

## Impact of ozone on monoterpene emissions and evidence for an isoprene-like antioxidant action of monoterpenes emitted by *Quercus ilex* leaves

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**Summary** *Quercus ilex* (L.) leaves emit monoterpenes, particularly  $\alpha$ -pinene,  $\beta$ -pinene and sabinene. Apart from the monoterpene pools that are stored in specialized structures and have a clear defensive or attractive role, the function of monoterpenes in *Q. ilex* leaves is unknown. We tested whether monoterpenes have an antioxidant role, as has previously been found for isoprene in isoprene-emitting leaves. We exposed *Q. ilex* leaves to either mild and repeated ozone exposure (Experiment I) or to a single acute ozone exposure (Experiment II) at temperatures ranging between 20 and 32 °C. Both ozone treatments rapidly stimulated monoterpene synthesis, but had no effect on photosynthesis and caused no visible damage to leaves maintained at 25, 30 or 32 °C. Ozone inhibited both photosynthesis and monoterpene synthesis in leaves maintained at 20 °C. To characterize the relationship between monoterpenes and ozone-induced damage, we fed detached leaves fosmidomycin, a selective inhibitor of isoprene synthesis. Fosmidomycin caused rapid and complete inhibition of monoterpene emissions in leaves maintained at 30 °C, confirming that monoterpenes are synthesized by the same biochemical pathway as isoprene. However, over the experimental period, fosmidomycin did not affect concentrations of compounds that are formed from chloroplastic isoprenoids and that might have conferred antioxidant protection, either directly (carotenoids) or indirectly (chlorophylls, xanthophylls). In leaves whose monoterpene synthesis had been inhibited by fosmidomycin, ozone rapidly and significantly inhibited photosynthesis and increased the production of hydrogen peroxide and malondialdehyde. We conclude that monoterpenes produced by *Q. ilex* leaves share the same biosynthetic pathway and function as isoprene. Furthermore, all volatile isoprenoids may have similar antioxidant properties and may be stimulated by the same stress-inducing conditions.

**Keywords:** chlorophyll fluorescence, fosmidomycin, isoprenoids, photosynthesis, reactive oxygen species.

### Introduction

Isoprenoid emission is widespread in the plant kingdom (Kesselmeier and Staudt 1999). There are two isoprenoid biosynthetic pathways: the classic mevalonic acid pathway produces isoprenoids extra-chloroplastically, whereas the recently discovered methyl-erythritol-phosphate (MEP) pathway (Lichtenthaler et al. 1997) produces isoprenoids in chloroplasts. “Volatile” isoprenoids are produced through both pathways, although isoprene and several monoterpenes, considered to be the most important biogenic volatile organic compounds, are formed only through the MEP pathway in the main isoprenoid-emitting species (Lichtenthaler et al. 1997). Monoterpenes are also stored in large quantities in extra-chloroplastic reservoirs where they have defensive or attractive functions (Gershenzon and Croteau 1991). Isoprene is not stored in large pools and its role is poorly understood. Some experimental evidence indicates that it has a protective role against heat stress (Sharkey and Singsaas 1995, Singsaas et al. 1997, reviewed by Sharkey and Yeh 2001). More recently, it has been demonstrated that isoprene may protect against a range of stresses, including ozone-induced oxidative stress (Loreto et al. 2001, Loreto and Velikova 2001), or singlet oxygen accumulation (Affek and Yakir 2002).

Some Mediterranean *Quercus* species emit isoprene-like monoterpenes, which are light-dependent and are rapidly labeled with <sup>13</sup>C, indicating that the monoterpene pool is small and that the emitted monoterpenes are synthesized from carbon recently fixed through photosynthesis, as found for isoprene (Loreto et al. 1996a, 1996b). Is the similarity in biochemistry of isoprene and monoterpenes emitted by Mediterranean plant species indicative of similar functional roles for these compounds? There is evidence that monoterpenes improve thermotolerance at elevated temperatures (Loreto et al. 1998). The possible antioxidant role of monoterpenes has not been examined. This can be done by comparing in vivo and in vitro damage caused by oxidative stresses in leaves with different rates of isoprenoid emission. Isoprene emission can be

selectively and almost completely inhibited by fosmidomycin (Zeidler et al. 1998, Loreto and Velikova 2001, Sharkey et al. 2001), or it can be consistently reduced by exposing plants to low temperatures (Loreto and Sharkey 1990). Low temperature has the same effect on monoterpene emissions (Loreto et al. 1996a), but the effect of fosmidomycin on monoterpene synthesis is unknown.

