Linking photorespiration, monoterpenes and thermodurance in *Quercus*

Josep Peñuelas and Joan Llusià

Unitat d'Ecofisiologia CSIC-CEAB-CREAF, CREAF (Center for Ecological Research and Forestry Applications), Edifici C, Universitat Autònoma de Barcelona, E–08193 Bellaterra, Barcelona, Catalonia, Spain

**Summary**

- The functions of two important plant processes, photorespiration and monoterpenes production remain controversial. Here, we investigated one possible function, that of protection of plants from photodamage at high temperatures.
- Fluorescence, reflectance, monoterpenes concentrations and visual leaf damage were measured in *Quercus ilex* seedlings exposed to temperature increases from 25 to 50°C (in 5°C steps) under photorespiratory (21% O₂) or nonphotorespiratory (2% O₂) atmospheres, and under control or terpene fumigation conditions.
- Lower variable to maximum fluorescence ratio (Fv : Fm: potential photochemical efficiency of photosystem II, PSII) and electron transport rate (ETR) were found in nonphotorespiratory conditions at temperatures greater than 35°C. Monoterpene concentrations were also lower, and leaf damage greater, in the low O₂ atmospheres. Monoterpene fumigation, which increased the foliar terpene concentrations by two- to four-fold, increased the photochemical efficiency between 35°C and 50°C, and decreased leaf damage, only under the nonphotorespiratory conditions.
- These results provide evidence that: photorespiration decreases photodamage, especially at high temperatures; photorespiration increases monoterpenes production; plants are able to acquire exogenous monoterpenes and the acquisition response to temperature follows the stomatal conductance response; and monoterpenes can replace photorespiration in protection from photodamage at high temperatures, possibly by scavenging oxygen-reactive species, but they do not provide additional thermodurance.

**Key words:** Fv : Fm, electron transport rate (ETR), photochemical reflectance index (PRI), α-pinene, limonene, fumigation, photorespiration, monoterpenes, thermodurance.


**Introduction**

High temperature may represent an important constraint for plants, restricting growth and productivity (Boyer, 1982) and influencing the distribution of species (Grace, 1987). Models of global climate predict a further 1.5–5.5°C warming for this century as a result of the increased atmospheric concentrations of CO₂ and other trace gases (IPCC, 2001). Therefore, plants are likely to experience increasing high-temperature stress.

Plants thus need to protect themselves against the damaging effects of high temperature. Photorespiration, which lowers the energy efficiency of photosynthesis, particularly in C₃ plants (Ogren, 1984), seems to protect these plants from photoinhibition by dissipation of excess light energy (Kozaki & Takeba, 1996). While chloroplasts have superoxide dismutase to detoxify oxygen radicals, it is apparently insufficient to protect the leaf in high light intensity when CO₂ fixation is limited by heat (or drought) stress, stomata close and CO₂ cannot enter the leaf. Photorespiration can continue in leaves with external and internally produced oxygen. Photorespiration thus provides internal CO₂ for re-fixation, and the Calvin cycle keeps running. This photoprotective role for photorespiration is, however, somewhat controversial since sometimes has been
not demonstrated (Powles et al., 1979; Ogren, 1984; Wu et al., 1991; Demmig-Adams & Adams, 1992; Aro et al., 1993; Long & Humphries, 1994; Brestdt et al., 1995; Heber et al., 1996; Migge et al., 1999; Niyogi, 1999).

Temperature increases the production and emission rates of most terpenes exponentially up to a maximum by enhancing the synthesis enzymatic activities, raising the terpene vapor pressure and decreasing the resistance of emission pathway (Tingey et al., 1991; Loreto et al., 1996; Peñuelas & Llusia, 2001). Even for nonstored volatile organic compounds (VOCs) such as \( \alpha \)-pinene in *Quercus ilex*, emission has been found to increase threefold when temperature raises from 20 to 30°C (Loreto et al., 1996). Terpenes serve several functions, including deterring herbivores and attracting pollinators, but the function of terpene production and emission by plants that do not store them remains elusive. One potential function of isoprene and terpenes is the stabilization and protection of plant membranes against high temperatures (Sharkey & Singsaas, 1995; Loreto et al., 1998; Singsaas, 2000) even described in neighbor nonemitting species, although at unlikely high air concentrations (Delfine et al., 2000). However, such thermotolerance enhancement has not always been found (Logan & Monson, 1999). A second hypothesis of a protective role of isoprene and monoterpenes is that they serve as antioxidants in leaves. However, this hypothesis has little evidence to support or reject it (Sharkey & Yeh, 2001).

