*Rapid report*

# Isoprene emission rates under elevated $CO_2$ and $O_3$ in two field-grown aspen clones differing in their sensitivity to $O_3$

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## Summary

• Isoprene is the most important nonmethane hydrocarbon emitted by plants. The role of isoprene in the plant is not entirely understood but there is evidence that it might have a protective role against different oxidative stresses originating from heat shock and/or exposure to ozone ($O_3$). Thus, plants under stress conditions might benefit by constitutively high or by higher stress-induced isoprene emission rates.
• In this study, measurements are presented of isoprene emission from aspen (*Populus tremuloides*) trees grown in the field for several years under elevated $CO_2$ and $O_3$. Two aspen clones were investigated: the $O_3$-tolerant 271 and the $O_3$-sensitive 42E.
• Isoprene emission decreased significantly both under elevated $CO_2$ and under elevated $O_3$ in the $O_3$-sensitive clone, but only slightly in the $O_3$-tolerant clone.
• This study demonstrates that long-term-adapted plants are not able to respond to $O_3$ stress by increasing their isoprene emission rates. However, $O_3$-tolerant clones have the capacity to maintain higher amounts of isoprene emission. It is suggested that tolerance to $O_3$ is explained by a combination of different factors; while the reduction of $O_3$ uptake is likely to be the most important, the capacity to maintain higher amounts of isoprene is an important factor in strengthening this character.

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## Introduction

Isoprene is the most abundant hydrocarbon emitted by vegetation, and this emission may have large consequences for the chemistry of the atmosphere (Guenther *et al.*, 1995; Wang & Shallcross, 2000; Karl *et al.*, 2004; Yokouchi & Ambe, 2007). The genus *Populus* includes some of the highest isoprene-emitting species (Lenz *et al.*, 2001). *Populus* species are widely used in short-rotation plantations that are becoming common in agroforestry (FAO, 2005), thus contributing heavily to the global isoprene pool released into the atmosphere. Global change is expected to influence heavily isoprene emission from plants. Global warming is expected to stimulate isoprene emission, but the effects of rising

atmospheric $CO_2$ and tropospheric ozone ($O_3$) episodes remain uncertain, in particular because of the indirect and feedback effects that might occur at the ecosystem level (Arneth *et al.*, 2008). A series of experiments showed a decrease of basal isoprene emission under elevated $CO_2$ (Rosenstiel *et al.*, 2003; Possell *et al.*, 2004; Scholefield *et al.*, 2004). Using different models, it was estimated that the positive effect of warming on global isoprene emission might be balanced by the negative effect of rising $CO_2$ concentration (Arneth *et al.*, 2008), with crucial repercussions on tropospheric ozone and aerosols (Liao *et al.*, 2006). While we are aware of the important role of isoprene on the formation of $O_3$, very little is known about the effect of elevated tropospheric $O_3$ on the emission of isoprene or volatile organic compounds. In a few laboratory studies it was observed that isoprene emission might increase following exposure to a high level of $O_3$ (Velikova *et al.*, 2005a), although the effect seems to be modulated by the position and the age of the leaf (Fares *et al.*, 2006). Different responses to $O_3$ in terms of monoterpene emission rates were observed in various Mediterranean species (Llusià *et al.*, 2002). The role of isoprene in protection against oxidative stress deriving from $O_3$ exposure has been more thoroughly investigated (Loreto & Velikova, 2001; Velikova *et al.*, 2005b). In particular it has been shown that when isoprene is provided to nonemitting plants, $O_3$ damage is considerably reduced, whereas $O_3$ damage is induced when isoprene synthesis is inhibited in isoprene emitters (Loreto & Fares, 2007). Lerdau (2007) suggested that increasing the concentration of atmospheric $O_3$ will favour isoprenoid-emitting species over nonemitting species because of the protective role of isoprenoids on $O_3$ stress.

We measured isoprene emission in an $O_3$-tolerant clone (271) and an $O_3$-sensitive clone (42E) of aspen grown for several years under elevated $O_3$ and elevated $CO_2$, alone or in combination, at the AspenFACE facility. In a previous manuscript we showed that isoprene synthase (ISPS), the enzyme responsible for isoprene synthesis, was inhibited at both transcriptional and translational levels by treatment with $O_3$. In this study we present data deriving from a large campaign carried out in early summer and aimed to establish whether the different $O_3$ sensitivity of clones is related to $O_3$ uptake and isoprene emission.

