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The influence of light environment on photosynthesis and basal methylbutenol emission from *Pinus ponderosa*

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ABSTRACT

Methylbutenol is a 5-carbon alcohol that is produced and emitted by several species of pine in western North America, and may have important impacts on the tropospheric chemistry of this region. In the present study the response of methylbutenol basal emission rate (measured at a constant light intensity of 1500 μmol m^{-2} s^{-1} and temperature of 30 °C) to the light and temperature conditions of the growth environment was examined, using field-grown plants shielded with shade cloth of various densities. Methylbutenol basal emission rates increased linearly with the temperature of the growth environment but did not respond to the shading of foliage during growth and development. Both photosynthesis and basal methylbutenol emission rate declined in older needles; however, these declines appear to result from parallel but independent processes and not from basal MBO emission rate directly tracking photosynthetic rates. Older needles did not occupy cooler microenvironments within the canopy; and thus differing thermal microenvironment could not explain the reduced MBO emission in older needles.

Key-words: light; methylbutenol; needle age; photosynthesis; ponderosa pine; shade; temperature; volatile organic compounds (VOCs).

INTRODUCTION

Through the production and release of volatile organic compounds (VOCs), plants exert profound influences on the trace gas composition of the atmosphere and the chemical processes taking place in the troposphere. Phytogenic VOCs have been implicated in the production of tropospheric ozone (Brasseur & Chatfield 1991; Chameides *et al*. 1992; Fehsenfeld *et al*. 1992), carbon monoxide (Zimmerman *et al*. 1978; Seiler & Conrad 1987), and acetone (Goldstein & Schade 2000). They also play important roles in regulating OH radical concentration in the troposphere (Ehhalt, Dorn & Poppe 1991), and in aerosol formation (Andreae & Crutzen 1997). While generally not considered to be strong greenhouse gases, phytogenic VOCs may indirectly exacerbate the greenhouse effects of methane by increasing its residence time in the atmosphere (Jacob & Wofsy 1988; Wuebbles *et al*. 1989). Thus, identifying and understanding the processes regulating VOC emission to the atmosphere, and predicting what emission will be under various environmental conditions is an important prelude to understanding and predicting the chemical behaviour of the lower atmosphere.

Globally, isoprene and monoterpenes are the most important and well-studied phytogenic VOCs (Tingey *et al*. 1979; Zimmerman 1979; Tingey, Evans & Gumpertz 1981; Monson & Fall 1989; Harley *et al*. 1994; Monson *et al*. 1994; Harley, Guenther & Zimmerman 1996). However, many pines in western North America emit large amounts of the C-5 alcohol 2-methyl-3-buten-2-ol (methylbutenol or MBO); and subsequent studies have shown MBO to have impacts on atmospheric chemistry rivalling those of isoprene and monoterpenes in large portions of this region (Goldan, Kuster & Fehsenfeld 1993; Harley *et al*. 1998; Lamanna & Goldstein 1999; Schade *et al*. 2000; Gray, Lerdau & Goldstein 2003; Lerdau & Gray 2003). Yet, in contrast to the extensive history of research on isoprene and monoterpenes, methylbutenol emission has received little study.

The emission of MBO into the atmosphere is regulated at two temporal scales. On short time scales (seconds to minutes) MBO emission responds to both light and temperature in a fashion similar to that observed for isoprene emission and the emission of monoterpenes from the Mediterranean oaks (Sanadze & Kursanov 1966; Tingey *et al*. 1979; Monson & Fall 1989; Loreto & Sharkey 1990; Monson *et al*. 1992; Staudt & Seufert 1995; Harley *et al*. 1996; Harley, Guenther & Zimmerman 1997; Harley *et al*. 1998; Staudt & Bertin 1998; Singsaas *et al*. 1999; Singsaas 2000; Schade & Goldstein 2001; Gray *et al*. 2003). MBO emission increases with light intensity in a pattern that parallels increases in photosynthetic carbon assimilation (Harley *et al*. 1998; Gray *et al*. 2003). In response to temperature, MBO emission exhibits an Arrhenius response in which

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emission increases exponentially with temperature to an optimum, above which emission rates decline precipitously (Harley *et al*. 1998; Gray *et al*. 2003). These light and temperature responses are consistent with the emission behaviour of VOCs that are not stored within the plant's tissues.

Although much of the variation in MBO emission can be explained by the short-term ('instantaneous') influences of light and temperature on MBO emission rates, MBO emission also appears to be regulated at longer time scales. MBO exhibits a pronounced seasonal pattern of emission (Harley *et al*. 1998; Schade *et al*. 2000; Schade & Goldstein 2002; Gray *et al*. 2003) in which emission rates are highest during the middle of the growing season and lower at the beginning and end. There is also a developmental pattern in which MBO emission rates decline with needle age (Harley *et al*. 1998; Gray *et al*. 2003). Similar seasonal patterns have been observed for isoprene emission (Schnitzler, Lehning & Steinbrecher 1997; Goldstein *et al*. 1998; Sharkey *et al*. 1999); and for both isoprene and MBO, these seasonal patterns appear to be explained by the temperature history experienced by the plant (Sharkey & Loreto 1993; Monson *et al*. 1994; Hanson & Sharkey 2001b; Gray *et al*. 2003). The 'longer term' effects of ambient light environment on basal emission rate have been examined for isoprene-emitting species but are less dramatic than those of temperature; however, including ambient light intensity in addition to temperature improved predictions of isoprene emission at the ecosystem scale (Harley *et al*. 1997; Sharkey *et al*. 1999; Geron *et al*. 2000; Hanson & Sharkey 2001b; Funk *et al*. 2003).

