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GLOBAL CHANGE ECOLOGY - ORIGINAL PAPER

Enhanced isoprene-related tolerance of heat- and light-stressed photosynthesis at low, but not high, CO_2 concentrations

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Abstract The principal function of isoprene biosynthesis in plants remains unclear, but emission rates are positively correlated with temperature and light, supporting a role for isoprene in maintaining photosynthesis under transient heat and light stress from sunflecks. Isoprene production is also inversely correlated with CO_2 concentrations, implying that rising CO_2 may reduce the functional importance of isoprene. To understand the importance of isoprene in maintaining photosynthesis during sunflecks, we used RNAi technology to suppress isoprene production in poplar seedlings and compared the responses of these transgenic plants to wild-type and empty-vector control plants. We grew isoprene-emitting and non-emitting trees at low (190 ppm) and high (590 ppm) CO_2 concentrations and compared their photosynthetic responses to short, transient periods of high light and temperature, as well as their photosynthetic thermal response at constant light. While there was little difference between emitting and non-emitting plants in their photosynthetic responses to simulated sunflecks at high CO_2, isoprene-emitting trees grown at low CO_2 had significantly greater photosynthetic sunfleck tolerance than non-emitting plants. Net photosynthesis at 42°C was 50% lower in non-emitters than in isoprene-emitting trees at low CO_2, but only 22% lower at high CO_2. Dark respiration rates were significantly higher in non-emitting poplar from low CO_2, but there was no difference between isoprene-emitting and non-emitting lines at high CO_2. We propose that isoprene biosynthesis may have evolved at low CO_2 concentrations, where its physiological effect is greatest, and that rising CO_2 will reduce the functional benefit of isoprene in the near future.

Keywords Poplar · Photosynthesis · Carbon gain · Sunflecks

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Introduction

Isoprene (2-methyl-1,3-butadiene) is a volatile organic compound emitted by a number of plant species and with important effects on atmospheric chemistry (Fuentes et al. 2000; Monson and Holland 2001; Carlton et al. 2009). Isoprene biosynthesis has been proposed as an important adaptation for plants, potentially contributing to tolerance of high leaf temperatures, high photon flux densities and/or metabolic homeostasis (Loreto and Schnitzler 2010). Isoprene emission rates increase with rising temperatures (Monson and Fall 1989; Monson et al. 1994; Sharkey et al. 1999; Singsaas et al. 1999; Petron et al. 2001), but decline with increasing CO_2 concentrations (Sanadze 1964; Rosenstiel et al. 2003; Wilkinson et al. 2009). Thus, the potential for isoprene to function in an adaptive role may have changed during past geologic epochs and may differ in the future as the atmosphere and climate continue to change.

One proposed biological function for isoprene (as well as mono- and sesquiterpenes) is abiotic stress protection. Isoprene acts as a thermoprotective molecule, potentially stabilizing chloroplast membranes during high temperature events (e.g., Singsaas et al. 1997; Singsaas and Sharkey 2000; Behnke et al. 2007). Because isoprene production is enhanced by both high temperatures and light, it has been proposed to maintain photosynthetic capacity during rapid leaf temperature fluctuations caused by sunflecks in the canopy (Sharkey and Singsaas 1995; Behnke et al. 2010). Indeed, an in-silico experiment has demonstrated that isoprene can interact with the phospholipid bilayer of membranes to maintain membrane stability during high temperature events (Siwko et al. 2007). Isoprene also appears to act as an antioxidant compound, reducing damage caused by ozone (Loreto and Velikova 2001; Vickers et al. 2009) and reactive oxygen species (Affek and Yakir 2002; Velikova et al. 2004). All these functions should be more important during simultaneous light and heat stress, when thermotolerance and reactive oxygen quenching mechanisms are needed. Isoprene production has also been proposed as a means of maintaining metabolic homeostasis in the face of past changes in atmospheric CO_2 concentration (Rosenstiel et al. 2004). In this role, isoprene production may facilitate the metabolic breakdown of excess pyruvate in chloroplasts of certain plants. It was proposed that, as atmospheric CO_2 concentration declined in past geologic epochs, the import of phosphoenolpyruvate (PEP) into chloroplasts may have increased due to reduced cytosolic PEP carboxylase activity, thus creating a build-up of chloroplastic pyruvate that could not be accommodated through the highly regulated biosynthetic processes that normally utilize pyruvate in the chloroplast.

While predicting how rising CO_2 concentrations will impact isoprene production is an important scientific goal, the biological importance of isoprene may be best evaluated under low CO_2 conditions. This suggestion is because: (1) emission rates are highest at low CO_2 (thereby maximizing both potential benefits and costs); (2) atmospheric CO_2 concentrations have been low for much of the recent evolutionary history of plants (Petit et al. 1999; Zachos et al. 2001; Siegenthaler et al. 2005); and (3) the need for photosynthetic tolerance of heat and light stress should be of greatest importance at low CO_2 (Cowling and Sage 1998; Sage and Kubien 2007).

The goal of our study was to determine how growth at both low and elevated atmospheric CO_2 affected photosynthetic tolerance to simulated sunflecks and photosynthesis above the thermal optimum in isoprene-emitting and non-emitting plants. We examined stress tolerance in wild-type (and empty-vector control) poplar trees in which isoprene was produced at high rates, as well as in mutants in which isoprene biosynthesis was suppressed by silencing the formation of the isoprene synthase (ISPS) protein. We hypothesized that isoprene would provide a greater level of stress tolerance to photosynthesis in trees grown and measured at low CO_2 concentrations than in high CO_2-grown trees. In light of our hypothesis, we then discuss the potential implications of our results for the evolution of isoprene biosynthesis and the future utility of isoprene in a high CO_2 atmosphere.

Materials and methods

We used four lines of the poplar hybrid *Populus* × *canescens* (syn. *Populus tremula* × *P. alba*): wild-type plants (WT); two well-characterized mutants where ISPS expression was silenced by RNA interference (RNAi) (R2 and R22); and a line transformed with the empty vector (C) to act as a control for the transgenic manipulation; for more details on the plant lines, see Behnke et al. (2007). The four lines were grown from cuttings placed in small pots filled with sterile sand. Cuttings were grown at 24°C and 200 μmol photons m^{-2} s^{-1} photosynthetic photon flux density (PPFD) with a 12-h photoperiod and ambient CO_2 in misting rooms with 70% relative humidity. Once roots formed, each plant was transferred to a 10 × 10 × 36 cm pot filled with 1:1:1 (v:v:v) sand:perlite:peat and transferred to one of two growth chambers (Model M-13; Environmental Growth Chambers, Chagrin Falls, OH, USA). Plants were grown at 27:23°C day:night temperatures and 16:8 h day:night photoperiods with 700 μmol photons m^{-2} s^{-1} PPFD at canopy level from parallel sets of metal halide lamps and incandescent bulbs (Phillips MH400 and A19 100 W bulbs). At least five trees from each line were grown for 3 months at either low (190 ppm) or high (590 ppm) CO_2 concentrations. CO_2 concentrations were measured with an infra-red gas analyzer (LI-COR 6252, Lincoln, NE, USA) every 2–5 min. Elevated CO_2 was achieved by injecting pure CO_2 into the ambient air-stream as needed, while low CO_2 concentrations were maintained by using soda lime to scrub CO_2 from the incoming air. Treatments were rotated between chambers every 3 weeks to minimize chamber effects.