## Materials and Methods

### Site description

The experiment was carried out at the AspenFACE facility located in Rhinelander (Northern Wisconsin, USA). The AspenFACE experiment started in 1998 when 12 experimental plots were planted that underwent four different treatments, as follows: ambient $CO_2$ and $O_3$ (control); elevated $CO_2$ and ambient $O_3$ ($CO_2$); ambient $CO_2$ and elevated $O_3$ ($O_3$); and elevated $CO_2$ and elevated $O_3$ ($CO_2 + O_3$). The target for elevated $CO_2$ at the AspenFACE is 560 ppm and for elevated $O_3$ is 1.5× ambient concentration. Details on the layout of the plots and of the fumigation performances are given in the AspenFACE website (http://aspenface.mtu.edu). In our study, samples were collected only within the sectors occupied by the trembling aspen (*Populus tremuloides* Michx.) clones. Two clones were investigated, namely 271 ($O_3$ tolerant) and 42E ($O_3$ sensitive) (Isebrands *et al.*, 2001; Karnosky *et al.*, 2003). These two clones exhibited the most contrasting responses to $O_3$ in a previous campaign focusing on the isoprene synthase gene and isoprene synthase protein (Calfapietra *et al.*, 2007).

### Leaf gas-exchange measurements

An intensive campaign was carried out from 26 June to 12 July 2006 to measure leaf gas exchange in six sun-exposed leaves from the upper canopy of each clone in each plot. Measurements were carried out on sunny days and from 10:00 to 16:00 h every day in order to measure parameters under homogeneous environmental and physiological conditions.

A LI-COR 6400 (LI-COR Inc., Lincoln, NE, USA) infrared gas analyzer was used for measurements of photosynthesis, stomatal conductance to water vapor diffusion ($g_s$) and emission of isoprene under environmental conditions that were previously standardized (basal emission, Guenther *et al.*, 1991). Leaf temperature was set at 30°C and photosynthetic photon flux density (PPFD) at 1000 μmol $m^{-2}$ $s^{-1}$. Measurements were carried out at the $O_3$ and $CO_2$ concentrations at which plants were growing. Peak hourly $O_3$ concentrations during the campaign were 46 ppb and 65 ppb in the ambient and elevated $O_3$ plots, respectively. To avoid $CO_2$ oscillations during measurements, a fixed $CO_2$ concentration of 560 and 370 ppm, simulating growth conditions in the different plots, was set with the LI-COR 6400 equipment. The outlet of the LI-COR 6400 cuvette was connected to a Fast Isoprene Sensor (Hills Scientific, Boulder, CO, USA) for online measurements of isoprene emission, as described by Hanson & Sharkey (2001).

Ozone uptake was calculated by multiplying data of $O_3$ concentration and stomatal conductance to $O_3$ during leaf gas-exchange measurements (Emberson *et al.*, 2000). Stomatal conductance to water vapor diffusion ($g_s$) was converted to $O_3$ conductance by dividing the $g_s$ value by the coefficient of molecular diffusivity of $O_3$ in water vapor (1.68), and assuming that the concentration of $O_3$ inside the intracellular spaces of the leaf is approximately zero (Laisk *et al.*, 1989).

After measurements were taken, leaves were immediately frozen in dry ice and then stored at −80°C until used in biochemical assays.

### Biochemical assays

The assay for dimethylallyl diphosphate (DMADP) was carried out on tissue samples previously ground and subjected

 

**Table 1** Values of leaf gas exchange, isoprene emission, and dimethylallyl diphosphate (DMADP) and carotenoid contents of the leaves in the aspen (*Populus tremuloides*) clones 271 ($O_3$-tolerant) and 42E ($O_3$-sensitive) in the four treatments