Two ozone fumigation experiments were carried out on *Quercus ilex* (L.) leaves to determine if: (a) fosmidomycin inhibits monoterpene synthesis in Mediterranean oaks; (b) ozone affects monoterpene emission; and (c) monoterpenes have an antioxidant action that protects plants against oxidative stresses. We provide evidence for a common biochemical origin, biosynthetic pathway and functional role for volatile isoprenoids, and show that monoterpenes and isoprene emitted by Mediterranean oaks have an isoprene-like protective role against ozone.

## Materials and methods

### Plant material

*Quercus ilex* plants were grown at the Botanical Garden of the University of Roma I, in 3-l pots filled with commercial sandy soil. Plants were regularly irrigated and fertilized to avoid nutritional stress and drought. All plants were grown for 3 years under tall pine trees in typical Mediterranean ecosystem conditions characterized by moderate illumination of the understory vegetation before the treatments were imposed.

### Experiment I: Whole-plant ozone fumigation

Four 3-year-old plants were assigned to each of four groups. Two groups (controls) were grown for 1 week in two growth chambers (Model CT15, LABCO, Califon, NJ) under controlled conditions similar to those experienced in the botanical garden. Photosynthetic photon flux was  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ , photoperiod was 12 h and relative humidity was 65%. One control group was grown at a day/night temperature of 25/20 °C, another at 32/25 °C. The two groups of treated plants were grown under the same conditions as the control plants except that, after 2 days of acclimation in the growth chambers, they were fumigated with an increasing ozone concentration for 5 days, to simulate rising ozone concentration in the atmosphere during hot and still summer days. Ozone was provided at 100, 120, 130, 150 and 200 ppb on Days 3–7 from the beginning of the experiment, respectively, and fumigation lasted for 4 h per day. The total ozone dose at the end of the experiment was  $2.8 \text{ ppm h}^{-1}$ . Ozone was generated by passing oxygen through a UV source. Ozonated air was then introduced into the chambers through each chamber's air exchange system. The amount of ozone generated was regulated by changing the output of the UV source and was measured photometrically with an ozone detector (Model 1008-RS, Dasibi Environmental, Glendale, CA) at the chamber outlet. Care was taken to minimize boundary layer effects (total air flow  $> 30 \text{ l min}^{-1}$ ), and heterogeneity of ozone distribution in the cham-

bers ( $< 10\%$  of the mean concentration, as assessed by placing the detector inlet in different parts of the chambers).

Ozone damage was assessed immediately after ending fumigation and 2 days after the fumigation period by monitoring visible damage and measuring physiological parameters indicating alteration of photosynthetic carbon metabolism ( $\text{CO}_2$  exchange, chlorophyll fluorescence) and stomatal function ( $\text{H}_2\text{O}$  exchange). Physiological measurements were made with a CIRAS-1 portable system (PP Systems, Hertfordshire, U.K.) immediately after the ozone treatment, and with the laboratory gas exchange/fluorescence system described by Loreto and Velikova (2001) 2 days after the ozone treatment. During this second round of measurements, photochemical yield in dark-adapted and in illuminated leaves was measured by fluorescence. Monoterpene emissions were measured by gas chromatography as described by Loreto and Velikova (2001). This system allows highly sensitive ( $< 0.1 \text{ ppb}$ ) measurements of both isoprene and monoterpene emissions. Because complete detection of the emitted monoterpene profile could be made over a 21-min time step, all gas chromatography measurements of monoterpene emissions were carried out over this time period. Measurements were made on single leaves attached to the plants and enclosed in the gas exchange cuvette. During measurements, leaves were maintained under environmental conditions (irradiance, day temperature, relative humidity) similar to those experienced in the growth chambers. Synthetic air representing ambient  $\text{CO}_2$  (350 ppm) and  $\text{O}_2$  (20%) concentration, but free of ozone and other contaminants, was used during measurements as explained previously (Loreto and Velikova 2001).