While the instantaneous responses of MBO emission to light and temperature have been well characterized and longer term responses to temperature and needle age have been documented (Harley *et al*. 1998; Gray *et al*. 2003), longer term responses of MBO emission to light have not yet been addressed and the mechanisms underlying the decline in MBO emission in older needles are currently unknown. Furthermore, the 'long-term' response of basal isoprene emission to the light environment observed in broadleaf deciduous plants may not be a good model for predicting the behaviour of MBO emission from pines. Unlike the leaves of deciduous trees, which develop and spend most of their lives within a single light environment, the foliage of pines initially develops in the un-shaded outer portion of the canopy, and by virtue of being retained for many years experiences a light environment that becomes progressively more shaded through time. If the basal MBO emission rate responds to light environment, this could explain the observed pattern of declining basal MBO emission with needle age (Harley *et al*. 1998; Gray *et al*. 2003). Alternatively, these declines may be mediated indirectly through effects of shading on needle temperature. Understanding the role of light and temperature in regulating basal MBO emission and the causes of declining basal emission with needle age is important both for understanding the biology surrounding MBO emission as well as for developing better models for predicting MBO emission.

In this study we present the results of an experiment in which the ambient light environment of *P. ponderosa* branches was manipulated with shade cloth. We examine the effect this shading had on photosynthetic capacity, chlorophyll content, and MBO basal emission rates in current flush, 1-year-old, and 2-year-old needles over the course of the season. By monitoring needle temperatures in shaded and un-shaded branches we also address the question of whether the differences in basal emission amongst needle age classes could be due directly to self-shading in the canopy, correlated with changes in photosynthetic capacity, or the result of older needles residing in more shaded and hence cooler microenvironments within the canopy.

METHODS

Study site and species

Experiments were conducted in the central Sierra Nevada Mountains of California at the UC Berkeley Blodgett Forest Research Station. Blodgett Forest Research Station is located east of the town of Georgetown, CA at an elevation of 1300 m (38°53′ 42.9″ N, 120°37′ 57.9″ W). This region experiences a Mediterranean climate with hot dry summers and cool wet winters. Studies took place in an even-aged plantation of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), aged 10–15 years, 4–6 m in height, and located in compartment 400 of Blodgett Forest. This plantation had an open canopy, was located on a south-east-facing slope, and in addition to *P. ponderosa* contained scattered individuals of sugar pine (*Pinus lambertiana* Dougl.), Douglas fir (*Pseudostuga menziesii* (Mirb.) Franco), white fir (*Abies concolor* (Gord. & Glend.) Hildebr.), and incense cedar (*Calocedrus decurrens* Torr.). We conducted all experiments on ponderosa pine because it produces large quantities of MBO, covers extensive areas of the forested western United States, and is the most commercially important MBO-emitting species.

Experimental designs

In order to examine the effects that the ambient light environment had on MBO basal emission rates, we performed an experiment in which the light environment of developing (expanding) and fully expanded foliage was manipulated with shade cloth. Four trees were selected, each with at least three branches receiving full sun for at least half the day. Branches on a tree were randomly assigned to one of three shade treatments (full sun, 50% sun, or 20% sun). Alteration of light environment was achieved by draping shade cloth of the appropriate density over a wooden scaffold. This scaffold was attached to the branch and held the shade cloth at least 5 cm away from the foliage. Shade cloth was tied to the base of the branch and fastened along its seam to completely encase the scaffold and branch on all sides. Shading was applied on 14 June 2000 prior to flushing of the current year's needles. Therefore Age 0 (season 2000) needles developed entirely under the respective

shade treatment; however, Age 1 (season 1999) and Age 2 (season 1998) needles developed under ambient light prior to application of the shade cloth.

Measurements of gas exchange parameters and methylbutenol emission were made on all available needle age classes (Age 0, Age 1, Age 2) five times between 10 June 2000 and 4 October 2000. Initial measurements took place prior to the flushing of new needles and shade treatments were applied on 14 June 2000 immediately following the first set of measurements. The same trees and branches were sampled during each sampling period over the season; however, measurements were made on different needles at each sampling period due to destructive sampling for chlorophyll content. At the end of each sampling period the needle pairs measured were excised from the tree and subsamples were dried for the determination of leaf mass per unit area or frozen in liquid nitrogen prior to determination of needle chlorophyll content. Needle temperatures were recorded for all trees, branches, and needle age classes throughout the season.