One of the primary effects of high-temperature stress is the damage to photosynthetic electron transport. A review by Berry & Björkman (1980) found the electron transport through photosystem II (PSII) to be the component of photosynthesis most susceptible to irreversible high-temperature damage. Chlorophyll fluorescence and reflectance methods allow the quantification of such stress effects on plant photosynthesis, with the advantage of being nondestructive and rapid, thereby only minimally affecting the object of study (Bolhar-Nordenkampf et al., 1989; Bilger et al., 1995; Peñuelas & Filella, 1998). Changes in chlorophyll fluorescence induced by high temperature have been widely used as an indicator of thermal damage (Bilger et al., 1984). These changes seem to be produced by dislocation between the light-harvesting complexes and PSII reaction centers because of excessive membrane fluidity (Armond et al., 1980) or/and denaturation of PSII reaction center proteins (Yamane et al., 1997). The xanthophyll cycle is another photoprotective mechanism for excess of energy that lowers the photochemical efficiency of PSII (Demmig-Adams & Adams, 1992; Long & Humphries, 1994; Peñuelas et al., 1995). PSII efficiency can be monitored by changes in reflectance at 531 nm (Gamon et al., 1992; Peñuelas et al., 1995; Peñuelas & Filella, 1998).

High-temperature stress is especially evident in Mediterranean area where high temperatures coincide with drought conditions in summer (Peñuelas & Llusia, 1999; Larcher, 2000). *Quercus ilex* is one of the most typical dominant Mediterranean forest species. Its leaves may suffer from thermal stress above 35°C (Larcher, 2000). Usually, \( \text{CO}_2 \) uptake suddenly decreases at 40–45°C, but *Q. ilex* still grows on sites where the maximum air temperatures reach 49°C (Seville, Spain). This species seems to present higher photorespiration rates than other co-occurring species under the high temperatures and dry conditions of the Mediterranean summer (Filipella et al., 1998; Peñuelas et al., 1998), and its emissions of biogenic terpenes seem to have an enhancement effect on the thermotolerance of leaves (Loreto et al., 1998; Peñuelas & Llusia, 1999).

We measured fluorescence, reflectance, monoterpane concentrations and visual leaf damage of *Q. ilex* whole seedlings exposed to temperature increases from 25 to 50°C in 5°C steps in atmospheres under photorespiratory (21% \( \text{O}_2 \)) or nonphotorespiratory conditions (2% \( \text{O}_2 \)), and under control or terpene fumigation conditions. Our general aim was to study the effects of (1) photorespiration, (2) monoterpenes, and (3) their interactions on thermotolerance. Our detailed aims were to determine (1) whether photorespiration protects from photodamage at high temperatures, (2) whether there is a link with monoterpane foliar concentrations, (3) whether plants are able to take up monoterpenes and increase the foliar concentrations, and (4) whether monoterpenes can replace or add to photorespiration in protection from photodamage at high temperature.

**Materials and Methods**

**Experimental system**

To avoid dysfunction and mismatching between the functioning of single leaf and the whole plant, we conducted the experiments with whole-seedling plants inside the fumigation chambers. Two-yr-old plants of *Q. ilex* L., previously grown in a nursery (Forestal Catalana, Breda, Spain) in Mediterranean-like environmental conditions, were transplanted to 5-l pots with a substrate composed of peat, Perlite and vermiculite (2 : 1 : 1). They were then exposed to a temperature ramp of 50°C steps from 25 to 50°C. The plants were maintained at each temperature for 30 min before measuring leaf chlorophyll fluorescence, leaf reflectance, and air terpene concentration and sampling leaves for VOC concentration analyses.