Physiological measurements were made on one leaf per plant. Differences among means ( $n = 4$ ) were separated statistically by the Student-Neuman-Keuls post hoc test for multiple comparisons.

### Experiment II: Single leaf ozone fumigation

Single leaves attached to the plant (Treatments 1 and 3) or cut and maintained with the petiole in water (Treatment 2) were enclosed in the laboratory gas exchange cuvette, and  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and monoterpene exchange and fluorescence emission were measured as described for Experiment I (cf. Loreto and Velikova 2001). Leaves were exposed to the same environmental conditions as described for Experiment I except that the leaf temperature was changed as specified for each treatment. After monitoring steady-state gas exchange ( $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and monoterpenes), leaves were exposed for 4 h to 250 ppb of ozone which was generated, regulated and monitored as explained by Loreto and Velikova (2001).

In Treatment 1, the effects of ozone fumigation on monoterpene emissions and on the physiological parameters were followed daily on leaves ( $n = 4$ ) maintained at 30 °C. Monoterpene emissions were first measured 4 h after the end of ozone fumigation and then once a day for 3 days.

In Treatment 2, leaves ( $n = 4$ ) were cut, maintained with the petiole in water and fed with  $30 \mu\text{M}$  fosmidomycin, an inhibitor of isoprene synthesis (Zeidler et al. 1998), as described by Loreto and Velikova (2001). All other environmental param-

ters were the same as those in Treatment 1. The effects of fosmidomycin feeding on monoterpene emissions and on the other physiological parameters were followed for 2 h. Leaves were then fumigated with ozone for 4 h as previously described. The effects of ozone fumigation on monoterpene emissions and other physiological parameters of fosmidomycin-fed leaves were compared with values obtained for leaves ( $n = 4$ ) that were cut and maintained in water but not fed with fosmidomycin. Gas exchange measurements were carried out 2 h after the end of the ozone treatment, whereas fluorescence measurements were carried out during the ozone treatment. After the physiological measurements, leaves were rapidly frozen in liquid nitrogen and analyzed for hydrogen peroxide and malonyldialdehyde (MDA) as described by Loreto and Velikova (2001). To test whether fosmidomycin affected nonvolatile isoprenoids, further batches of fosmidomycin-fed and control leaves ( $n = 4$  for each treatment) were used. Leaves were exposed to the same environmental conditions as in the previous experiments but were not exposed to ozone. Fosmidomycin feeding (30  $\mu\text{M}$ ) was carried out for 8 h to simulate the time-course of the ozone treatment and to ensure complete inhibition of monoterpene synthesis. Leaf disks (2.3  $\text{cm}^2$ ) were then punched and the tissue powdered in liquid nitrogen. Concentrations of chlorophylls and total carotenoids were determined spectrophotometrically after pigment extraction with 80% acetone and centrifugation for 20 min at 10,000  $g$ . Details of the methods and coefficients for pigment calculation are given in Wellburn (1994). Xanthophyll de-epoxidation status was determined as described by Brugnoli et al. (1994). Briefly, after extraction with acetone and centrifugation, the filtered supernatant was injected onto an HPLC column (Perkin Elmer series 4), and xanthophylls were separated on a non-end-capped column (Alltech Alltima C18 5 $\mu$ ). The de-epoxidation status was calculated as the ratio:  $(Z + A)/(Z + A + V)$ , where Z is zeaxanthin, A is antheraxanthin and V is violaxanthin.

In Treatment 3, the effects of ozone fumigation on leaves maintained at 30 °C during fumigation were compared with leaves maintained at 20 °C during ozone fumigation.

In each treatment, leaves from different plants were chosen for measurement. Physiological parameters and monoterpene emissions were measured as described for Experiment I (cf. Loreto and Velikova 2001). Values are presented as means  $\pm$  standard errors. Differences among means ( $n = 4$ ) were separated statistically by the Student-Neuman-Keuls test ( $P \leq 0.01$ , 0.05, or 0.10).