| | Control | | $CO_2$ | | $O_3$ | | $CO_2 + O_3$ | | *P* values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 271 | 42E | 271 | 42E | 271 | 42E | 271 | 42E | $CO_2$ | $O_3$ | Clone |
| Assimilation (μmol $m^{-2}$ $s^{-1}$) | $12.9^{ab}$ (0.3) | $13.7^{ab}$ (0.3) | $17.4^{cd}$ (0.7) | $20.2^{d}$ (0.5) | $11.7^{b}$ (1.1) | $12.7^{ab}$ (1.6) | $15.4^{ac}$ (1.4) | $15.1^{ac}$ (1.8) | **0.000** | **0.010** | 0.184 |
| $g_s$ (mol $m^{-2}$ $s^{-1}$) | $0.20^{ab}$ (0.01) | $0.24^{ab}$ (0.02) | $0.15^{b}$ (0.01) | $0.26^{ab}$ (0.03) | $0.19^{ab}$ (0.04) | $0.28^{a}$ (0.08) | $0.16^{b}$ (0.03) | $0.20^{ab}$ (0.05) | 0.231 | 0.878 | **0.027** |
| $C_i$ (ppm) | $207^{a}$ (5) | $214^{a}$ (8) | $292^{b}$ (12) | $354^{b}$ (15) | $203^{a}$ (23) | $221^{a}$ (32) | $299^{b}$ (27) | $335^{b}$ (28) | **0.000** | 0.872 | 0.054 |
| Ozone uptake (nmol $m^{-2}$ $s^{-1}$) | $5.2^{a}$ (0.3) | $6.3^{a}$ (0.6) | $3.5^{a}$ (0.19) | $6.1^{a}$ (0.6) | $7.9^{ab}$ (2.5) | $11.7^{b}$ (4.4) | $6.3^{ab}$ (1.7) | $8.0^{ab}$ (2.2) | 0.233 | **0.044** | 0.137 |
| Isoprene emission (nmol $m^{-2}$ $s^{-1}$) | $47.1^{a}$ (0.7) | $44.5^{a}$ (2.6) | $41.6^{ab}$ (3.6) | $34.4^{c}$ (3.4) | $40.4^{abc}$ (3.1) | $35.5^{bc}$ (0.7) | $36.2^{bc}$ (1.5) | $23.9^{d}$ (1.3) | **0.000** | **0.000** | **0.001** |
| DMADP (ng $mg^{-1}$ FW) | $2.6^{a}$ (0.2) | $2.5^{a}$ (0.2) | $1.8^{b}$ (0.2) | $1.8^{b}$ (0.2) | $1.9^{b}$ (0.1) | $2.0^{b}$ (0.1) | $1.8^{b}$ (0.1) | $2.1^{ab}$ (0.1) | **0.003** | 0.173 | 0.561 |
| Carotenoids (mg $g^{-1}$ FW) | $0.73^{a}$ (0.03) | $0.61^{a}$ (0.08) | $0.69^{a}$ (0.06) | $0.56^{a}$ (0.03) | $0.69^{a}$ (0.10) | $0.53^{a}$ (0.03) | $0.70^{a}$ (0.01) | $0.56^{a}$ (0.04) | 0.806 | 0.805 | **0.043** |

The effect of $CO_2$, $O_3$ and clone is indicated by the *P* values in the last three columns at the right of the table (a significant effect is shown in bold). The interactions between these factors are never significant and therefore are not reported. Letters in superscript indicate differences identified from the *post hoc* multiple comparison using the Fischer's test. For each parameter, values with the same letter are not significantly different. Values are means (SE) (*n* = three plots). FW, fresh weight.

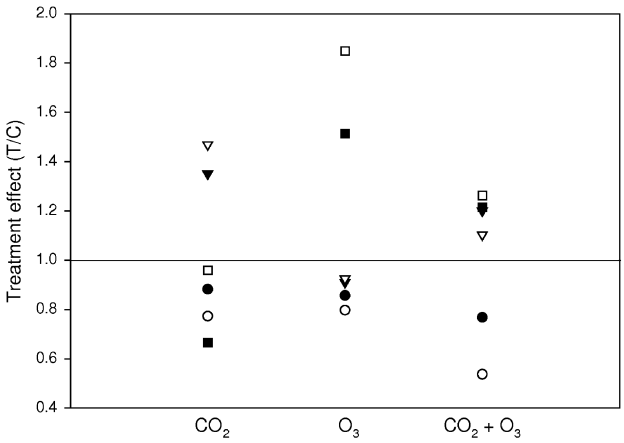


**Fig. 1** Treatment effect calculated as treated (T) over control (C) for $O_3$ uptake (squares), assimilation rates (triangles) and isoprene emission (circles) by aspen (*Populus tremuloides*) trees. Closed symbols, $O_3$-tolerant clone 271; open symbols, $O_3$-sensitive clone 42E.

to acid hydrolysis with 1 M $H_3PO_4$ at 60°C for 30 min. The powdered leaf samples were incubated with 500 μl of $H_2O$ and 500 μl of $H_3PO_4$ in 4-ml vials sealed with inert caps. Pure $N_2$ was continuously flushed in the vials at a flux of 100 ml $min^{-1}$. The air exiting the vial was injected directly into the Proton Transfer Reaction-Mass Spectrometer (PTR-MS; Ionicon, Innsbuck, Austria). The amount of DMADP was calculated after measuring the amount of isoprene evolved by the hydrolysis (protonated *m/z* 69), as indicated by Fisher *et al.* (2001) and Brüggeman & Schnitzler (2002).