This experimental system (Fig. 1) is similar to the systems described by McCree (1986) and Smart et al. (1994). It consists of two independent Plexiglas chambers (0.5 × 0.5 × 0.5 m, 0.125 m³ volume) whose environmental conditions (photosynthetic photon flux density (PPFD), relative humidity and temperature) can be programmed independently by an automated control mechanism (Fig. 1). Light was supplied...
by three 500 W halogen lamps (Model 64702; Osram, Madrid, Spain) per chamber, each supplying a PPFD of about 500–860 µmol m$^{-2}$ s$^{-1}$ on a plane at half the height of the plant during a 10-h photoperiod. The lamps were placed about 50 cm above the plants and 7 cm of deionized water and 1 cm of Plexiglas in the container filtered the radiation. Air temperature and relative humidity in the chambers were measured with two HMP 112Y sensors (Vaisala Oy, Helsinki, Finland). The relative humidity in each chamber was regulated by controlling the water content of the air entering the chamber with a bypass control valve (Fig. 1). Air entered the chambers at about 355 µmol mol$^{-1}$ CO$_2$. This concentration was controlled by using an infrared gas analyzer (IRGA) and a manometer to regulate CO$_2$ flux from a CO$_2$ tank (Fig. 1). Air entering the chambers was cooled at 4°C to reduce its water content and a regulated proportion (independently determined for each chamber) passed through a bubble tank at 18°C before entering the chamber. For each chamber, the amount of air passed through the bubbler was regulated by a progressive valve (Model VXG41.15R; Landis & Gyr, Geneva, Switzerland) controlled by computer. The air temperature in each chamber was automatically regulated by adjusting the temperature bath where the air incoming to the chambers circulated. The airflow was measured with a mass flow meter (Model 5811N; Brooks Instruments, Veenendaal, The Netherlands) attached to each chamber. The temperature difference between the air and a chosen leaf of each plant was measured with a pair of thermocouples (Model LI-6000TC; Li-Cor, Lincoln, NE, USA). Data acquisition and process control of the entire system were carried out with the aid of a computerized data logger (Model HP3412A; Hewlett Packard, Palo Alto, CA, USA).

O$_2$ atmospheres and terpene fumigation

Four different atmospheres were assayed as result of the combination of 21% and 2% O$_2$ and of terpene-fumigation and non-fumigation. Monoterpene fumigation was applied each time to one of the Plexiglas chambers with a peristaltic pump that fluxed air through a flask containing 1 cm$^3$ of α-pinene and 1 cm$^3$ of limonene (99% purity; Fluka, Buchs, Switzerland). These monoterpenes are two of the most abundant monoterpenes produced by *Q. ilex* (Peñuelas & Llusia, 1999; Llusia & Peñuelas, 2000). This flask with
α-pinene and limonene was placed in a thermostatic bath at 20°C (Fig. 1). The concentration in the fumigated chamber was 2.6 ± 0.3 µg l⁻¹ (n = 10) for α-pinene and 1.9 ± 0.19 µg l⁻¹ (n = 10) for limonene, whereas the concentrations in the control chamber were 0.019 ± 0.006 µg l⁻¹ for α-pinene and 0.013 ± 0.0048 µg l⁻¹ for limonene. The system was allowed to equilibrate for 1 h before starting the measurements. For each one of these four different atmospheres, the temperature ramp experiment was repeated five or six times (five or six different plants).

Terpene analysis

Part of the air exiting the chamber flowed through a T-system to a glass tube (11.5 cm long and 0.4 cm internal diameter) manually filled with terpene adsorbents Carbotrap C (300 mg), Carbotrap B (200 mg), and Carbosieve S-III (125 mg) (Supelco, Bellefonte, PA, USA) separated by plugs of quartz wool. The hydrophobic properties of the tubes were supposed to minimize sample displacement by water. In these tubes, terpenes did not suffer chemical transformations, as checked by analysing replicate samples immediately after 48-h storage. Terpene analyses were conducted by gas chromatography–mass spectrometry (GC-MS) (Hewlett Packard HP59822B, California). Trapped monoterpenes were desorbed (Thermal Desorption Unit, Model 890/891; Supelco) at 250°C over 2 min and injected into a 30-m 0.25 × 0.25 mm film thickness capillary column (Supelco HP-5, Crosslinked 5% pH Me Silicone). After sample injection, the initial temperature (46°C) was increased at 30°C min⁻¹ up to 70°C, and thereafter at 10°C min⁻¹ up to 150°C, which was maintained for further 5 min. Helium flow was 1 cm³ min⁻¹. The identification of monoterpenes was conducted by GC-MS and comparison with standards from Fluka (Chemie AG, Buchs, Switzerland), literature spectra, and GCD Chemstation G1074A HP. Internal standard dodecane, which did not mask any terpene, together with frequent calibration with common terpene standards (α-pinene, β-pinene, 3-carene, β-myrcene, p-cymene, limonene and sabine) once every three analyses were used for quantification. Terpene calibration curves (n = 4 different terpene concentrations) were always highly significant (r² > 0.97) in the relationship between signal and terpene concentration. The most abundant terpenes had very similar sensitivity (differences were 5%).