## Results

In Experiment I, plants exposed to ozone showed no visible damage, no reductions in  $\text{CO}_2$  and  $\text{H}_2\text{O}$  exchange, and no photochemical damage as monitored by fluorescence, irrespective of the temperature treatment (data not shown). *Quercus ilex* leaves emitted 14 monoterpenes, as previously observed (Loreto et al. 1996b). Because about 75% of the total emissions comprised three compounds ( $\alpha$ -pinene,  $\beta$ -pinene and

sabinene), we show results only for these monoterpenes, for which detection was simple and straightforward and the impact of possible measurement errors was negligible. Monoterpene emissions were strongly reduced by low temperature (20 °C) in control plants (Figure 1). Two days after the ozone fumigation period, the ozone-treated plants showed significantly higher emission of all monoterpenes at both 25 °C (Figure 1a) and 32 °C (Figure 1b) than controls unexposed to ozone.

In Experiment II, Treatment 1 confirmed that exposure to ozone stimulated emission of every monoterpene (Figure 2), but had no effect on photosynthesis and other physiological parameters measured (e.g., Figure 4). Monoterpene emissions increased significantly within a few hours after ozone treatment and continued at a higher rate for at least 3 days after the fumigation period.

Feeding the leaves with fosmidomycin inhibited the emission of monoterpenes (Figure 3). The emissions declined after 21 min and were virtually undetectable 1 h after feeding with fosmidomycin. Over the duration of the experiment, fosmidomycin had no effect on concentrations of chlorophyll and total carotenoids, or on the de-epoxidation status of xanthophylls (Table 1). Although fosmidomycin had no effect on photosynthetic rate or the photochemistry of photosynthesis (Fig-

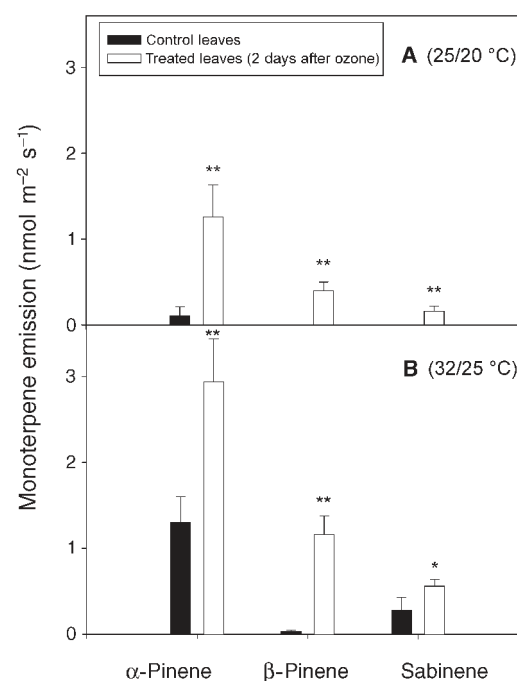


Figure 1. Emissions of the three most abundant monoterpenes from leaves of *Quercus ilex* plants in the absence of ozone (controls) or after repeated exposure to ozone (Experiment I). Emissions from ozone-treated plants were measured 2 days after the end of the ozone treatment. Plants were treated and measured at day/night temperatures of 25/20 °C (A) or 32/25 °C (B). Means  $\pm$  SE ( $n = 4$ ) are shown. Statistically significant differences between emissions of each monoterpene from leaves of the control and ozone-treated plants ( $P \leq 0.05$  or  $P \leq 0.10$ ) are shown by \*\* and \*, respectively.

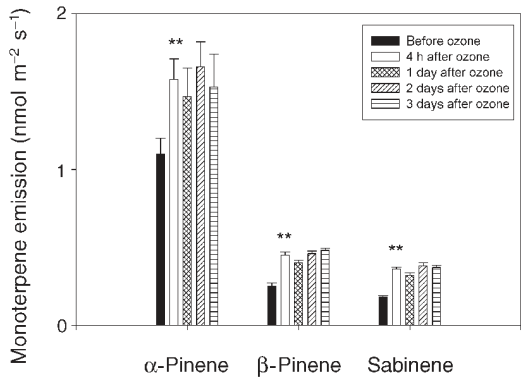


Figure 2. Emissions of the three most abundant monoterpenes from leaves of *Quercus ilex* before and after exposure to a single acute ozone treatment (Experiment II). Emissions from ozone-treated plants were measured from 4 h to 3 days after the end of the ozone treatment. Means  $\pm$  SE ( $n = 4$ ) are shown. The asterisks indicate statistically significant differences between emissions of each monoterpene before and immediately after ozone treatment ( $P \leq 0.05$ ). Differences during recovery were not statistically significant.