Temperature measurements

Measurements of needle temperature were made using four single channel automated data loggers (MicroDAQ, Warner, NH, USA) equipped with 0.005 inch (0.127 mm) fine wire type E thermocouples (Omega Engineering Inc., Stamford CT, USA). To ensure accurate measurement of needle temperature, each thermocouple was wrapped tightly around its associated needle such that the thermocouple junction was tightly appressed to the needle surface. The integrity of this configuration was checked weekly, adjusted as needed, and proved very robust to disturbances caused by wind-induced branch movements. One data logger was dedicated to measuring needle temperatures for a single set of 1-year-old, un-shaded needles over the course of the season and the remaining three data loggers were rotated among the remaining study trees, branches, and age classes. This allowed us to determine daily temperature profiles for each measured tree, branch, and age class, and to correct for differences in temperature micro-environment caused by the spatial positioning of trees, branches, and needles, or the influence of altered light and air circulation patterns caused by application of the shade cloth. Ambient air temperatures were measured within the canopy of each study tree using a thermistor integrated within the casing of each data logger.

Gas exchange and chromatography protocols

Leaf level measurements of photosynthetic parameters and MBO emission rates were made using a LiCor LI-6400 portable gas exchange system (LiCor Inc., Lincoln, NE, USA) and a Voyager portable gas chromatograph (GC) (PE Photovac Inc., Norwalk, CT, USA). The LI-6400 allows good control of the light, temperature, and CO_2 environment experienced by the foliage while it is being measured. Light control in this system is achieved with a series of red and blue light-emitting diodes (peak irradiance 665 and 470 nm) mounted in the top of the leaf cuvette. Temperature is regulated using thermoelectric (Peltier) coolers mounted on the sides of the cuvette. The Peltier coolers supplied by the manufacturer allow temperature control of the cuvette in a range of ±6 °C of ambient temperature; however, by adding heating elements in a cardboard shroud enclosing the lower portions of the cuvette, we were able to operate at temperatures as much as 15 °C above ambient. Leaf temperatures were calculated based on the energy balance equations implemented in the LI-6400 software (LI-COR 1995).

A pair of three-needle fascicles (six needles) were clamped into the LI-COR cuvette, and the cuvette was flushed with 100 μmol s^{-1} (approximately 150 mL min^{-1}) of ambient air scrubbed of hydrocarbons by passage through an activated charcoal filter. The effectiveness of this trap for hydrocarbon removal was checked daily by running chamber blanks. This trap reduced the hydrocarbon content of air entering the measurement system below the 5 p.p.b. detection level for the GC and did not require replacement for the duration of the study. Exhaust gases from the cuvette were routed via Teflon® (DuPont, Wilmington, DE, USA) tubing past the sample inlet port on the GC. The Voyager GC contains three columns and a 1 mL sample loop, operates isothermally, and uses a photo-ionization detector for detection and quantification of volatiles. The GC sample loop was loaded via an internal sampling pump; and a 1 mL aliquot was injected onto a methyl silicone capillary column (15 m length, ID 0.32 mm, coating thickness 12 μm). The GC was operated isothermally at 65 °C and 103.42 kPa column pressure of ultra high purity nitrogen carrier gas. The detection limit for this system was 5 p.p.b., and precision was within 10%. Chamber air typically had mixing ratios between 10 and 100 p.p.b. Five point standard curves diluted from a 100 p.p.m. MBO gas standard (Scott-Marrin, Inc. Riverside, CA, USA) were run at the end of each sampling day.

Light and temperature response curves

Light response curves were developed by holding two fascicles at 30 °C, 380 p.p.m. CO_2, and a saturating photosynthetic photon flux density (PPFD) of 2000 μmol photons m^{-2} s^{-1} (400–700 nm) until steady-state MBO flux was attained. Steady state was defined as three successive methylbutenol measurements differing by <5% or 1 mV, whichever was less. It typically took 30–40 min to reach steady state (equilibrium) but could take as long as 2 h in rare cases. Holding temperature and CO_2 constant, light was subsequently reduced in discrete steps allowing the MBO flux to come to equilibrium prior to the next light reduction. Temperature response curves were developed by holding two fascicles at a constant light level of 1500 μmol photons m^{-2} s^{-1}, 380 p.p.m. CO_2, and the lowest temperature possible under prevailing ambient conditions until MBO flux reached steady state. Holding light and CO_2 constant, temperature was subsequently increased in 2 °C steps

allowing the MBO flux to come to equilibrium prior to the next temperature increase. Temperature was increased until MBO emission began to decline precipitously.

Instantaneous emission rate versus basal emission rate/production capacity

Since MBO emission is both light and temperature dependent, these factors must be held constant to allow comparison amongst measurements and investigation of factors regulating emission at longer time scales. We measured MBO emission and photosynthetic rates under constant conditions (380 p.p.m. CO_2, 1500 μmol photons m^{-2} s^{-1} PPFD, 30 °C) in order to control for the effects of light and temperature. This 'basal' emission rate is an index of the plant's capacity to produce MBO (see Monson *et al*. 1995 for the analogous argument with respect to isoprene production). Comparison of production capacities obtained by holding light and temperature constant factors out the short-term influence of light and temperature and allows one to investigate physiological and biological changes with respect to MBO production that occur over longer-term periods and in response to experimental manipulations. All measurements of basal MBO emission and photosynthesis were made under steady-state conditions. Steady state was defined as three successive methylbutenol measurements differing by <5% or 1 mV, whichever was less.