Sunfleck simulations

Gas exchange was measured using an open photosynthesis system (Walz GFS-3000; Effeltrich, Germany) with a dual LED/PAM (pulse amplitude modulation) fluorometer module, programmed to provide light and leaf temperature conditions as outlined below. Simultaneous isoprene emission rates were measured by diverting a fraction of the outgoing air from the leaf cuvette to a chemi-luminescence based fast isoprene sensor (Hills Scientific, Boulder, CO, USA; described in Hills et al. 1991). Measurements on the eighth to tenth leaf from the top of each sapling were made on five trees from each line (WT, C, R2 and R22) from both CO_2 treatments at growth CO_2 (190 or 590 ppm) and a constant water concentration of 15,000 ppmv (50% relative humidity at 30°C). Leaves were placed in a dark cuvette programmed to provide a leaf temperature of 30°C for 30 min to measure dark respiration (measurement R_d). At the end of the 30-min dark period, leaves were exposed to a 0.8-s saturating light pulse (4,500 μmol photons m^{-2} s^{-1}) to measure F_v/F_m (the maximum efficiency of photosystem II). Light was then increased to representative growth levels (700 μmol photons m^{-2} s^{-1}) with a constant leaf temperature of 30°C for another 30 min to assess unstressed net CO_2 assimilation rates (measurement AU). Gas exchange was then measured while exposing leaves to two sequential, 10-min light- and heatflecks designed to simulate a sunfleck: leaf temperatures were rapidly increased to 38–39°C while PPFD was simultaneously raised to 1,600 μmol photons m^{-2} s^{-1} (measurement AS_1). We maintained leaf temperature below 40°C to study reversible heat stress effects and avoid thermal damage. Samples were then given a 15-min recovery period at 30°C and 700 μmol photons m^{-2} s^{-1} PPFD (measurement AR_1). After the recovery period, leaves were exposed to a second sunfleck stress (measurement AS_2) and then left for a second recovery period of 25 min (measurement AR_2). Electron transport rates $[J = (F_m' - F/F_m') \times \text{PPFD} \times 0.5 \times 0.8]$ (from Genty et al. 1990, where 0.5 accounts for the fraction of light to photosystem II and 0.8 accounts for leaf absorptivity) were assessed before the first sunfleck and at the end of both stress events and both recovery periods. Non-photochemical quenching ($\text{NPQ} = (F_m - F_m')/F_m'$) was calculated according to Bilger and Björkman (1990).

Photosynthetic temperature response curves

The temperature response of net CO_2 assimilation rates (A_{net}) was assessed with an open photosynthesis system [either a LI-COR 6400 (Lincoln, NE, USA) or a Walz GFS-3000] for six isoprene-emitting trees and six non-emitting plants from each growth CO_2 treatment (three trees from each line from each CO_2 treatment). The cuvette was maintained at growth CO_2 concentrations (190 or 590 ppm), saturating light (1,000 μmol photons m^{-2} s^{-1}) and a constant water concentration (15,000 ppmv) to examine conditions similar to the sunfleck experiment. A_{net} was measured on the eighth to tenth leaf from the top of the plants at 3°C intervals from 30 to 42°C with a 30-min acclimation time to achieve stable readings. Simultaneous isoprene emission rates were measured for three isoprene emitters and non-emitters from both CO_2 treatments as described above; isoprene emissions were not measured from the LI-COR system due to equipment constraints.

Statistics

Because there were no differences in measured variables between WT and C lines or between R2 and R22 lines (Student's t test, all $p > 0.05$), data were pooled into two groups: isoprene emitters and non-emitters. Temperature response curves of photosynthesis were analyzed using a repeated-measures ANOVA. Gas exchange from the sunfleck experiment was analyzed with ANOVAs, and differences within a CO_2 treatment were compared with Student's t tests. The specific gas-exchange measurements analyzed were dark respiration rates after 30 min of dark acclimation (R_d), as well as net CO_2 assimilation rates (A_{net}): (1) after 30 min of unstressed light acclimation (AU); (2) at the end of the first light- and heatfleck stress (AS_1); (3) at the end of the first recovery period (AR_1); (4) at the end of the second fleck stress (AS_2); and (5) at the end of the second recovery period (AR_2) (see Fig. 1). We also ran a repeated-measures ANOVA on points from the simulated sunfleck experiment that were taken at the same measurement conditions (unstressed, 30°C, 700 μmol photons m^{-2} s^{-1} PPFD: AU, AR_1 and AR_2; and stressed, 38–40°C, 1,600 μmol photons m^{-2} s^{-1} PPFD: AS_1 and AS_2). The goal of the repeated-measures ANOVA was to determine if repeated temperature and light stress affected A_{net}, J and NPQ over time. All statistics were performed in JMP 7.0.1 (SAS, Cary, NC, USA).

Results

Sunfleck simulations

Light levels increased rapidly during light- and heatflecks (sunflecks) while leaf temperature continued to increase over the 10-min stress (Fig. 1a). Suppressed lines showed no detectable isoprene emissions at any temperature; for isoprene-emitting trees, plants from low CO_2 generally exhibited twice the isoprene emission rates of plants from high CO_2 ($p < 0.001$; Fig. 1b, c). Isoprene emissions were suppressed in the dark, began when light was provided, and