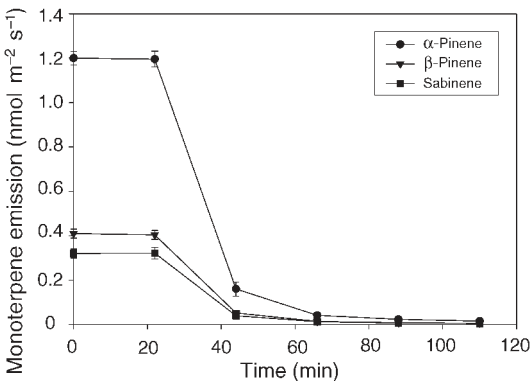


Figure 3. Effects of fosmidomycin feeding on the three most abundant monoterpenes emitted by leaves of *Quercus ilex*. At time 0, cut leaves with steady-state rates of photosynthesis and monoterpene emissions were fed with 30  $\mu$ M fosmidomycin. The inhibitory effect of fosmidomycin on monoterpenes was followed until emissions became undetectable. Means  $\pm$  SE ( $n = 4$ ) are shown.

ure 4), it increased the sensitivity of the leaf to subsequent ozone exposure. Photosynthesis and the quantum yield of photosystem II (PSII), estimated by the fluorescence param-

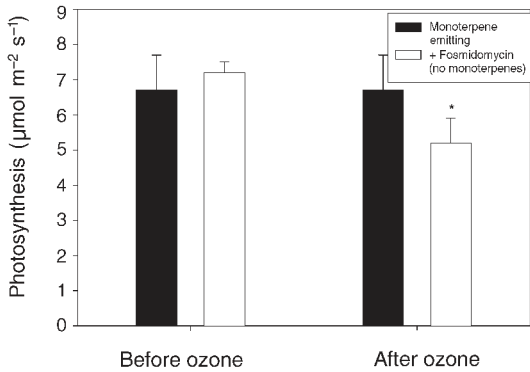


Figure 4. Effect of a single acute ozone treatment (Experiment II) on photosynthesis of leaves emitting monoterpenes and on leaves in which monoterpene emissions were previously inhibited by fosmidomycin as shown in Figure 3. Means  $\pm$  SE ( $n = 4$ ) are shown. The asterisk indicates a statistically significant difference between photosynthesis of control leaves that were emitting monoterpenes and fosmidomycin-fed leaves that were not emitting monoterpenes ( $P \leq 0.10$ ).

ter  $\Delta F/F_m'$ , were significantly reduced in leaves fed with fosmidomycin and subsequently exposed to ozone compared with every other treatment. This was particularly evident when the leaf-to-leaf intrinsic photosynthetic differences were cancelled out by expressing the ozone treatment effect as a percent decrease in each parameter (photosynthesis and quantum yield of PSII) with respect to the values measured on the same leaf before the ozone treatment (Figure 5). The inhibition was rapid and progressive. The fluorescence trace showed a significant reduction in  $\Delta F/F_m'$  in fosmidomycin-fed leaves within 1 h after beginning the exposure to ozone (Figure 6) and the reduction increased steadily during the 4-h ozone fumigation (Figure 6). Fosmidomycin-fed leaves also showed significantly higher accumulations of hydrogen peroxide and MDA following ozone treatment compared with leaves treated with ozone alone ( $P \leq 0.05$  in both cases; Table 2).

Between leaves maintained at 20 or 30  $^{\circ}$ C, photosynthesis did not differ (Figure 7a), but the fluorescence yield (Figure 7b) was considerably lower at 20  $^{\circ}$ C, and emission of monoterpenes was almost undetectable (data not shown but see the low emission from leaves kept at 25  $^{\circ}$ C, Figure 1). Photosynthesis and  $\Delta F/F_m'$  in leaves maintained at 20  $^{\circ}$ C were significantly reduced by exposure to ozone (Figure 7).