Standard DMADP (1 mg $ml^{-1}$; Sigma-Aldrich, St Louis, MO, USA) was used at amounts of 2.5, 5, 10 and 20 μl for calibrations of the PTR-MS.

Total carotenoids were extracted in 96% ethanol and detected spectrophotometrically, as described in Calfapietra *et al.* (2003).

### Statistics

Analysis of variance (ANOVA) was carried out to determine the effects of elevated $CO_2$, elevated $O_3$ and clone. The SYSTAT 11 software (Systat Software Inc., Richmond, CA, USA) general linear models procedure was used. Thereafter, the significance within each combination of clone, $CO_2$ and $O_3$ treatments was calculated using Fischer's multiple comparison procedure. Differences between means were considered significant at a *P* value of $< 0.05$.

## Results

Plants grown at elevated $CO_2$ had higher photosynthesis but lower isoprene emission than controls grown at ambient $CO_2$. The $CO_2$ effect on photosynthesis was significant for both clones, whereas the effect on isoprene was only significant for the $O_3$-sensitive clone 42E (Fig. 1, Table 1). Under conditions of elevated $CO_2$, DMADP concentrations dropped considerably in both clones compared with the control, whereas the amount of carotenoids was only slightly affected.

Stomatal conductance to water vapor diffusion ($g_s$) and $O_3$ uptake were not significantly affected by elevated $CO_2$, although a decrease was observed in clone 271 (Fig. 1, Table 1).

 

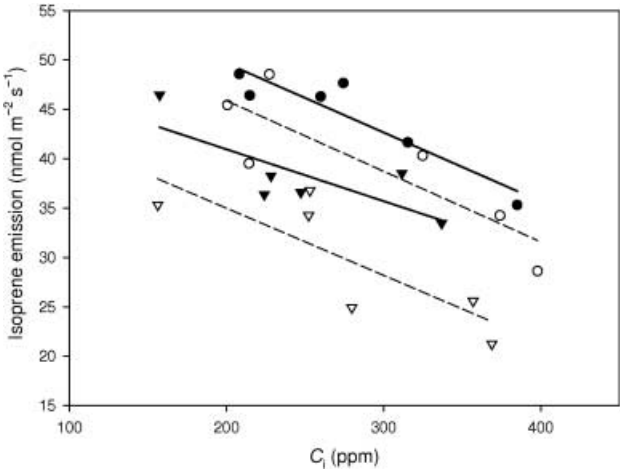


**Fig. 2** Relationship between intercellular $CO_2$ concentration ($C_i$) and isoprene emission rates in aspen (*Populus tremuloides*) $O_3$-tolerant clone 271 (circles) and in the $O_3$-sensitive clone 42E (triangles) under ambient (closed symbols) or elevated (open symbols) $O_3$. Each symbol represents the value of one plot. $R^2$ values range from 0.60 and 0.85 and $P$ values from 0.04 to 0.008.

Elevated $O_3$ induced a drop in both photosynthesis and isoprene emission, the latter being more affected. The DMADP concentration also decreased under conditions of elevated $O_3$, but to a lesser extent than under elevated $CO_2$. Ozone uptake was largely increased under elevated $O_3$, especially in clone 42E which also showed a slight $O_3$-induced increment of $g_s$.

When elevated $O_3$ was combined with elevated $CO_2$, the stimulating effect of $CO_2$ on photosynthesis was almost completely suppressed, especially in the case of clone 42E. The combination of elevated $CO_2$ and $O_3$ induced a large decrease in the isoprene emission rate, especially in clone 42E. The concentrations of DMADP were also reduced in comparison with the control, whereas the amount of carotenoids did not change significantly with treatments, but were lower in clone 42E than in clone 271.

Isoprene emission rates decreased linearly with the increase of intercellular $CO_2$ concentration ($C_i$) for both clones and in both $O_3$ treatments. At a given value of $C_i$, isoprene emission was lower in elevated $O_3$ than in ambient $O_3$, and was also lower in clone 42E than in clone 271 (Fig. 2).