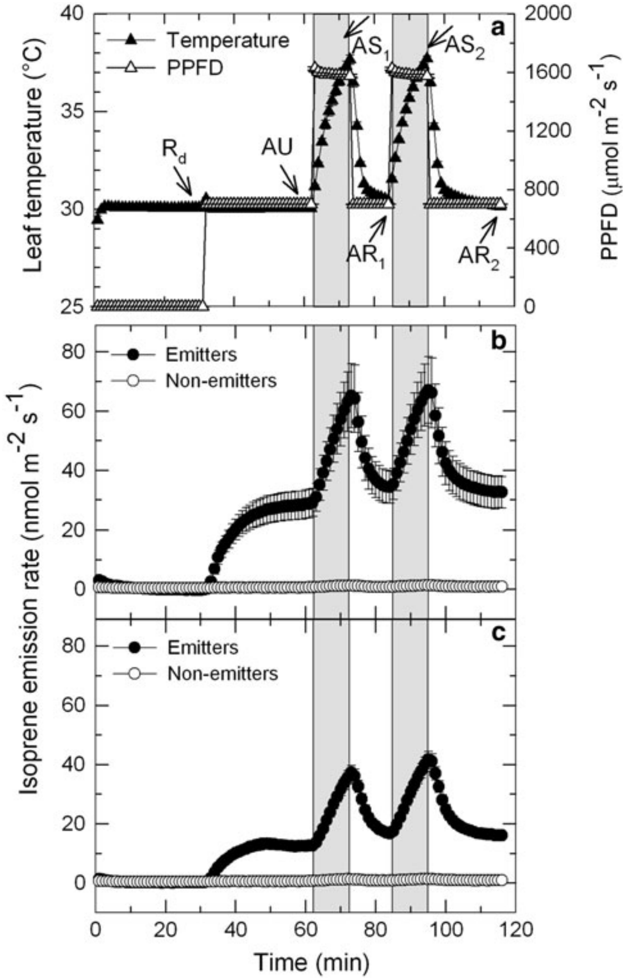

Fig. 1 **a** Leaf temperature and light levels during the simulated sunfleck experiment, and isoprene emission rates in isoprene-emitting (*filled symbols*) and non-isoprene-emitting (*empty symbols*) leaves, measured at **b** low (190 ppm) and **c** high (590 ppm) growth CO_2 concentrations. Measurements began at 30°C in the dark and light was increased to 700 μmol photons m^{-2} s^{-1} after 30 min. These conditions were maintained except for the two flecks (*gray bars*) where leaf temperature was increased to <40°C and light to 1,600 μmol photons m^{-2} s^{-1}. *Letters* and *arrows* indicate times where representative gas exchange measurements were made for dark respiration (R_d), unstressed net CO_2 assimilation rates (AU), the first light- and heatfleck stress (AS_1), recovery from the first fleck (AR_1), the second light- and heatfleck stress (AS_2) and recovery from the second fleck (AR_2). Mean ± SE, $n = 20$ for (**a**), 10 for (**b** and **c**)

showed rapid increases during the transient high light and heat stresses of the simulated sunflecks. In both CO_2 treatments, the maximum isoprene emission rate increased from the first to the second sunfleck.

Elevated CO_2 increased A_{net} at all temperatures (Fig. 2a, b; Table 1). Isoprene emitters had higher pre-stress A_{net} (measurement AU) than non-emitting leaves as a group (Table 1), but especially at low CO_2 (Fig. 2a, b). At low CO_2, A_{net} declined during the first sunfleck, increased during the recovery period, decreased more sharply during the second fleck, and rose again during the last recovery phase (Fig. 2a). At high CO_2, A_{net} increased during both flecks, declining slightly during the first recovery period compared to the initial measurement (AU), but remaining stable after the second fleck, such that measurements AR_1 and AR_2 were similar (Fig. 2b). Compared to A_{net} measured at the end of the first sunfleck (AS_1), A_{net} after the second sunfleck (AS_2) was reduced by an extra 0.5 μmol CO_2 m^{-2} s^{-1} in isoprene-emitting leaves, but by twice this amount (1.05 μmol m^{-2} s^{-1}) in non-emitting leaves, regardless of the CO_2 concentration (Fig. 2a, b).

Photosynthesis was reduced proportionally more at low CO_2 than high CO_2 when isoprene synthesis was suppressed. Because of differences between A_{net} at different CO_2 levels, A_{net} values were normalized relative to pre-stress A_{net} (measurement AU) to generate relative net CO_2 assimilation rates (A_{rel}) (Fig. 2c, d). By the end of the first 10-min sunfleck (AS_1), A_{rel} was reduced by 20% in both emitters and non-emitters at low CO_2 (Fig. 2c). However, A_{rel} continued to decline after the first sunfleck was finished, so that while leaf temperatures recovered towards 30°C, it eventually fell by 29 and 38% in isoprene-emitting and non-isoprene-emitting leaves, respectively (data not shown). By the end of the second sunfleck (AS_2), A_{rel} was reduced by 27% in emitters and 47% in non-emitters from low CO_2 (Fig. 2c). At high CO_2, the first fleck (AS_1) increased A_{rel} by 9 and 7% in isoprene-emitting and non-isoprene-emitting leaves, respectively, but by the second fleck (AS_2), A_{rel} was increased by 4% in emitting trees and reduced by 5% in non-emitters (Fig. 2d). After recovering from both flecks (AR_2), A_{rel} in low CO_2-grown trees was only reduced by 4% in isoprene-emitting leaves, but was 14% lower in non-isoprene-emitting leaves (Fig. 2d); at high CO_2, A_{rel} was 4–7% lower in both groups at AR_2 (Fig. 2d).

Electron transport rates (J) and non-photochemical quenching (NPQ) recovered better from sunflecks in isoprene-emitting leaves (Fig. 2e, f, i, j). Electron transport rates were lower in non-isoprene-emitting trees than in emitters (Table 2), and while J was reduced during sunflecks at low CO_2, J increased in non-emitting leaves during each sunfleck at high CO_2 (AS_1 and AS_2; Fig. 2e, f). Non-emitting poplars lost a greater proportion of their initial J capacity (relative J, J_{rel}) after recovering from sunflecks, but this effect was exacerbated at low CO_2; while J_{rel} in isoprene-emitters from both CO_2 concentrations was reduced by less than 4% at point AR_2, J_{rel} in non-isoprene-emitters was 8% lower at high CO_2 and 14% lower at low CO_2 (Fig. 2g, h). At both CO_2 concentrations, isoprene-emitters showed lower NPQ values than non-emitters at 30°C (points AU, AR_1 and AR_2) (Fig. 2i, j; Table 2). When normalized relative to pre-stress NPQ (NPQ_{rel}), non-isoprene emitters had higher