Table 1. Effects of fosmidomycin on the concentrations of chlorophylls and carotenoids, the de-epoxidation status of xanthophylls, and monoterpene emissions. Means ( $n = 4$ )  $\pm$  SE are shown for controls and leaves 8 h after feeding with 30  $\mu$ M fosmidomycin. The asterisks indicate statistically significant ( $P \leq 0.01$ ) differences between controls and fosmidomycin-fed leaves. Abbreviations: Z = zeaxanthin; A = antheraxanthin; and V = violaxanthin.

Treatment	Monoterpenes (nmol m <sup>-2</sup> s <sup>-1</sup> )	Chlorophylls (μg cm <sup>-2</sup> )	Carotenoids (μg cm <sup>-2</sup> )	Xanthophyll de-epoxidation (Z + A)/(V + A + Z)
Control	2.0 $\pm$ 0.3	53.1 $\pm$ 1.4	4.0 $\pm$ 0.2	0.15 $\pm$ 0.02
Fosmidomycin	0.03 $\pm$ 0.05 ***	55.4 $\pm$ 2.5	4.2 $\pm$ 0.3	0.16 $\pm$ 0.04

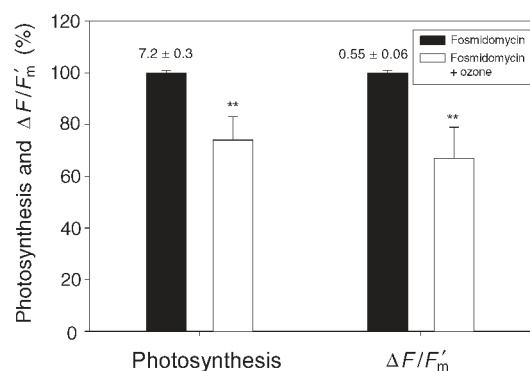


Figure 5. Photosynthesis and the quantum yield of photosystem II as monitored by the fluorescence parameter  $\Delta F/F_m'$  in leaves with monoterpene emissions inhibited by fosmidomycin before and after a single acute ozone exposure. Both parameters are expressed as a percent of the same parameters measured on the same leaves before fosmidomycin feeding. Actual values are also shown for leaves before the ozone treatment (numbers above filled bars). Means  $\pm$  SE ( $n = 4$ ) are shown. The asterisks indicate a statistically significant difference between measurements before and after the ozone treatment ( $P \leq 0.05$ ).

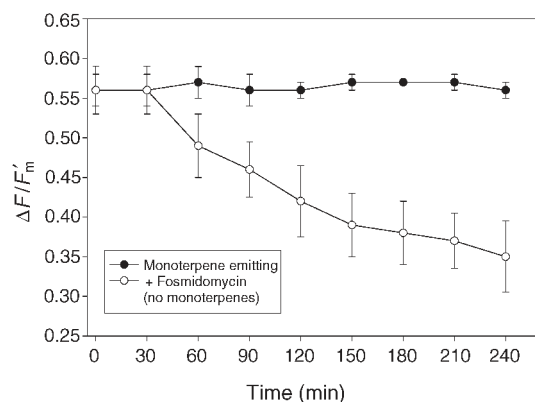


Figure 6. Effects of a single acute ozone treatment on the quantum yield of photosystem II as monitored by the fluorescence parameter  $\Delta F/F_m'$  in leaves emitting monoterpenes (●) and in leaves in which monoterpene emissions were previously inhibited by fosmidomycin as shown in Figure 3 (○). Fluorescence measurements were made every 30 min during the 4-h ozone treatment. Means  $\pm$  SE ( $n = 4$ ) are shown.

Table 2. Effects of ozone on concentrations of hydrogen peroxide ( $H_2O_2$ ) and malonyldehydro (MDA) in *Quercus ilex* leaves emitting monoterpenes and in leaves in which monoterpene emissions were previously inhibited by fosmidomycin as shown in Figure 3. Means ( $n = 4$ )  $\pm$  SE are shown. The asterisks indicate statistically significant differences ( $P \leq 0.05$ ) between treatments and control values.