Area versus mass-based measurements

Flux measurements were expressed either per unit of biomass or per unit needle surface area (all sided area). Subsequent comparisons made in the lab showed that projected leaf area is approximately one half the calculated needle surface area in *P. ponderosa*. Total leaf surface area inside the cuvette was calculated using caliper measurements made on each needle. Each needle of a three-needle fascicle was assumed to have a cross-section shaped like a pie slice taken from a circle with a radius equal to the average caliper measured radius of all three needles. The perimeter of each needle was calculated according to the following equation

$$P = 2/3\ \pi r_{ave} + 2r_n \quad (1)$$

where r_{ave} equals the average needle radius, $2/3\ \pi r_{ave}$ equals the length of the curved surface of each needle, r_n equals the radius of each individual needle, and $2r_n$ equals the length of the sides of each pie slice. The surface area for the foliage enclosed in the chamber was calculated by summing the perimeter of each needle and multiplying by the 3 cm length of the needle enclosed in the cuvette. The needle segments placed in the chamber were marked, excised following the measurement, dried at 60 °C for 72 h or until no further loss of mass was recorded, and used to calculate a mass per unit area. Gas exchange parameters and MBO fluxes were expressed on both an all sided area basis and on a unit mass basis as described in Gray *et al*. (2003).

Chlorophyll analysis

Needle samples designated for analysis of chlorophyll content were flash frozen and stored in liquid nitrogen until chlorophyll extractions and measurements were conducted. Chlorophyll was extracted with dimethyl sulphoxide (DMSO) using the method of Hiscox & Israelstam (1979), and the chlorophyll *a* and chlorophyll *b* content were determined spectrophotometrically using the equations of Wellburn (1994) developed for use with DMSO as the extraction solvent. Samples were extracted in 6 mL DMSO incubated at 65 °C for 6 h, then frozen until absorbance was measured 12 h later. Absorbance of extracts was measured at 649 and 665 nm (1 nm band centred on these values) using a spectrophotometer (Ocean Optics, Inc., Dunedin FL, USA).

Statistical analysis

The instantaneous responses of photosynthesis and MBO emission to changes in light intensity were compared for shaded and un-shaded Age 0 needles with the Kolmogorov–Smirnov test (SYSTAT 1997). The experimental design employed for the shading experiment necessitated a non-standard statistical model because of the expectation that branches are biologically integrated within a tree and that needles are biologically integrated within a branch. Data from the shading experiment were analysed as a four-factor ANOVA in which all factors (Date, Tree, Shade, Age) were crossed with each other. The design of the shading experiment further incorporated a split-plot treatment structure for the experimental factors Shade and Age because branches as experimental units are nested within trees, and needles as experimental units are nested within branches (Oehlert 2000). In this design branches were treated as the whole plot to which various levels of the Shade treatment were applied and individual needles were treated as the split plot to which different levels of the Age treatment were applied. Sampling dates were treated as a main effect rather than incorporated as a repeated measurement in a repeated measures design because different needles were measured during each sampling period. All analyses of the shading experiment were carried out using the SAS procedure GLM (SAS 1990).

RESULTS

Influence of ambient light environment on instantaneous light response curves

To determine whether the shape of the instantaneous light response curves for photosynthetic carbon assimilation and MBO emission differed depending on the light environment under which *P. ponderosa* needles developed, we measured light response curves in Age 0 (year 2000) needles grown in full sun and Age 0 (year 2000) needles grown in the 20% sun treatment. This comparison showed that instantaneous photosynthetic rates and MBO emission

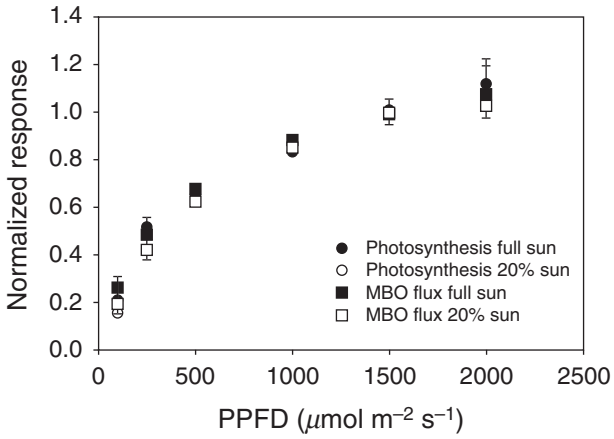

Figure 1. Response of methylbutenol emission (squares) and photosynthesis (circles) to light intensity for Age 0 (current flush) *Pinus ponderosa* needles grown under full sun (closed symbols) or 20% sun (open symbols). Measurements of methylbutenol emission and photosynthesis were made under constant temperature conditions (30 °C) on July 30–31, 2000 (DOY 211–212). Data were normalized by dividing each light response measurement by the value obtained at a photosynthetic photon flux density (PPFD) of 1500 μmol photons m^{-2} s^{-1}. Error bars represent ±1 standard deviation with $n = 3$.

rates changed in parallel with increasing light, but that photosynthetic and MBO emission responses did not differ between treatments (Fig. 1).