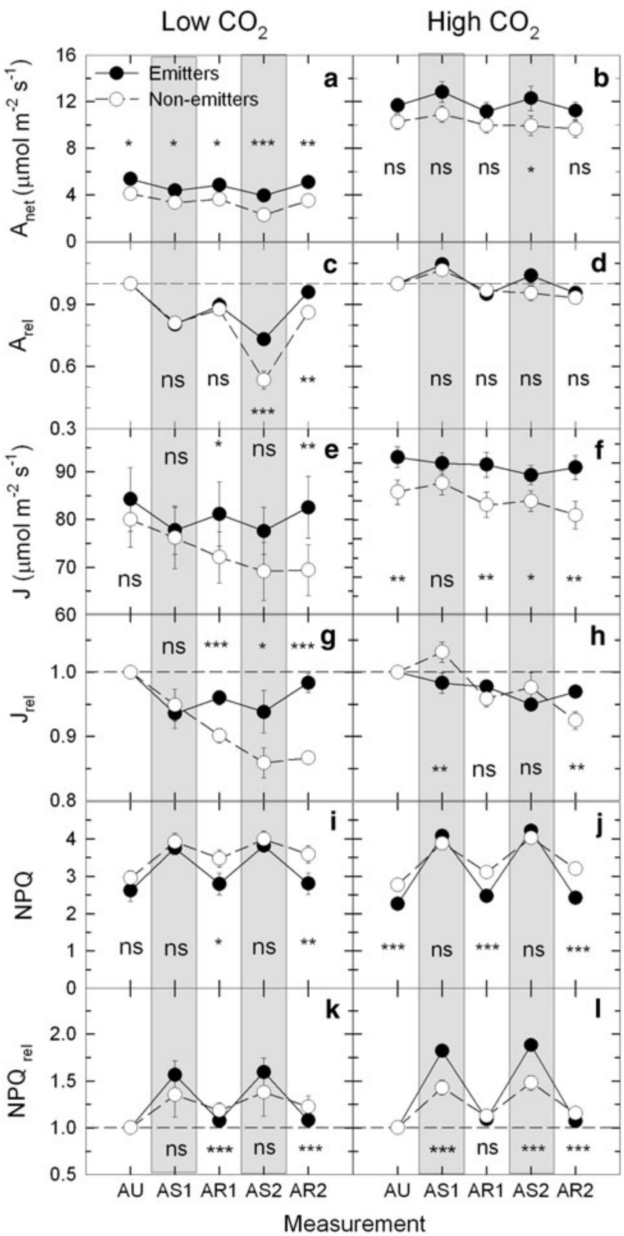

Fig. 2 The effect of two sequential simulated sunflecks on: **a**, **b** net CO_2 assimilation rates (A_{net}); **c**, **d** net CO_2 assimilation rates relative to rates measured at point AU (A_{rel}); **e**, **f** electron transport rates (J); **g**, **h** electron transport rates relative to rates measured at point AU (J_{rel}); **i**, **j** non-photochemical quenching (*NPQ*); **k**, **l** non-photochemical quenching relative to NPQ measured at point AU (NPQ_{rel}) in isoprene-emitting (*filled symbols*) and non-isoprene-emitting (*empty symbols*) leaves, measured at their growth CO_2 concentration (low, 190 ppm; high, 590 ppm). Measurements taken at points as shown in Fig. 1. *AU* unstressed conditions, AS_1 sunfleck 1, AR_1 recovery 1, AS_2 sunfleck 2, AR_2 recovery 2. Mean ± SE, $n = 10$. *ns* non-significant, *$p < 0.10$, **$p < 0.05$, ***$p < 0.01$

NPQ_{rel} values after recovering from sunflecks (points AR_1 and AR_2) and less of an increase in NPQ during heat stress (points AS_1 and AS_2) than isoprene-emitting plants (Fig. 2k, l; Table 2). There was no difference in F_v/F_m between CO_2 treatments or isoprene lines (data not shown).

Table 1 ANOVA results for the response of net CO_2 assimilation (A_{net}) during simulated sunflecks for isoprene-emitting and non-emitting poplars grown at either low (190 ppm) or high (590 ppm) CO_2 concentrations

	CO_2	Emit	CO_2 × Emit
Absolute values			
R_d	**0.035**	0.082	**0.0090**
AU	**<0.0001**	**0.017**	0.89
AS_1	**<0.0001**	**0.027**	0.50
AR_1	**<0.0001**	0.053	0.99
AS_2	**<0.0001**	**0.0092**	0.62
AR_2	**<0.0001**	**0.013**	0.99
Relative values			
R_d	**0.0003**	**0.0004**	**0.0010**
AS_1	**<0.0001**	0.58	0.37
AR_1	**0.0071**	0.99	0.48
AS_2	**<0.0001**	**0.0003**	0.12
AR_2	0.19	**0.018**	0.13

The column labels refer to: growth CO_2 concentration (*CO_2*); isoprene emitting or non-emitting line (*Emit*); dark respiration rate after 30-min dark acclimation (*R_d*); unstressed A_{net} after 30-min light acclimation (*AU*); A_{net} after the first and second 10-min fleck stress, respectively (*AS_1* and *AS_2*); A_{net} measured at the end of the first and second recovery period, 15 and 25 min, respectively (*AR_1* and *AR_2*). See Fig. 1 for further information on the measurements

$p < 0.05$ are indicated in bold

Dark respiration rates (R_d) were reduced in isoprene-emitting leaves from low CO_2 compared to non-isoprene-emitting leaves and to both groups at high CO_2 (Fig. 3a, c; Table 1). When normalized to account for differences in A_{net}, relative dark respiration rates were greater ($p < 0.01$) in non-isoprene emitting leaves from low CO_2 than low CO_2 emitters or either group from high CO_2 (Fig. 3b, d).

Photosynthetic temperature response curves

Isoprene-emitting leaves from low CO_2-grown plants produced two to three times more isoprene across all leaf temperatures than leaves grown and measured at high CO_2 (Fig. 4a; Table 3). Isoprene emission, however, peaked at 39°C at low CO_2, whereas it increased linearly in plants grown and measured at high CO_2. As in the sunfleck experiment, no detectable isoprene was produced by the suppressed lines. Leaves from elevated CO_2 displayed higher A_{net} than low CO_2 leaves, with A_{net} declining above a leaf temperature of 30–33°C (Fig. 4b; Table 3). Both isoprene-emitting and non-emitting lines had similar A_{net} across the temperature range measured (Fig. 4b; Table 3). At 42°C, A_{net} was 50% lower in non-isoprene-emitters than in emitting leaves at low CO_2, while at high CO_2, A_{net} was

Table 2 Results from repeated measures ANOVAs from simulated sunflecks for A_{net}, A_{rel}, J, J_{rel}, NPQ and NPQ_{rel}

	CO_2	Time	Emit	CO_2 × Time	CO_2 × Emit	Time × Emit	CO_2 × Time × Emit
A_{net} 30°C	**<0.0001**	**<0.0001**	**0.022**	0.49	0.97	**0.076**	0.88
A_{rel} 30°C	**<0.0001**	0.72	**0.026**	0.24	0.99	**0.023**	0.94
A_{net} 40°C	**<0.0001**	**<0.0001**	**0.015**	0.85	0.56	**0.0061**	0.71
A_{rel} 40°C	**<0.0001**	**<0.0001**	**0.0028**	**0.0063**	0.42	**0.0002**	**0.026**
J 30°C	0.35	**<0.0001**	**0.011**	**0.026**	0.80	**<0.0001**	**0.051**
J_{rel} 30°C	**0.0090**	**0.014**	**<0.0001**	0.16	**0.013**	**0.0002**	0.14
J 40°C	**0.043**	**0.0001**	0.080	0.96	0.75	**0.025**	0.18
J_{rel} 40°C	**0.0027**	**0.0002**	0.88	0.98	0.091	**0.0098**	0.10
NPQ 30°C	0.75	**<0.0001**	**0.026**	**0.00031**	0.16	**<0.0001**	0.10
NPQ_{rel} 30°C	0.18	**0.0088**	**0.0001**	0.14	0.10	**0.0004**	0.51
NPQ 40°C	0.28	**<0.0001**	0.94	**<0.0001**	0.29	0.93	0.86
NPQrel 40°C	0.072	**<0.0001**	**0.032**	**0.0001**	0.35	0.29	0.58