Treatment	$H_2O_2$ ( $\mu\text{mol g}_{FM}^{-1}$ )	MDA ( $\mu\text{mol g}_{FM}^{-1}$ )
Control	$3.6 \pm 0.2$	$0.12 \pm 0.01$
Control + ozone	$3.9 \pm 0.3$	$0.13 \pm 0.01$
Fosmidomycin + ozone	$4.8 \pm 0.5^{**}$	$0.18 \pm 0.03^{**}$

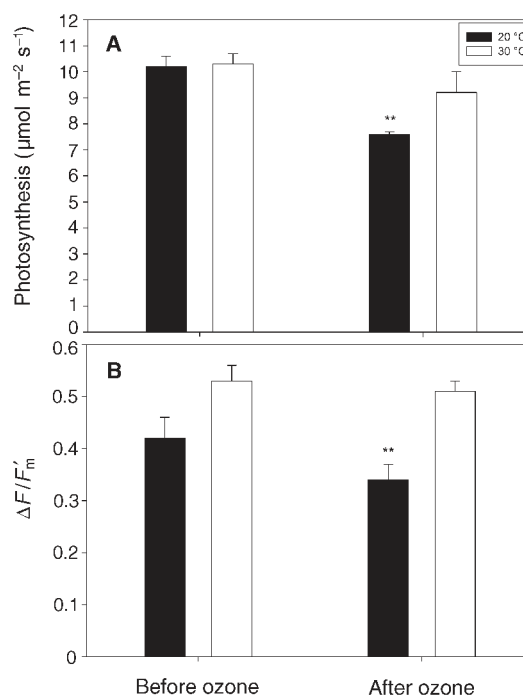


Figure 7. Photosynthesis (A) and the quantum yield of photosystem II as monitored by the fluorescence parameter  $\Delta F/F_m'$  (B) before and after a single acute ozone exposure. During the ozone treatment, leaves were maintained at 20 or 30 °C to significantly change monoterpene emissions but not photosynthesis. Means  $\pm$  SE ( $n = 4$ ) are shown. The asterisks indicate a statistically significant difference between measurements at the same temperature before and after ozone treatment ( $P \leq 0.05$ ).

## Discussion

We demonstrated that leaves fumigated with ozone are stimulated to emit monoterpenes. The stimulation was not caused by increased carbon availability (e.g., increased photosynthesis) or by reduced resistance to monoterpene emissions (e.g., increased stomatal conductance) because photosynthesis and stomatal conductance were unaffected by ozone (Figures 4 and 7), except under conditions where monoterpene synthesis was also inhibited. Because increased rates of isoprene emission have been observed in plants recovering from water stress (Sharkey and Loreto 1993), it is likely that synthesis of volatile isoprenoids is activated in response to stress. We have shown that this activation occurs rapidly and may persist for days after the stress is relieved (Figure 2). Monoterpene emissions were stimulated more, however, in leaves repeatedly exposed to ozone than in leaves exposed to a single acute treatment (cf. Figures 1 and 2), suggesting that emissions continue to increase in response to chronic stresses. Thus, it may be necessary to consider the effects of stresses when modeling isoprenoid emissions by plants.

The amounts of chlorophylls and carotenoids, perhaps the two main products of the MEP pathway, generally decrease in response to ozone treatment (Oksanen and Saleem 1999). Thus, the MEP pathway is not activated by ozone; only monoterpene synthesis is enhanced by ozone. Polyamines are also

induced by ozone, especially in ozone-tolerant plants (Langebartels et al. 1991). Polyamines are thought to scavenge reactive oxygen species (Langebartels et al. 1991) with an action similar to that suggested for isoprene (Loreto et al. 2001).