Influence of ambient light environment on basal photosynthesis

Analysis of the shading experiment by ANOVA revealed significant effects of sampling Date and needle Age and non-significant effects of Tree and Shade treatment on photosynthetic rates (Table 1). However, the interpretation of these results is complicated by statistically significant Age × Shade, Date × Shade, and Date × Age interactions. A closer examination of the data reveals that the Age × Shade and Age × Date interactions are driven by photosynthetic differences across shade treatments occurring only during the final sampling period day of year (DOY) 275–278. On DOY 275–278 photosynthetic rates differed with shade only in Age 0 and Age 1 needles (Age × Shade interaction).

Photosynthesis consistently declined with Age in all sampling periods; however, the quantitative differences between needle ages differed slightly through time, resulting in the Age × Date interaction (data not shown). Pooling data across sampling periods and re-analysing the data via ANOVA shows a significant decline in photosynthetic rates (Fig. 2a) with needle age (ANOVA, $P < 0.0001$), but no significant effect of shading for any needle age class (Fig. 2a). This result is consistent with the significant Age and non-significant Shade main effects obtained from the full analysis shown in Table 1. A closer examination of the Date effect shows that photosynthesis increased over the first two sampling periods and then declined over the rest of the season (Fig. 3a). This seasonal pattern was consistent for all needle age classes (data not shown). Photosynthetic rates differed across Shade treatments only during the final sampling period resulting in a Date–Shade interaction (Fig. 3a), however, shading did not influence photosynthetic rates in a consistent fashion across needle ages during this final sampling period (data not shown).

Influence of ambient light environment on basal MBO emission

Analysis of the shading experiment by ANOVA revealed significant effects of sampling Date, Tree, and Age on MBO basal emission rate, but no significant effect of Shade (Table 1). However, the interpretation of these results is complicated by statistically significant Date × Age and Date × Age × Shade interactions. Examining the pattern of MBO emission more closely shows that MBO emission

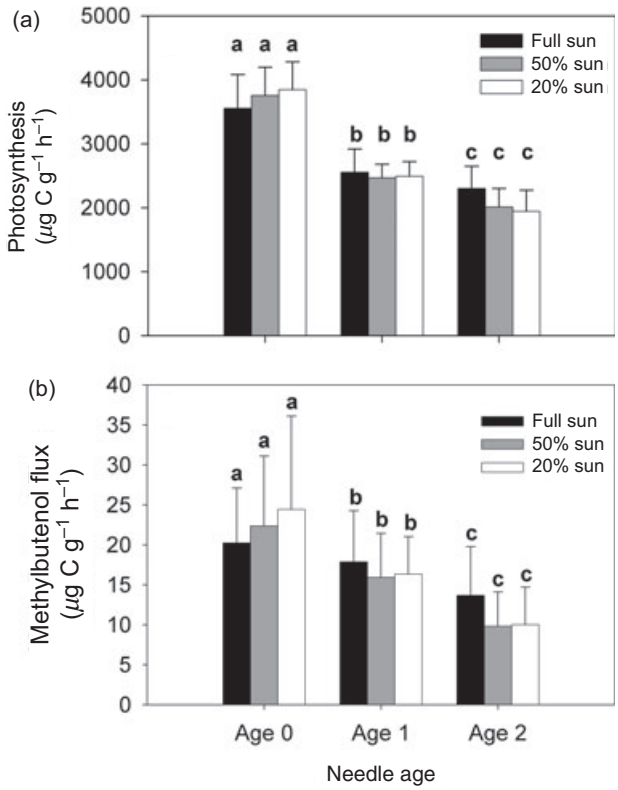

Figure 2. Response of basal photosynthetic rate (a) and basal MBO emission rate (b) to growth under full sun (black bars), 50% sun (grey bars), or 20% sun (white bars) in Age 0, Age 1, and Age 2 needles of *Pinus ponderosa*. Age 0 needles developed under the indicated shading treatment, while Age 1 and Age 2 needles developed under full sun and received shading treatment either 1 or 2 years after completing development. Data were collected at standard measurement conditions of 1500 μmol photons m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and 30 °C needle temperature. Error bars represent standard deviations of measurements pooled across sampling dates. Significant differences (Tukey test $P < 0.05$) amongst shading treatments within a sampling date are indicated by different letters above bars.