Values for 30°C include measurement points AU (for absolute values only), AR_1 and AR_2; value for ~40°C include points AS_1 and AS_2

CO_2 growth CO_2 concentration, *Time* changes between values as sunflecks and recovery periods occurred, *Emit* isoprene emitting or non-emitting line

$p < 0.05$ are indicated in bold

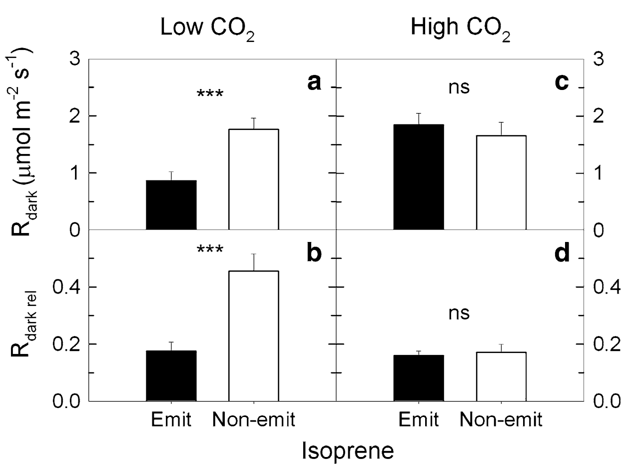

Fig. 3 Dark respiration rates in isoprene-emitting (emit, *filled bars*) and non-isoprene-emitting leaves (non-emit, *empty bars*), measured at their growth CO_2 concentration (low, 190 ppm; high, 590 ppm). **a**, **c** Dark respiration rates; **b**, **d** dark respiration rates relative to net CO_2 assimilation rates measured at point AU. Mean ± SE, $n = 10$. *ns* non-significant, *$p < 0.10$, **$p < 0.05$, ***$p < 0.01$

only 22% lower in non-isoprene-emitters than in isoprene-emitters (Fig. 4b). Measurements of A_{net} were normalized relative to A_{net} measured at 30°C to generate A_{rel}. A_{rel} decreased with increasing leaf temperature more at low CO_2 than high CO_2, and more in non-emitting leaves than in isoprene-emitting lines (Fig. 4c; Table 3). While maintaining a constant water vapor content resulted in an increase in vapor pressure deficit at the higher temperatures, there were no significant differences in stomatal conductance (g_s) between emitters and non-emitters in general or between groups over the temperature response curve measurements (data not shown).

Discussion

Our analysis shows that isoprene provides greater heat and light stress tolerance to photosynthesis at low CO_2 than at high CO_2. Leaves with suppressed isoprene formation lost a greater fraction of their photosynthetic capacity than isoprene-emitting leaves at high temperatures (>40°C), especially at low CO_2 concentrations. Even at more moderate leaf temperatures, isoprene-emitting leaves maintained higher A_{net} than non-emitting leaves at low CO_2. When heat stress was applied concurrently with light stress to mimic sunflecks, isoprene-emitting leaves from low CO_2 lost less and recovered more of their pre-stress photosynthetic capacity than non-emitting leaves. This photosynthetic response was mirrored by the ability of isoprene-emitting plants to recover pre-stress levels of J and NPQ after two sunfleck events.

Low CO_2-grown leaves emitted twice as much isoprene as those from high CO_2-grown plants. Since the effect of isoprene on thermotolerance depends on the dose of isoprene at ambient CO_2 (Singsaas et al. 1997), the greater effect of isoprene at low than at high CO_2 likely relates to differences in emission rates. If isoprene stabilizes protein–protein interactions in membranes at high temperatures (Sharkey and Singsaas 1995; Sharkey and Yeh 2001), increased isoprene emission rates may yield greater protection from stress-induced electron transport declines. At low CO_2, declines in A_{net} with rising leaf temperature are generally caused by increasing photorespiration and decreasing Rubisco carboxylation capacity, rather than by impairment of photosynthetic electron transport, but photosynthesis can be electron transport-limited at low CO_2 as

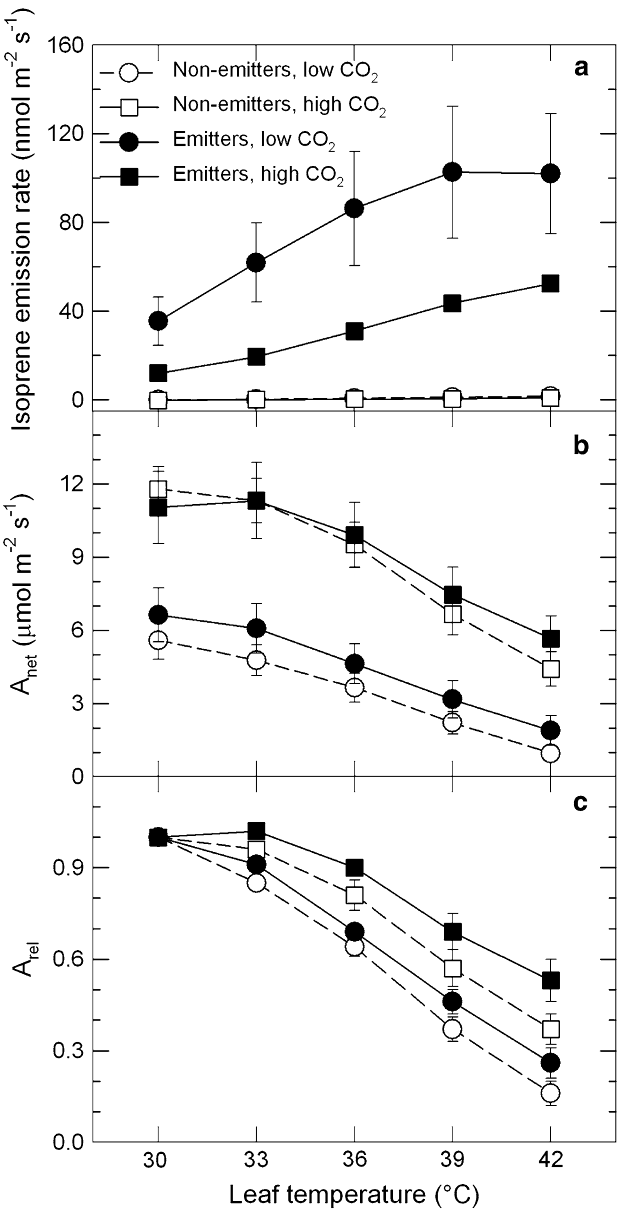

Fig. 4 Response of **a** isoprene emission rates, **b** net CO_2 assimilation rates, and **c** net CO_2 assimilation rates relative to 30°C to leaf temperature in isoprene-emitting (*filled symbols*) and non-emitting (*empty symbols*) lines measured at their growth CO_2 concentration (low, 190 ppm, *circles*; high, 590 ppm, *squares*). Mean ± SE; **a** $n = 3$ trees, **b**, **c** $n = 6$ trees