Fluorescence measurements

The variable to maximum fluorescence ratio, Fv : Fm in the nonenergized state after darkness is a reliable measure of the potential efficiency of PSII photochemistry. It is used as an estimate of the functional state of the photosynthetic apparatus at a given environmental situation. A decrease in Fv : Fm indicates photoregulation of PSII (Oliveira & Peñuelas, 2001). An indicator of the actual photochemical activity is the electron transport rate (ETR). Genty et al. (1989) have shown that PSII radiation-use efficiency may be determined with the expression:

\[ \Delta F/F\text{m} = (F\text{m} - F)/F\text{m} \]  
Eqn 1

derived from measurements of leaf fluorescence under ambient light (F), and under saturating light pulses (Fm). This ΔF/Fm can be used to calculate relative ETR, which represents the apparent photosynthetic electron transport rate in µmol electrons m⁻² s⁻¹ (Schreiber et al., 1995), by multiplying the quantum yield of PSII (ΔF/Fm) by leaf absorbance (A) and by half PPFD, since it is assumed that radiant energy is equally absorbed by the two photosystems:

\[ \text{ETR} = \Delta F/F\text{m} \times \text{PPFD} \times 0.5 \times A \]  
Eqn 2

At each temperature of the experimental ramp, chlorophyll fluorescence of apical mature leaves was determined using a portable modulated fluorometer PAM-2000, including the leaf clip holder part 2030-B (Heinz Walz, Effeltrich, Germany). Minimal fluorescence of a dark adapted leaf with all PSII reaction centers fully open (Fo), maximal fluorescence yield of a dark-adapted leaf with all PSII reaction centers fully closed (Fm), maximal (potential) photochemical efficiency of PSII (given by the Fv : Fm ratio) after dark adaptation for 20 min, and actual photochemical efficiency of PSII (Fm – Fs)/Fm or ΔF/Fm were measured, as well as leaf temperature and incident PPFD.

Reflectance measurements

Another indirect optical measure of photosynthetic performance with similar nondestructive and speed properties can be derived from the reflectance changes near 531 nm (Gamon et al., 1992; Peñuelas et al., 1995; Peñuelas & Filella, 1998). These changes are associated with xanthophyll pigment interconversion and chloroplast conformational changes, and correlate with both epoxidation state of the xanthophyll cycle pigments and photosynthetic radiation-use efficiency.
The de-epoxidation state of the xanthophyll cycle, DPS = (A + Z)/(V + A + Z), is an additional indicator of the functional state of chloroplasts. When light becomes excessive, violaxanthin (V) is de-epoxidized to zeaxanthin (Z) through antheraxanthin (A).

Leaf spectral radiance was measured by directing a fiber optic probe to the leaf surface at the same angle of the leaf clip holder of the PAM-2000 fluorometer. The other end of the fiber optic was attached to a spectroradiometer (Spectral analysis VIS/NIR portable equipment UniSpec; PP-Systems, Haverhill, MA, USA). Reflectance was calculated by normalizing leaf radiance by the radiance of a 99% reflective standard (Spectralon; Labsphere, North Sutton, NH, USA).

To follow the reflectance changes at 531 nm associated with the photosynthetic radiation-use efficiency and with the dissipation of excess energy, we used the ‘photochemical reflectance index’ PRI (Gammon et al., 1992; Peñuelas et al., 1995; Peñuelas & Filella, 1998), calculated as follows:

\[
PRI = \frac{(R531 - R570)}{(R531 + R570)} \quad \text{Eqn 3}
\]

where R is reflectance and the numbers for the wavelength in nm.

We also measured other reflectance indices such as normalized difference vegetation index (NDVI) (R900 – R680)/(R900 + R680), indicative of greenness, water index (WI) (R900/R970) indicative of water content or normalized total pigment to chlorophyll ratio index (NPCI) (R485 – R680)/(R485 + R680) indicative of the ratio between carotenoids and chlorophyll (Peñuelas & Filella, 1998).