Table 1. Results of ANOVA from the shading experiment examining differences in basal photosynthetic rate and basal methylbutenol emission rate in *Pinus ponderosa* needles as a function of sampling date, tree identity, shading, and needle age

Variable	Source	d.f.	MS	Error Term	MSE	*F*	*P*
Methylbutenol flux							
	Main effects						
	Date	4	858.5	Date × Branch (Tree × Shade)	16.61	51.70	0.0001
	Tree	3	1339.4	Branch (Tree × Shade)	45.20	29.63	0.0005
	Shade	2	32.3	Branch (Tree × Shade)	45.20	0.71	0.5267
	Age	2	1529.3	Error	10.79	141.80	0.0001
	Interactions						
	Age × Shade	4	82.77	Age × Branch (Tree × Shade)	37.37	2.21	0.1082
	Date × Shade	8	26.87	Error	10.79	2.49	0.0646
	Date × Age	7	38.87	Age × Date × Branch (Tree × Shade)	12.80	3.04	0.0090
	Date × Age × Shade	14	32.03	Age × Date × Branch (Tree × Shade)	12.80	2.50	0.0079
Photosynthesis							
	Main effects						
	Date	4	12259628	Date × Branch (Tree × Shade)	137027	89.47	0.0001
	Tree	3	642192	Branch (Tree × Shade)	284768	2.26	0.1823
	Shade	2	67293	Branch (TreevShade)	284768	0.24	0.7966
	Age	2	38434404	Error	35910	1070.0	0.0001
	Interactions						
	Age × Shade	4	683609	Age × Branch (Tree × Shade)	130921	5.22	0.0057
	Date × Shade	8	467969	Error	35911	13.04	0.0001
	Date × Age	7	511646	Age × Date × Branch (Tree × shade)	121698	4.20	0.0009
	Date × Age × Shade	14	106146	Age × Date × Branch (Tree × Shade)	121698	0.87	0.5915

Photosynthesis and methylbutenol flux measured under standard conditions (1500 μmol m^{-2} s^{-1} PPFD and 30 °C needle temperature) as micrograms carbon fixed/released g^{-1} leaf dry weight h^{-1}.

rates declined with needle age in all shade treatments (Fig. 2b) This qualitative pattern of declining MBO emission with needle age was consistent across sampling dates, although quantitative differences in MBO emission rates resulted in a significant Date × Age interaction (data not shown). MBO basal emission rate increased during the first two sampling periods, and subsequently declined over the rest of the season (Fig. 3b). MBO emission did not differ amongst shade treatments in Age 1 needles and only on sampling days 202–205 in Age 0 needles and DOY 178–181 in Age 2 needles (data not shown). These differences resulted in a significant Date × Age × Shade interaction. Pooling all sampling dates reveals that basal MBO emission rate declines significantly with needle age; however, within a needle age class shading level does not significantly alter basal MBO emission rate (Fig. 2b). This result is consistent with the significant Age and non-significant Shade main effects obtained from the full analysis shown in Table 1.

Influence of needle age and shade treatment on chlorophyll content

Analysis of total needle chlorophyll content revealed significant effects of both Tree and needle Age on total chlorophyll content and non-significant effects of both Date and Shade and significant Date × Shade and Date × Age interactions. Examining the changes in total chlorophyll content through time reveals that chlorophyll content was the same in all treatments prior to the application of shade cloth and that chlorophyll content diverged in the shade treatments following the application of shade cloth (Fig. 4a). This observation explains the significant Date × Shade interaction and further indicates that increased shade caused an increase in total chlorophyll. The significant Date × Age interaction results from an elevated total chlorophyll content in older needles at later sampling dates that is not present during the first sampling period (Fig. 4b). However, this observation does not alter the general pattern in which Age 0 needles have lower total chlorophyll contents than Age 1 and Age 2 needles (Fig. 4b).

Analysis of the chl *a*/*b* ratio revealed significant effects of Date and needle Age, non-significant effects of Tree and Shade, and no significant interactions (Table 2). Pooling measurements from all shading levels reveals that chl *a*/*b* ratio declines with needle Age (Fig. 5).

Influence of needle temperature on basal MBO emission

Basal MBO emission rates showed a consistent positive correlation with both ambient air temperature (Fig. 6) and needle temperature (data not shown) for all four trees measured. This relationship was well described by linear models ($r^2 = 0.432$–0.752). Comparing the slopes of the temperature/MBO regressions indicates that MBO basal emission changed by 3.2% for every degree by which ambient air temperature differed from 30 °C.