leaf temperatures rise (Sage and Kubien 2007). Thus, isoprene-induced maintenance of J would allow isoprene-emitters to quickly recover CO_2 fixation after sunfleck stresses. Because isoprene emission rates are largely independent of g_s (low g_s causes intercellular isoprene concentrations to build up, increasing the diffusive gradient, while high g_s allows for rapid diffusion, but a low build-up of isoprene concentrations in the intercellular airspace; Niinemets et al. 2004; Loreto and Schnitzler 2010), any changes in g_s from changing light levels, temperatures, or vapor pressure deficits will have little effect on differences in isoprene emission rates and, therefore, photosynthetic sunfleck tolerance. The higher maximum isoprene emission rates achieved in the second sunfleck, compared to the first, are consistent with increases seen in successive heat stresses or simulated sunflecks in other studies (Behnke et al. 2007, 2010), and may reflect incomplete recovery of pre-stress isoprene emission rates prior to a rapid reapplication of the stress.

Table 3 Results from a repeated-measures ANOVA for the temperature response (30–42°C) of A_{net} in isoprene-emitting and non-emitting poplar leaves grown at either low (190 ppm) or high (590 ppm) CO_2 concentrations

	Isoprene	A_{net}	A_{rel}
CO_2	0.071	**<0.0001**	**<0.0001**
T_{leaf}	**0.0033**	**<0.0001**	**<0.0001**
Emit	**0.0013**	0.44	**0.011**
$CO_2 \times T_{leaf}$	**0.0037**	**0.0098**	**0.0036**
$CO_2 \times$ Emit	0.075	0.69	0.57
$T_{leaf} \times$ Emit	**0.0049**	**0.044**	0.58
$CO_2 \times T_{leaf} \times$ Emit	**0.0020**	0.57	0.65

CO_2 growth CO_2 concentration, *T_{leaf}* leaf temperature, *Emit* isoprene-emitting or non-emitting line, *Isoprene* isoprene emission rate, *A_{net}* net CO_2 assimilation rate, *A_{rel}* net CO_2 assimilation rate relative to 30°C

$p < 0.05$ are indicated in bold

In previous work, pre-stress A_{net} at ambient CO_2 was similar in these emitting and non-emitting mutants (Behnke et al. 2007, 2009). This result is consistent with our high CO_2 data, but at low CO_2, isoprene-emitting leaves had higher A_{net} and J than non-emitters in our study. The slightly higher A_{net} in isoprene-emitters at low CO_2 implies either an alleviation of Rubisco carboxylation limitations on photosynthesis, which limit gross CO_2 assimilation (A_{gross}) rates at low CO_2 concentrations, or lower day respiration rates (R_{day}), since $A_{net} = A_{gross} - R_{day}$. Emitting leaves operated at lower intercellular CO_2 concentrations (C_i) than non-emitters (data not shown), so they did not increase photosynthesis by operating at a higher C_i. The possibility of a difference in R_{day} is plausible, given that emitters have 50% lower dark respiration rates at low CO_2 and that day and dark respiration rates are usually correlated (Loreto et al. 2007; Way and Sage 2008). If R_{day} was 10–30% lower than R_{dark} (as it was in *Populus alba* grown at ambient and high CO_2; Loreto et al. 2007), this would reduce, but not fully account for, the difference in A_{net}; isoprene-emitters at low CO_2 had 31% higher A_{net} than non-emitters, but would still have 8–12% higher A_{gross} if differences in R_{day} were taken into account. Regardless of the cause for lower carbon fixation rates in non-emitters, their ability to recover from sunflecks was not inherently hindered. Individual leaves with the lowest pre-stress photosynthetic rates (2.6 μmol m^{-2} s^{-1} for both emitters and non-emitters at low CO_2) were fully capable of recovering pre-stress A_{net} values, suggesting that non-emitting poplar leaves, with mean A_{net} rates of 4.1 μmol m^{-2} s^{-1}, had more than sufficient capacity to fully recover from sunflecks.

In isoprene-emitting lines, high CO_2 increased respiration rates but suppressed isoprene emissions compared to low CO_2 (Griffin et al. 2001; Wang et al. 2001; Wilkinson et al. 2009). However, isoprene-emitters had lower dark respiration rates at low CO_2 than non-emitters, while there was no such effect at high CO_2. Following current metabolic theory (Rosenstiel et al. 2004), competition for phosphoenolpyruvate (PEP) between the cytosol and chloroplast is mediated by PEP carboxylase. Reduced isoprene emission rates at high CO_2 might result from higher consumption rates of cytosolic PEP through PEP carboxylase, thus restricting the chloroplast import of PEP (and thus pyruvate).

Monson and co-workers (Monson et al. 2009; Wilkinson et al. 2009) proposed a metabolic control scheme for C_3 plants with competition between cytosolic PEP carboxylase and Rubisco controlling carbon flow to the MEP (methylerythritol 4-phosphate) pathway in response to changes in CO_2 concentration. In line with this scheme, increased mitochondrial respiration, as shown here, can constitute a growing sink for cytosolic PEP under rising CO_2 (as proposed by Loreto et al. 2007); higher respiration demand would then compete with the chloroplast import of PEP for isoprene biosynthesis and lower isoprene emission rates (Rosenstiel et al. 2003). This possibility is supported by our low CO_2 data, where non-emitting mutants exhibited increased absolute and relative respiration rates, implying that the lack of isoprene had diminished chloroplast requirements for PEP, freeing up more PEP substrate to be channelled to mitochondrial respiration. Thus, in both cases, lower respiration rates correlated with higher isoprene emission rates. In contrast to our data, Loreto et al. (2007) found that in mature *Populus alba* leaves, rates of dark respiration and isoprene emission were positively correlated; however, the two rates were negatively correlated in developing leaves.

Competition between cytosolic and plastidic demands for substrate likely contributed to differences in isoprene emissions at high temperatures between treatments. At low CO_2 concentrations, isoprene emission rates of emitting lines leveled off at temperatures above 39°C, but continued to increase linearly at high CO_2. Under these conditions—low A_{net} and high metabolic demand of the MEP pathway—the metabolic flux to dimethylallyl diphosphate (DMAPDP) is likely limited, resulting in a substrate limitation of the ISPS enzyme. In contrast to other terpene synthases, ISPS enzymes display Michaelis constants for their substrate in the millimolar range (Schnitzler et al. 2005), making this catalytic reaction in vivo highly sensitive to a depletion of the plastidic DMADP pool (shown for poplar in Magel et al. 2006).