Differences between clones became more evident when different isoprene emission rates and $O_3$ uptake were compared. Ozone uptake was higher in clone 42E than in clone 271, whereas isoprene emission was higher in clone 271 than in clone 42E at all isoprene emission levels (Fig. 3). Interestingly, differences in $O_3$ uptake between clones increased with the increase of $O_3$ uptake (slope significantly different from 1, intercept not significantly different from 0). Differences in isoprene emission rates between clones were larger at low rates (corresponding to values under elevated $O_3$) than at higher rates, as shown by the intercept being significantly different from 0.

## Discussion

Both elevated $CO_2$ and $O_3$ induced a decrease in isoprene emission rates in closed canopy free air $CO_2$ enrichment (FACE)-grown trees. However, the effect of elevated $CO_2$ was significant for the $O_3$-sensitive clone but not for the $O_3$-tolerant clone. A reduction of isoprene emission under elevated $CO_2$ was previously found in experiments in closed environments (Rosenstiel *et al.*, 2003; Possell *et al.*, 2004), in natural springs

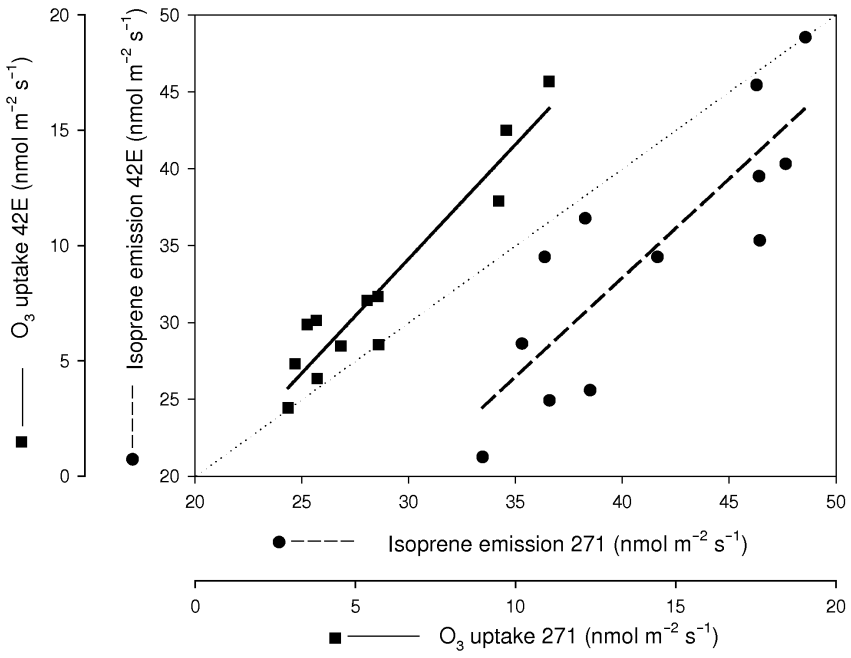


**Fig. 3** Comparison between the aspen (*Populus tremuloides*) $O_3$-tolerant clone 271 and the $O_3$-sensitive clone 42E in $O_3$ uptake (squares) and isoprene emission (circles). Each point represents the values of the two clones in each plot. The dotted line represents the 1:1 line. Fit line for $O_3$ uptake has $R^2 = 0.91$ and $P < 0.0001$, whereas for isoprene emission $R^2 = 0.72$ and $P < 0.001$.

 

(Scholefield *et al.*, 2004) and at different FACE sites (Monson *et al.*, 2007), but no significant effect of elevated $CO_2$ on isoprene emission was observed for *Populus alba* in the POP-EUROFACE experiment (Loreto *et al.*, 2007). A decrease of isoprene emission with increasing $C_i$ has been measured previously (Loreto *et al.*, 2007; Monson *et al.*, 2007). Here we show that this relationship exists in different clones and also under conditions of elevated $O_3$. This confirms the observed $CO_2$ sensitivity of isoprene synthesis, probably because the cytosolic source of carbon for isoprene is more efficiently competed for by respiratory processes under high $CO_2$ (Rosenstiel *et al.*, 2003; Loreto *et al.*, 2007). For each value of $C_i$, isoprene emission was higher in the $O_3$-tolerant clone than in the $O_3$-sensitive clone, and higher under ambient $O_3$ than under elevated $O_3$.