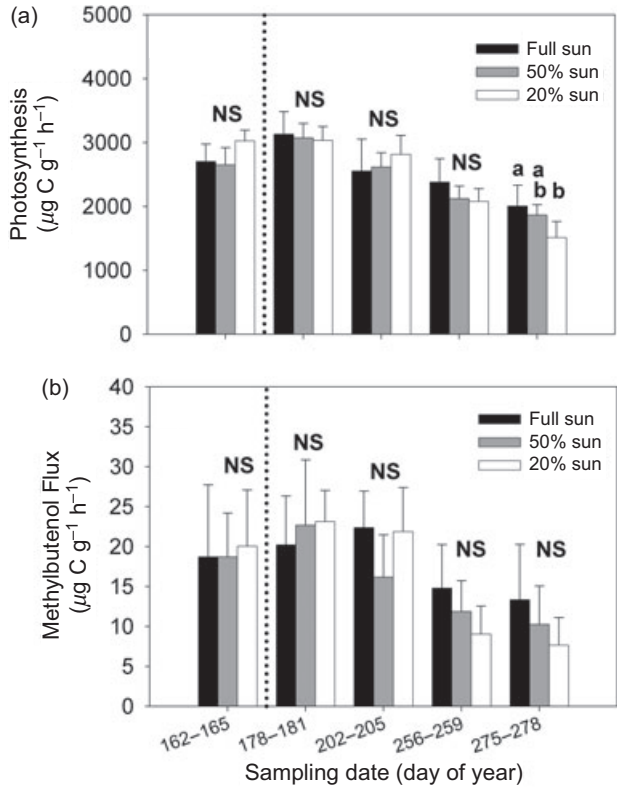

Figure 3. Seasonal patterns of basal photosynthetic rates (a) and basal MBO emission rates (b) in Age 1 needles growing in full sun (black bars), 50% sun (grey bars), or 20% sun (white bars). The date shading was applied (14 June 2000) is indicated by the vertical dotted line. Data were collected at standard measurement conditions of 1500 μmol photons m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and 30 °C needle temperature. Error bars represent standard deviations with sample size $n = 4$. Sampling dates represent Julian Calendar days where 1 January 2000 is day 1 and 31 December 2000 is day 366. Significant differences (Tukey test $P < 0.05$) amongst shading treatments within a sampling date are indicated by different letters above bars.

Influence of air temperature, needle age, and shade treatment on needle temperature

To determine whether older needle age classes inhabited cooler micro-environments deeper within the canopy, diurnal temperature profiles of current flush (Age 0), 1-year-old (Age 1), and 2-year-old (Age 2) needles were measured on a full sun branch over the course of several days (Fig. 7). Needle temperatures of full sun branches tracked air temperatures very closely throughout the day and needle temperatures did not differ amongst needle age classes (Kolmogorov–Smirnov test; $P > 0.5$).

Influence of photosynthetic rate on MBO basal emission rate

We use the seasonal changes in basal photosynthetic rate and MBO emission rate to examine whether the basal rate of photosynthesis controls the basal rate of MBO emission. Pooling data from all needle age classes reveals a significant positive correlation between basal photosynthesis and basal MBO emission. However, this relationship is not supported when each needle age class is analysed separately (Fig. 8), because within an age class there is no relationship between basal photosynthesis and MBO emission.

DISCUSSION

Shade responses

A reduction in sunlight to 20% of ambient light had relatively little effect on *P. ponderosa* needles in comparison with needles that developed in full sun. Shade did not have

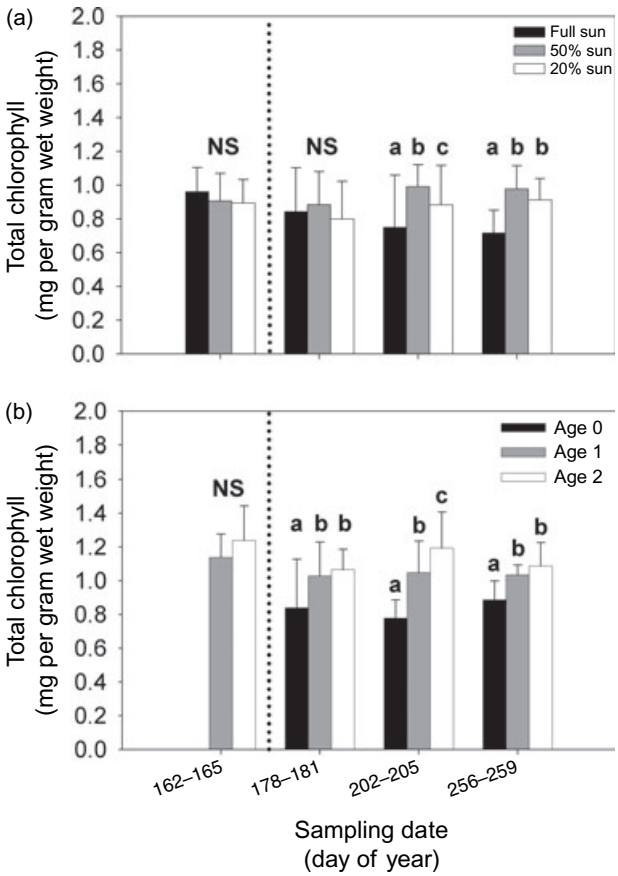

Figure 4. (a) Seasonal changes in total chlorophyll content to growth under full sun (black bars), 50% sun (grey bars), or 20% sun (white bars) in needles of *Pinus ponderosa*. (b) Seasonal changes in total chlorophyll content in Age 0 (black bars), Age 1 (grey bars), and Age 2 (white bars) needles of *P. ponderosa*. Chlorophyll content was determined spectrophotometrically using the equations of Wellburn (1994). The date shading was applied (14 June 2000) is indicated by the vertical dotted line. Error bars represent standard deviations of measurements pooled across needle ages. Sampling dates represent Julian Calendar days where 1 January 2000 is day 1 and 31 December 2000 is day 366. Significant differences (Tukey test $P < 0.05$) amongst shading treatments within a sampling date are indicated by different letters above bars.