In non-isoprene-emitting poplar leaves, neither J nor NPQ showed full recovery to pre-stress levels within 25 min, implying a reduced ability to recover from the two sunflecks imposed. In a heat stress experiment performed at ambient CO_2, J_{rel} and NPQ of wild-type poplars fully recovered from these events, while the J_{rel} of non-emitting trees decreased and NPQ increased with each successive heatfleck imposed (Behnke et al. 2007). Also, measurements at ambient CO_2 indicated that while J in isoprene-emitting leaves recovered within 30 min, non-emitting grey poplar needed 90 min or longer to recover from a series of six sunflecks following the cessation of the stress (Behnke et al. 2010). While we did not examine longer recovery periods, since J and A_{net} are usually correlated (for example, a 14% decrease in both A_{rel} and J_{rel} at point AR_2 in non-emitting, low CO_2 trees), photosynthesis is likely to remain inhibited in these trees for up to an hour and a half after sunfleck-type stress in the absence of isoprene production.

Potential evolutionary implications regarding atmospheric CO_2

It appears that isoprene production evolved independently in various groups of higher plants (Harley et al. 1999; Sharkey et al. 2005), a degree of convergent evolution implying a common selective pressure. The recent evolutionary history of land plants took place in a low CO_2 atmosphere: although atmospheric CO_2 concentrations were generally above 1,000 ppm for most of the last 600 million years, they decreased to modern, low levels of ~300 ppm by the early Miocene (20–24 mya) (Zachos et al. 2001; Tipple and Pagani 2007). CO_2 levels have stayed low since, ranging from 180 ppm during the last glacial maximum 21,000 years ago to 280 ppm before the industrial revolution (Jansen et al. 2007).

We propose that the evolutionary pressure for isoprene synthesis in higher plants may have been partly attributable to the relatively low CO_2 concentrations of the past 25 million years. Since photosynthesis is more susceptible to heat and high light damage at low CO_2 (Cowling and Sage 1998), plants that emit isoprene may have had a competitive advantage over non-emitting species at low CO_2 in environments.

Our proposal for the importance of a low CO_2 environment for the evolution of isoprene biosynthesis is also consistent with other researchers' theories regarding why plants emit isoprene. Isoprene may act as a

ROS-scavenging molecule, when excess light energy absorption at the thylakoid membranes cannot be channeled into photosynthesis (Affek and Yakir 2002; Peñuelas et al. 2005; Vickers et al. 2009); this role should be more important at low CO_2 concentrations, where low substrate availability reduces the photosynthetic sink for light energy. In the metabolic homeostasis hypothesis, isoprene biosynthesis was already proposed to have evolved in low CO_2 environments because of a decrease, relative to prior high CO_2 regimes, in the rate at which cytosolic PEP carboxylase used PEP substrate (Rosenstiel et al. 2004). In the absence of control over PEP partitioning between the cytosol and chloroplast at the level of the PEP/P_i antiporter in the chloroplast envelope, a shift toward higher chloroplastic PEP influx at low CO_2 may have led to pyruvate accumulation in chloroplasts (Rosenstiel et al. 2004). Enhanced isoprene biosynthesis at low CO_2 may provide a means of metabolizing accumulated pyruvate, similar to the role proposed for the alternative mitochondrial oxidase (Plaxton and Podesta 2006).

Our results also provide insight into how rising atmospheric CO_2 concentrations might alter any photosynthetic stress-tolerance advantage of isoprene biosynthesis in the future. We found few differences in photosynthesis between emitting and non-emitting poplar leaves at high CO_2. There was no significant difference at high CO_2 in pre-stress A_{net} between emitters and non-emitters, although emitters had slightly higher A_{net} at 30°C in general (Table 2), and both groups showed similar abilities to recover from sunflecks. While J and NPQ both recovered from the second sunfleck (AR_2) more fully in isoprene-emitting than non-emitting leaves at high CO_2, the relative difference between the two groups was much smaller than at low CO_2. Since the benefit of producing isoprene is substantially reduced at high CO_2, isoprene emission may be less adaptive in a future, high CO_2 atmosphere.

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References

Affek HP, Yakir D (2002) Protection by isoprene against singlet oxygen in leaves. Plant Physiol 129:269–277

Behnke K, Ehlting B, Teuber M, Bauerfeind M, Louis S, Hänsch R, Polle A, Bohlmann J, Schnitzler JP (2007) Transgenic, non-isoprene emitting poplars don't like it hot. Plant J 51:485–499

Behnke K, Kleist E, Uerlings R, Wildt J, Rennenberg H, Schnitzler JP (2009) RNAi-mediated suppression of isoprene biosynthesis in hybrid poplar impacts ozone tolerance. Tree Physiol 29:725–736

Behnke K, Loivamäki M, Zimmer I, Rennenberg H, Schnitzler JP, Louis S (2010) Isoprene emission protects photosynthesis in sunfleck exposed grey poplar. Photosynth Res 104:5–17

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth Res 25:173–185

Carlton AG, Wiedinmyer C, Kroll JH (2009) A review of secondary organic aerosol (SOA) formation from isoprene. Atmos Chem Phys 9:4987–5005

Cowling SA, Sage RF (1998) Interactive effects of low atmospheric CO_2 and elevated temperature on growth, photosynthesis and respiration in *Phaseolus vulgaris*. Plant Cell Environ 21:427–435

Fuentes JD, Lerdau M, Atkinson R, Baldocchi D, Bottenheim JW, Ciccioli P, Lamb B, Geron C, Gu L, Guenther A, Sharkey TD, Stockwell W (2000) Biogenic hydrocarbons in the atmospheric boundary layer: a review. Bull Am Meteorol Soc 81:1537–1575

Genty B, Wonders J, Baker NR (1990) Non-photochemical quenching of F0 in leaves is emission wavelength dependent: consequences for quenching analysis and its interpretation. Photosynth Res 26:133–139

Griffin KL, Anderson OR, Gastrich MD, Lewis JD, Lin G, Schuster W, Seemann JR, Tissue DT, Turnbull MH, Whitehead D (2001) Plant growth in elevated CO_2 alters mitochondrial number and chloroplast fine structure. Proc Natl Acad Sci USA 98: 2473–2478

Harley P, Lerdau M, Monson RK (1999) Ecological and evolutionary aspects of isoprene emission. Oecologia 118:109–123

Hills AJ, Fall R, Monson RK (1991) Methods for the analysis of isoprene emission from leaves. In: Liskin H, Jackson J (eds) Plant toxin analysis. Modern methods of plant analysis, new series, volume 13. Springer, Berlin, pp 297–313