Leaf sampling and terpene concentration analysis

Individual leaves were sampled at each temperature after the measurement of fluorescence and reflectance and were immediately submerged in liquid nitrogen. For extraction of terpenes in the atmospheres of the different treatments, the absence of breakthrough was checked by placing two traps in series and by verifying that no monoterpenes were collected in the second one. Standards of the different monoterpenes were also frozen and extracted with the same method to check for absence of losses. Monoterpenes trapped in the adsorbent tubes were desorbed and measured by GC as described above.

Leaf measurements: damage, area and dry weight

The percentage of damaged (dicolored or brown) leaves was measured immediately at the end of each temperature-ramp experiment between 25°C and 50°C by measuring the damage area of all the plant leaves. Leaf area (separating damaged and healthy) was measured in the laboratory using a Li-Cor LI-3100 area meter. The leaf dry mass was determined after drying at 60°C until constant mass.

Statistical analyses

All the temperature-ramp experiments described were repeated five or six times on different plants. Repeated measures analyses of variance (ANOVAs), and regression analyses were conducted using STATISTICA version 5.0 for Windows (StatSoft, Tulsa, Ok, USA). To test for treatment effects at each temperature (or at each O2 concentration and at each fumigation treatment when analysing leaf visual damage), one-way ANOVAS were also conducted.

Results and Discussion

Photorespiration and thermotolerance

Under photorespiratory conditions, the Fv/Fm index, fluorescence measure of the potential photochemical efficiency of PSII (Oliveira & Peñuelas, 2001) was maximum at 25°C, decreased slowly up to 40°C, and thereafter it decreased rapidly (Fig. 2). A similar pattern was found for ETR (Fig. 2) although at 45°C it presented a stronger decrease. This temperature of 45°C is close to the critical temperature of 47–48°C derived from chlorophyll fluorescence in other studies of Q. ilex (Méthy et al., 1997). These results confirm that one of the primary effects of high-temperature stress is the damage to photosynthetic electron transport through PSII (Berry & Björkman, 1980). Heat damage arises from inactivation of the highly sensitive water-splitting reaction, disconnection of PSII centers from the bulk pigments, thermal uncoupling of photophosphorylation, and biomembrane lesions (Berry & Björkman, 1980). The greatest damage was found close to 50°C. Necrosis appeared at this temperature, as has also been reported by Larcher (2000). This was also indicated by NDVI and WI values in the visually undamaged leaves, which strongly decreased from 0.83 ± 0.01 to 0.7 ± 0.06, and from 1.03 ± 0.003 to 1.01 ± 0.007 (data not shown), respectively, indicating foliar loss of green pigments and water (Peñuelas & Filella, 1998).

When photorespiration was inhibited (under 2% O2 atmospheres), Fv/Fm decreased (indicating a decrease of potential photochemical efficiency of PSII) markedly already at 35°C, and Fo was always greater (indicating reduction of the energy trapping by PSII centers) and ETR was always smaller than under 21% O2 atmospheres, as expected from the inhibition of photorespiration (Fig. 2). The photochemical reflectance index, the reflectance index of photochemical efficiency linked to zeaxanthin formation, did not change in the whole range of temperatures tested in control atmospheres.

However, PRI abruptly decreased at 50°C under nonphotorespiratory conditions (Fig. 3), indicating enhancement of the dissipation of excess of energy through the xanthophyll de-epoxidation cycle (Gamon et al., 1992; Peñuelas et al., 1995; Peñuelas & Filella, 1998). Finally, the inhibition of photorespiration increased leaf visual damage threefold after the temperature treatment (Fig. 4).

These results confirm the decreased ETR and increased dissipation as heat found in other studies under nonphotorespiratory conditions. Moreover, these other studies reported accumulation of photorespiratory metabolites, decreased number of starch grains in chloroplasts (among other changes in plant morphology) and lower growth under nonphotorespiratory conditions (Kozaki & Takeba, 1996; Migge et al., 1999; Niyogi, 1999). It is likely that when the supply of CO₂ to leaves is cut off by stomatal closure at high temperatures, and photorespiration does not work, the Calvin–Benson C₃ cycle will not operate. Under those conditions, light energy may be diverted to produce active oxygen, which damage the photosynthetic apparatus (Aro et al., 1993; Kozaki & Takeba, 1996).
Monoterpenes and thermotolerance