The high $C_i$ value measured in clone 42E was associated with a high $g_s$ value, which is typical of this clone (Darbah, 2007). The effect of clone on $C_i$ was not fully significant ($P = 0.054$) because of the high variability of measurements. However, $O_3$ uptake was higher in clone 42E than in clone 271 in all plots. In addition, the $g_s$ value of clone 42E plants was not reduced under elevated $O_3$, whereas this is usually the case in other aspen clones (Karnosky *et al.*, 2003). High $C_i$ might be also the consequence of inefficient photosynthesis (Evans & Loreto, 2000). However, photosynthesis in the $O_3$-sensitive and $O_3$-tolerant clones was similar within each treatment and does not explain the differences observed of $C_i$. Variability in $C_i$ was quite high and differences were strong both among clones and $CO_2$ treatments, where values of the $C_i/C_a$ ratio (where $C_a$ is the ambient $CO_2$ concentration) were found to be lower under ambient $CO_2$ than under elevated $CO_2$. This might be attributed to the onset of a hot and dry period that reached a peak later in the season and that affected trees under ambient $CO_2$ to a major extent, mainly as a result of generally higher stomatal conductance.

Clone 42E was more sensitive to $O_3$ than clone 271 in several experiments, showing decreased photosynthesis, decreased growth rates and increased visible symptoms (Isebrands *et al.*, 2001; Wustman *et al.*, 2001; Karnosky *et al.*, 2003). In this study, however, the expected decline of photosynthesis under $O_3$ was not observed. This may be attributed to the fact that the campaign was carried out quite early in the season. Noormets *et al.* (2001) showed that the negative effect of $O_3$ becomes evident only in leaves with a high leaf plastochrone index (i.e. on older leaves undergoing a long exposure to $O_3$). This finding is particularly important because it shows that the effect of $O_3$ on isoprene synthesis is greater, or occurs earlier, than that on assimilation. Unfortunately, data on specific leaf area are not available for all clones and for all treatments and therefore it is not possible to assess whether variations observed on a leaf area basis for these clones would have been different on a leaf mass basis.

High $O_3$ uptake is probably the main reason why clone 42E is very sensitive to $O_3$ (Karnosky *et al.*, 2003). However, isoprene emission may also play a role in $O_3$ sensitivity. It has been demonstrated that plants emitting higher amounts of isoprenoids are more protected against oxidative stress, probably because isoprenoids can increase the cohesion between cellular structures and can scavenge $O_3$ inside leaves (Loreto & Velikova, 2001; Loreto & Fares, 2007). This second hypothesis might lead to the idea that decreased isoprene emission rates under elevated $O_3$ are a result of the fact that isoprene is reacting with $O_3$ before exiting the leaf. However, it has been explained that the lifetime of isoprene in our measurement conditions is *c.* 8 h (Calfapietra *et al.*, 2007) and therefore the contribution to $O_3$ uptake as a result of scavenging by isoprenoids is important for monoterpenes but negligible for isoprene (Fares *et al.*, 2008).

Isoprene emission was considerably lower in the $O_3$-sensitive clone, particularly when the emission was reduced by elevated $O_3$. Therefore, we suggest that the capacity to maintain higher levels of isoprene emission contributes to the $O_3$ tolerance of clone 271.

Moreover, carotenoid content was higher in clone 271 leaves than in clone 42E leaves. Carotenoids are formed through the same biochemical pathway as isoprene (Laule *et al.*, 2003) and are able to quench singlet oxygen and other reactive oxygen species (Asada, 2006). The finding that both isoprene and carotenoids are higher in clone 271 suggests that resistance to $O_3$ is related to the activation of the entire metabolic pathway of isoprene formation, including both volatile and nonvolatile compounds with antioxidant action. However, our experiments do not rule out that isoprene emission and the amount of carotenoids remain higher in $O_3$-tolerant lines because of independent $O_3$ tolerance mechanisms that preserve isoprene emission capacity and carotenoids amount independently.

In a previous paper (Calfapietra *et al.*, 2007) we showed that the level of isoprene synthase gene expression and amount of isoprene synthase protein were reduced in aspen clones grown under elevated $O_3$, especially in sensitive clones. This report, on the other hand, shows that DMADP, the substrate for isoprene synthesis, decreased significantly only under elevated $CO_2$. Therefore, the decline of isoprene emission observed when elevated $CO_2$ and elevated $O_3$ co-occur may be exacerbated by a combination of substrate limitation and enzyme limitation. Moreover the amount of DMADP was very similar between clones, suggesting that differences in isoprene emission between clones might be caused more by differences in the enzyme amount or activity rather than by the substrate concentration.

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