means  $\pm$  SEs ( $n = 2\text{--}3$ ). Only the data from the control treatments on 26, 27 and 29 September were used, assuming that the steady-state had been attained after three months under relatively constant summer conditions. Solid lines = regressions, dashed lines = 95% CIs.

## Discussion

Despite the limited number of replicates that we were able to measure due to practical and logistic reasons, we observed a coupling between the monoterpene emission capacity and photosynthetic pigment metabolism in *Q. ilex* seedlings growing at or acclimating to different light levels. An increase in the total leaf carotenoid contents tended to be associated with an increase in the capacity of leaf monoterpene emission (Fig. 6), therefore, leaves with larger pools of carotenoids presented larger monoterpene emission capacities (Fig. 7).

### Response to the light treatments

The measured photosynthetic capacities ranged between 5 and  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which is within reported levels for non-stressed potted seedlings of *Q. ilex* ( $8$  to  $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Bertin and Staudt 1996, Loreto *et al.* 1998), yet field grown *Q. ilex* trees have typical summer levels of  $3\text{--}6 \mu\text{mol m}^{-2} \text{s}^{-1}$  under full sunlight (Peñuelas and Llusià 1999). Neither the change in light environment nor the long-term effect of light environ-

ment induced significant differences in photosynthetic capacity among treatments. In addition, the high relative water contents on 5 October in all treatments indicated the absence of water stress (Serrano *et al.* 2005). The maximum quantum yields of photosystem II remained close to 0.8 in all the treatments and for the entire duration of the experiment, denoting the absence of photoinhibition of photosynthesis or water stress. However, we cannot rule out the possibility that some treatments underwent transient water limitations, which could explain the temporary decrease in photosynthetic capacity in the SUN treatment at the beginning of the experiment (Fig. 2). In spite of this, it is generally accepted that moderate water stress and decrease in photosynthesis do not influence monoterpene emissions (Bertin and Staudt 1996). On the other hand, the appearance of the first cold nights with temperatures around  $5^\circ\text{C}$  could also explain the decreasing trend in photosynthetic capacity during the first week.

Carotenoid contents, and especially carotenoid contents based on chlorophyll (which reflect the relative protection level per amount of chlorophyll), responded consistently to the light environment treatments, increasing in the seedlings transferred to full sunlight (SHADESUN), decreasing in the seedlings brought to shade (SUNSHADE), and remaining relatively constant in the control treatments. This represents the normal physiological response to a change in excitation pressure together with the photoprotective role of carotenoids (Adams and Demmig-Adams 1994, Ensminger *et al.* 2006, Porcar-Castell *et al.* 2008).

The total monoterpene emission capacity observed in the *Q. ilex* seedlings was comparable to the values reported in literature (Street *et al.* 1997, Peñuelas and Llusià 1999). In our data, a seasonal decrease in emission capacity was evident in both SUN and SHADE seedlings. This was not surprising, since large seasonal differences in emission rates have been reported in many perennial species (Peñuelas and Llusià 1999, Staudt *et al.* 2002, Tarvainen *et al.* 2005).

The emissions of seedlings grown at constant light levels, in either full sunlight (SUN) or in shade (SHADE) did not differ significantly from each other. In contrast to our results, Bertin *et al.*

(1997) found large differences in monoterpene emissions between shade and sun branches of *Q. ilex* under natural growth conditions, yet the differences were attributed mostly to differences in specific leaf weight and photosynthate availability, i.e. lower photosynthetic rates in shaded branches. In the present study, neither LMA nor photosynthetic capacity differed between the SUN and SHADE treatments, indicating that variations in emission capacity between different light conditions in this study were not related to leaf morphological properties. This is logical since the leaves that we measured were all fully developed before the beginning of the study and thus had all developed in a similar light environment. In particular, the emission capacity in the SHADESUN treatment appeared to be controlled by different factors on top of the ones controlling other treatments. Changing the light environment induced a transient effect on the emissions for the seedlings transferred from shade to higher light environment (SHADESUN): a hysteresis-type pattern — a rapid increase, followed by a decrease and again an increase — in the monoterpene emission rate was measured after the change in light level. Eventually, one month after the transfer to the new light environment, emissions were higher in the SHADESUN as compared with those in the SUN or SUNSHADE treatment. Emission capacity changes have previously been shown to follow a rapid kinetics, in particular, in up-regulation after a change in light and temperature: shade/cool to sun/warm transfer induced an increase in emissions within few hours, the effect lasting several days (Staudt *et al.* 2003). Transferring plants from full light to shade affected the emissions in a low temperature regime but not in warmer conditions (Staudt *et al.* 2003). The mechanisms behind the up- and down-regulation have been related to monoterpene synthase activities, enzyme turnover rates or presence of non-specific storage pools (Loreto *et al.* 2001, Fischbach *et al.* 2002, Niinemets *et al.* 2002). Similarly, interaction between adjustment in the carotenoid contents and the VOC emission capacity through the common precursor DMADP pool could equally explain part of the seasonal variability in the total monoterpene emission capacity, and is discussed below.