Loreto et al. (1998) found monoterpenene emissions to be stimulated by low atmospheric O₂ concentrations. Here, we found decreased leaf concentrations (a higher rate of emissions could lead to these lower concentrations, but this hypothesis should be tested in further studies with simultaneous measurements of concentrations and emissions). The leaf concentrations of α-pinene and limonene were smaller (less than half) in the absence of photorespiration, especially at the highest temperatures, when leaf concentrations were also the highest (Fig. 5). As the protective effect of photorespiration was accompanied by these two to three times greater foliar terpene concentrations at the highest temperatures, we experimentally increased the concentrations of α-pinene and limonene, two of the most abundant monoterpenes produced by Q. ilex (Peñuelas & Llusia, 1999). Under fumigation, the concentrations of α-pinene in the chamber air increased from 0.019 ± 0.006 to 2.6 ± 0.3 µg l⁻¹, and the concentration of limonene increased from 0.013 ± 0.0048 to 1.9 ± 0.19 µg l⁻¹.

As a result, α-pinene and limonene concentrations in the Q. ilex leaves increased between two- and four-fold relative to the endogenous concentrations (Fig. 6). The maximum increase occurred at 40°C and then decreased again in parallel with the stomatal conductance response of the seedlings of this species to temperature (Larcher, 1995; Ogaya & Peñuelas, unpublished; J. Llusia & J. Peñuelas, unpublished). These increases in leaf concentration showed that there was terpene acquisition by Q. ilex plants and that it probably occurred through stomata. Many factors affect stomatal conductance. Most of them (irradiance, air humidity and CO₂ partial pressure) were the same for the different treatments, but low O₂ concentrations and terpene fumigation may affect stomatal conductance. However, for the same O₂ and terpene fumigation conditions, the changes in terpene acquisition and previous measurements of stomatal conductance response to temperature (Larcher, 1995; Ogaya & Peñuelas, unpublished; J. Llusia & J. Peñuelas, unpublished) indicated that stomatal conductance changes were mostly driven by temperature changes.

Monoterpenene fumigation, and consequent two- to four-fold increased monoterpenene leaf concentrations, did not enhance thermotolerance of Q. ilex plants, as measured by Fo, Fv/Fm and ETR under normal atmospheres (Fig. 7). Delfine et al. (2000) did not find improved thermotolerance when internal content was similar to the endogenously formed. They only found thermotolerance when high fumigation doses yielded about fivefold the endogenous pool of Q. ilex. High concentrations might be needed to achieve thermotolerance under standard photorespiratory conditions. These high monoterpenene concentrations could be reached in field conditions under typical summer stress in the Mediterranean region, where drought closes stomata and favors monoterpenene accumulation because they are less volatile than other VOCs such as isoprene.

However, monoterpenene fumigation did increase thermotolerance (Fo was 25–35% lower, Fv/Fm was 0.1–0.2 units higher and ETR was 20–40 µmol electrons m⁻² s⁻¹ higher) under nonphotorespiratory conditions at temperatures higher than 35°C (or than 40°C for Fo) (Fig. 7). Under these nonphotorespiratory conditions, and with monoterpenene fumigation, PRI recovered the standard value at 50°C (Fig. 8) indicating that zeaxanthin formation was not enhanced. Terpenes would have membrane-stabilizing properties similar to this other isoprenoid, zeaxanthin, at high temperatures (Singsaas, 2000) and could substitute xanthophyll de-epoxidation protection under nonphotorespiratory conditions and very high temperatures (50°C). Under the nonphotorespiratory conditions, monoterpenene fumigation decreased the leaf visual damage after the temperature treatment from 77% to 50% (Fig. 9). This was also indicated by NDVI and WI reflectance indices, which did not decrease at 50°C. Values of 0.85 and 1.03 were maintained for NDVI and WI (data not shown), respectively, when under the nonphotorespiratory conditions plants were monoterpenene-fumigated.

Thus, fumigation with monoterpenenes at physiologically realistic concentrations was able to improve tolerance to temperatures higher than 35°C only when photorespiration was
Sharkey & Singsaas (1995) also found that the protective effect of exogenous isoprene from thermal stress was enhanced under conditions that inhibited endogenous formation. We speculate that monoterpenes may have, at least in part, a photorespiration-like protection role that is enhanced when photorespiration is absent.