Jansen E, Overpeck J, Briffa KR, Duplessy JC, Joos F, Masson-Delmotte V, Olago D, Otto-Bliesner B, Peltier WR, Rahmstorf S, Ramesh R, Raynaud D, Rind D, Solomina O, Villalba R, Zhang D (2007) Palaeoclimate. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp 433–498

Loreto F, Schnitzler JP (2010) Abiotic stresses and induced BVOCs: a review. Trends Plant Sci 15:154–166

Loreto F, Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol 127:1781–1787

Loreto F, Centritto M, Barta C, Calfapietra C, Fares S, Monson RK (2007) The relationship between isoprene emission rate and dark respiration rate in white poplar (*Populus alba* L.) leaves. Plant Cell Environ 30:662–669

Magel E, Mayrhofer S, Müller A, Zimmer I, Hampp R, Schnitzler JP (2006) Photosynthesis and substrate supply for isoprenoid biosynthesis in poplar leaves. Atmos Environ 40:S138–S151

Monson RK, Fall R (1989) Isoprene emission from aspen leaves—influence of environment and relation to photosynthesis and photorespiration. Plant Physiol 90:267–274

Monson RK, Holland E (2001) Biospheric trace gas fluxes and their control over tropospheric chemistry. Annu Rev Ecol Syst 32:547–576

Monson RK, Harley PC, Litvak ME, Wildermuth M, Guenther AB, Zimmerman PR, Fall R (1994) Environmental and developmental control over the seasonal pattern of isoprene emission from aspen leaves. Oecologia 99:260–270

Monson RK, Wilkinson MJ, Monson ND, Trahan N, Lee S, Rosenstiel TN, Fall R (2009) Biochemical controls of the CO_2

response of leaf isoprene emission: an alternative view of Sanadze's double carboxylation scheme. Ann Agrar Sci 7:21–29
Niinemets U, Tenhunen JD, Harley PC, Steinbrecher R (1999) A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. Plant Cell Environ 22:1319–1335
Niinemets U, Loreto F, Reichstein M (2004) Physiological and physico-chemical controls on foliar volatile organic compound emissions. Trends Plant Sci 9:180–186
Peñuelas J, Llusià J, Asensio D, Munné-Bosch S (2005) Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. Plant Cell Environ 28:278–286
Petit JR, Jouzel J, Raynaud D, Barkov NI, Barnola JM, Basile I, Bender M, Chappellaz J, Davis M, Delaygue G, Delmotte M, Kotlyakov VM, Legrand M, Lipenkov VY, Lorius C, Pepin L, Ritz C, Saltzman E, Stievenard M (1999) Climate and atmospheric history of the past 420, 000 years from the Vostok ice core, Antarctica. Nature 399:429–436
Petron G, Harley P, Greenberg J, Guenther AB (2001) Seasonal temperature variations influence isoprene emission. Geophys Res Lett 28:1707–1710
Plaxton WC, Podesta FE (2006) The functional organization and control of plant respiration. Crit Rev Plant Sci 25:159–198
Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R, Monson RK (2003) Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem. Nature 421:256–259
Rosenstiel TN, Ebbets AL, Khatri WC, Fall R, Monson RK (2004) Induction of poplar leaf nitrate reductase: a test of extrachloroplastic control of isoprene emission rate. Plant Biol 6:12–21
Sage RF, Kubien DS (2007) The temperature response of C3 and C4 photosynthesis. Plant Cell Environ 30:1086–1106
Sanadze GA (1964) Light-dependent excretion of isoprene by plants. Photosynth Res 2:701–707
Schnitzler JP, Zimmer I, Bachl A, Arend M, Fromm J, Fischbach RJ (2005) Biochemical properties of isoprene synthase from poplar (*Populus*×*canescens*). Planta 222:777–786
Sharkey TD, Singsaas EL (1995) Why plants emit isoprene. Nature 374:769
Sharkey TD, Yeh S (2001) Isoprene emission from plants. Annu Rev Plant Physiol Plant Mol Biol 52:407–436
Sharkey TD, Singsaas EL, Lerdau MT, Geron CD (1999) Weather effects on isoprene emission capacity and applications in emissions algorithms. Ecol Appl 9:1132–1137
Sharkey TD, Yeh S, Wiberley AE, Falbel TG, Gong D, Fernandez DE (2005) Evolution of the isoprene biosynthetic pathway in kudzu. Plant Physiol 137:700–712
Siegenthaler U, Stocker TF, Monnin E, Lüthi D, Schwander J, Stauffer B, Raynaud D, Barnola J-M, Fischer H, Masson-Delmotte V, Jouzel J (2005) Stable carbon cycle-climate relationship during the late Pleistocene. Science 310:1313–1317
Singsaas EL, Sharkey TD (2000) Regulation of isoprene synthesis during high temperature stress. Plant Cell Environ 23:751–757
Singsaas EL, Lerdau M, Winter K, Sharkey TD (1997) Isoprene increases thermotolerance of isoprene-emitting leaves. Plant Physiol 115:1413–1420
Singsaas EL, Laporte MM, Shi JZ, Monson RK, Bowling DR, Johnson K, Lerdau M, Jasentuliytana A, Sharkey TD (1999) Leaf temperature fluctuations affect isoprene emission from red oak (*Quercus rubra*) leaves. Tree Physiol 19:917–924
Siwko ME, Marrink SJ, de Vries AH, Kozubek A, Uitercamp AJMS, Mark A (2007) Does isoprene protect plant membranes from thermal shock? A molecular dynamics study. Biochim Biophys Acta 1768:198–296
Tipple BJ, Pagani M (2007) The early origins of terrestrial C4 photosynthesis. Annu Rev Earth Plan Sci 35:435–461
Velikova V, Edreva A, Loreto F (2004) Endogenous isoprene protects *Phragmites australis* against singlet oxygen. Physiol Plant 122:219–225
Vickers CE, Possell M, Cojocariu CI, Laothawornkitkul J, Ryan A, Mullineaux PM, Hewitt CN (2009) Isoprene synthesis protects tobacco plants from oxidative stress. Plant Cell Environ 32:520–531
Wang X, Lewis JD, Tissue DT, Seemann JR, Griffin KL (2001) Effects of elevated atmospheric CO_2 concentration on leaf dark respiration of *Xanthium strumarium* in light and in darkness. Proc Natl Acad Sci USA 98:2479–2484
Way DA, Sage RF (2008) Thermal acclimation of photosynthesis in black spruce (*Picea mariana* (Mill.) B.S.P.). Plant Cell Environ 31:1250–1262
Wilkinson MJ, Monson RK, Trahan N, Lee S, Brown E, Jackson RB, Polley HW, Fay PA, Fall R (2009) Leaf isoprene emission rate as a function of atmospheric CO_2 concentration. Glob Change Biol 15:1189–1200
Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms and aberrations in global climate 65 MA to present. Science 292:686–696