### Interaction between carotenoid contents and monoterpene emission capacity

We hypothesized that the seasonal changes in the pool size of leaf carotenoids is connected to the seasonality in emission capacity of VOCs, given that both carotenoids and monoterpenes share a common precursor. This hypothesis, and the opportunistic functional role of VOC emissions, has been introduced by Owen and Peñuelas (2005). Following the hypothesis, an increase in the total pool of leaf carotenoids would require up-regulation of the MEP pathway, increasing the availability of DMADP. Subsequently, increased availability of DMADP would enhance VOC synthesis capacity. This hypothesis can be interpreted at several time-scales, although our study focussed on the long-term or seasonal time scale (weeks).

At a diurnal time-scale, in many emitting species DMADP contents have been shown to vary with noon values several times higher as compared with night values (Rosenstiel *et al.* 2002, Brüggemann and Schnitzler 2002, Mayrhofer *et al.* 2005). Diurnal changes in volatile isoprenoid emissions have been linked to diurnal changes in DMAPP concentrations. In turn, not only net photosynthetic carbon assimilation (Brüggemann and Schnitzler 2002), but also higher chlorophyll and carotenoid turnover upon higher illumination at noon (Rosenstiel *et al.* 2002), have been suggested as controls of the diurnal DMAPP pattern.

Similarly, at longer time-scales (weeks), an increase in the leaf carotenoid contents would require a sustained increase in the pool of precursors (e.g. DMADP), in order to sustain a higher carotenoid turnover rate. Indeed, higher DMADP contents have been observed in *Q. ilex* in July as compared with those in May (Nogués *et al.* 2006), when one would expect to find higher carotenoid contents. The results presented here indicated that during the transient period when pigment contents are adjusting there was a directly proportional trend between the rate of change in pigment contents and the rate of change in monoterpene emission capacity, i.e. if pigment contents increased, total monoterpene emission capacity also tended to increase (Fig. 6). Furthermore, when looking at the rela-

tive steady-state conditions by the end of the summer, leaves with higher carotenoid contents presented higher monoterpene emission capacities (Fig. 7). This correlation was no longer significant when including all the data from the SUN and SHADE control treatments. It is crucial to note that the interaction reported here would only be responsible for a fraction of the seasonal variation in monoterpene emission capacity, the response of the emissions to the first autumn chilling temperatures (Fig. 1), or differences in the acclimation rates between individuals, would explain the lack of correlation when departing from the steady-state. Furthermore, the partitioning of DMAPP between VOCs and higher isoprenoids is likely controlled by a number of highly-regulated mechanisms. In isoprene emitting species, for example, the partitioning of DMADP between carotenoid and isoprene synthesis has been shown to be strongly controlled by leaf ontogenesis, where carotenoid biosynthesis dominates in leaves under development, while transcript levels of isoprene synthase are close to zero (Lehning *et al.* 2001, Mayrhofer *et al.* 2005). Further research is required (1) to study the coupling between carotenoid and VOC synthesis in evergreen foliage undergoing seasonal acclimation, involving gene transcription and translation studies, and (2) to study the potential implications of this coupling at larger spatial and temporal scales: in particular, to what extent VOC emissions from boreal evergreen vegetation during early spring might be associated to the simultaneous acclimation in carotenoid contents.

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