There is not enough evidence of the mechanism or mechanisms and the interactions involved in this phenomenon of enhanced PSII thermotolerance by photorespiration and monoterpenes. Some xanthophylls, which are also isoprenoids, are known to increase thermotolerance. Zeaxanthin, when present in the membranes, reduces photon leakage at high temperatures (Tardy & Havaux, 1997). The hypothesis is that zeaxanthin may act as a solute in the membrane, which stabilizes its structure at high temperatures. Although terpenes are extremely hydrophobic they could have similar membrane-stabilizing properties (Singsaas, 2000). It is likely that the thermotolerance achieved results from both the terpene-induced thermostable conformations of the membrane-connected thylakoid protein subunits and the terpene-induced adjustment of membrane lipid fluidity. However, our data for monoterpene enhancement of thermotolerance only under nonphotorespiratory conditions fits better with a mechanism linked to the monoterpene capacity to scavenge photosynthesis-derived reactive oxygen species. These reactive oxygen species raise their concentration inside the leaf during periods of high temperature and light, especially when photorespiration is not active in inhibiting their formation (Aro et al., 1993; Kozaki & Takeba, 1996). The monoterpenes would use their capacity to scavenge the radical oxygen species produced under high temperatures.

In addition to isoprene, monoterpenes and xanthophylls, several heat-shock proteins accumulate in vegetative tissues in response to heat stress (Vierling, 1991) and one of them, the small chloroplasts’ heat-shock protein, has been demonstrated to protect PSII from high-temperature damage (Heckathorn et al., 1998). However, unlike the relatively rapid reversibility of isoprene and terpene emission and xanthophyll epoxidation that should enhance thermotolerance during short periods of high temperature, protection from heat-shock proteins generally requires several hours for induction and the proteins are around for a long time (Vierling, 1991, Hanson & Sharkey, 2001). Thus, it is unlikely that these heat-shock proteins could mask some of the beneficial effects of photorespiration or terpenes in the short-period high temperatures studied. Moreover, under photorespiratory conditions and terpene fumigation plants suffered significant damage (Fig. 9), with no indication of possible protective role of heat-shock proteins.

Finally, and to summarize these results, several important conclusions can be drawn from this study. First, photorespiration seems to be necessary to avoid photochemical damage,
especially at high temperatures. Second, terpene concentrations are higher under photorespiratory than under non-photorespiratory conditions. Third, plants are able to acquire exogenous monoterpenes and the acquisition response to temperature follows stomatal conductance response. Fourth, since we did not find thermotolerance enhancement under standard photorespiratory conditions and instead found thermotolerance enhancement under non-photorespiratory conditions, monoterpenes seem to replace photorespiration in the plant’s protection against high temperatures. Fifth, the
monoterpene capacity to scavenge reactive oxygen species seems a better explanation for these results than membrane-stabilization capacity. Sixth, these results warrant further studies to deal with the biochemical processes that are involved in these terpene and photorespiration effects on thermotolerance (the competition for electrons between terpene synthesis pathways and photorespiration is a possible one) and the physiological and ecological role of differential terpene synthesis among species and conditions.

Acknowledgements

We thank F. Rodà and J. Terradas for insightful comments on the manuscript, and J. Casadesús and Servei Camps Experimentals Fac. Biologia University Barcelona for technical assistance. This research was supported by CICYT grants REN2000-0278/CLI and REN2001-0003/GLO, and Generalitat grant DURSI-DMA IMMPACTE 1999.

References


Logan BA, Monson RK. 1999. Thermodermality of leaf discs from four isoprene-emitting species is not enhanced by exposure to exogenous isoprene. Plant Physiology 120: 821–826.


---

About New Phytologist

New Phytologist is owned by a non-profit-making charitable trust dedicated to the promotion of plant science. Regular papers, Letters, Research reviews, Rapid reports and Methods papers are encouraged. Complete information is available at www.newphytologist.com

All the following are free – essential colour costs, 100 offprints for each article, online summaries and ToC alerts (go to the website and click on Synergy)

You can take out a personal subscription to the journal for a fraction of the institutional price. Rates start at £83 in Europe/$133 in the USA & Canada for the online edition (go to the website and click on Subscriptions)

If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 594691) or, for a local contact in North America, the USA Office (newphytol@ornl.gov; tel 865 576 5251)