Our experiments indicate that the involvement of monoterpenes in protective mechanisms against oxidative stresses likely parallels the protective role previously observed for isoprene (Loreto et al. 2001, Loreto and Velikova 2001, Affek and Yakir 2002). Ozone generally does not affect photosynthesis and photochemical properties of *Quercus* leaves either in mild and repeated doses (Experiment I) or in a single, short, acute dose (Experiment II). However, if monoterpene synthesis is reduced by exposure to low temperature, photosynthesis may be damaged by acute ozone treatments (Figure 7). Although our low temperature treatment did not affect net photosynthesis, it caused a considerable reduction in monoterpene emissions. Photorespiration was also reduced by the low temperature treatment, as indicated by the low  $\Delta F/F_m'$ , which in turn indicates reduced photorespiratory-driven electron transport (Cornic and Briantais 1991). Photorespiration may contribute to the protection of leaves under stressful conditions (Cornic and Briantais 1991, Osmond et al. 1997, Ort and Baker 2002). Thus, no unequivocal conclusion about the protective role of monoterpenes can be drawn from the temperature experiment. Peñuelas and Llusà (2002) have recently shown that monoterpene fumigation decreases photo-damage when photorespiration is inhibited and speculated that this may indicate antioxidant activity of these compounds. It remains to be determined whether exogenous monoterpenes protect leaves exposed to ozone at low temperatures.

In contrast to the low temperature treatment, fosmidomycin does not affect photosynthetic carbon reduction/oxidation but has a strong inhibitory effect on isoprene synthesis (Zeidler et al. 1998, Loreto and Velikova 2001, Sharkey et al. 2001). We show that fosmidomycin inhibits foliar monoterpene emissions to the same extent as and with a similar time course to its effect on foliar isoprene emissions. This confirms that monoterpenes emitted by Mediterranean oaks share the same biosynthetic pathway as isoprene emitted by plants, as previously suggested on the basis of the response to environmental factors (Loreto et al. 1996a) and monoterpene labeling with  $^{13}\text{C}$  (Loreto et al. 1996b). When monoterpene emissions were inhibited by fosmidomycin, *Quercus* leaves rapidly became highly susceptible to ozone, and photosynthesis was rapidly and progressively reduced (Figures 4–6), reactive oxygen species such as hydrogen peroxide accumulated, and membrane peroxidation, monitored by increasing concentrations of peroxidation products (MDA), occurred (Table 2). All these symptoms have been observed following inhibition of isoprene synthesis in isoprene-emitting plants (Loreto and Velikova 2001). We conclude, therefore, that volatile monoterpenes emitted by *Q. ilex* leaves not only share the same biosynthetic pathway with isoprene but may also have the same physiological role as isoprene in protecting leaves against oxidative damage.

Fosmidomycin may affect not only volatile isoprenoids but also nonvolatile compounds formed with the contribution of the chloroplastic isoprenoid pathway (Laule et al. 2003). Some of these compounds may also be involved in antioxidant protection directly (carotenoids) or indirectly, for instance by maintaining efficient photochemistry (chlorophylls and xanthophylls). Because fosmidomycin had no effects on the concentrations of chlorophylls and carotenoids or on the depoxidation status of xanthophylls (Table 1), we conclude that the involvement of nonvolatile isoprenoids in the protective mechanism is unlikely. Fosmidomycin may eventually inhibit de novo synthesis of nonvolatile isoprenoids, but not at the concentrations we used to inhibit nonvolatile isoprenoids (Laule et al. 2003).

The mechanism by which volatile isoprenoids protect plants against oxidative stress, and probably against a wide range of environmental stresses, resulting in accumulation of reactive oxygen species, is unclear. Isoprenoids may stabilize membranes, making them resistant to denaturation caused by heat (Sharkey 1996), ozone (Loreto and Velikova 2001) and perhaps other stresses, or they may react with reactive molecules in the mesophyll, indirectly decreasing the rate of formation of reactive oxygen species that can irreparably damage the photosynthetic apparatus (Loreto et al. 2001). We maintain the latter hypothesis is less likely because the calculated lifetime of isoprenoids with respect to gas phase reactions with ozone is too large to allow rapid scavenging of ozone in the absence of large isoprenoid pools in the leaves (Fuentes et al. 2000). Although more studies are necessary to clarify the mechanism of action of volatile isoprenoids, we have demonstrated both a common origin and a common functional role for isoprene and monoterpenes.

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