Table 2. Results of ANOVA from the shading experiment examining differences in total chlorophyll content and the chlorophyll *a*/*b* ratio in *Pinus ponderosa* needles as a function of sampling date, tree identity, shading, and needle age

Variable	Source	d.f.	MS	Error Term	MSE	*F*	*P*
Chl_{tot}							
	Main effects						
	Date	3	0.0162	Date × Branch (Tree × Shade)	0.0085	1.64	0.2146
	Tree	3	0.1265	Branch (Tree × Shade)	0.0181	7.00	0.0200
	Shade	2	0.0794	Branch (Tree × Shade)	0.01781	4.46	0.0599
	Age	2	0.4879	Error	0.0092	53.07	0.0001
	Interactions						
	Age × Shade	4	0.0235	Age × Branch (Tree × Shade)	0.0116	2.04	0.1181
	Date × Shade	6	0.0277	Error	0.0092	3.01	0.0214
	Date × Age	5	0.0399	Age × Date × Branch (Tree × Shade)	0.0092	4.34	0.0032
	Date × Age × Shade	9	0.0094	Age × Date × Branch (Tree × Shade)	0.0092	1.02	0.4469
Chl *a*/*b* ratio							
	Main effects						
	Date	3	1.4010	Date × Branch (Tree × Shade)	0.2592	5.41	0.0038
	Tree	3	1.2351	Branch (Tree × Shade)	0.4774	2.59	0.1439
	Shade	2	0.2237	Branch (Tree × Shade)	0.4698	0.48	0.6410
	Age	2	3.5842	Error	0.2610	13.74	0.0001
	Interactions						
	Age × Shade	4	0.4935	Age × Branch (Tree × Shade)	0.2436	2.03	0.1615
	Date × Shade	6	0.0503	Error	0.2610	0.19	0.9762
	Date × Age	5	0.3372	Age × Date × Branch (Tree × Shade)	0.2609	1.29	0.2927
	Date × Age × Shade	9	0.3984	Age × Date × Branch (Tree × Shade)	0.2609	1.53	0.1870

Total chlorophyll content measured spectrophotometrically using the equations of Wellburn (1994) and analysed on a unit fresh mass basis (mg chlorophyll g^{-1} fresh weight).

an effect on the instantaneous MBO light response patterns (Fig. 1); and comparison of basal MBO emission rates in needles that developed in shade and needles that developed in full sun did not reveal any difference in emission between the shade treatments (Fig. 2, Table 1). This contrasts sharply with similar investigations of the effect that shading has on basal isoprene emission rates. Harley *et al.* (1996) and Lerdau & Throop (2000) show that isoprene emission from shaded foliage reaches saturation at lower light intensity than isoprene emission from foliage grown

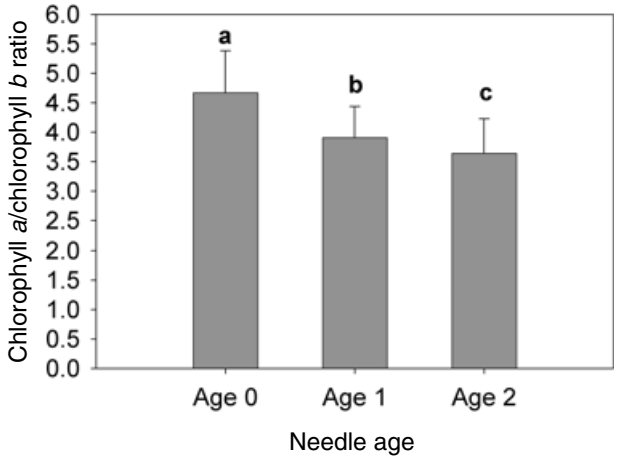

Figure 5. Influence of needle age on the ratio of chlorophyll *a* to chlorophyll *b*. Chlorophyll content was determined spectrophotometrically using the equations of Wellburn (1994). Error bars represent standard deviations of measurements pooled across sampling dates and shading treatments. Significant differences amongst needle ages are indicated by different letters above bars.

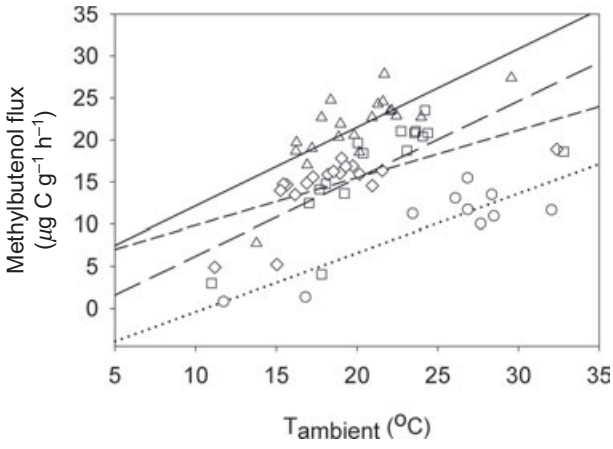

Figure 6. Relationship between basal methylbutenol emission rate and ambient air temperature at the time of emission measurement. Measurements were made between 10 June 2000 and 22 October 2000 and each measurement was made under constant cuvette environmental conditions of 1500 μmol photons m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and a needle temperature of 30 °C. Different symbols and regression lines represent data taken from four different trees. Regression lines are the results of least squares linear models. Solid line corresponds to triangles, long dash corresponds to squares, short dash corresponds to diamonds, dotted corresponds to circles.

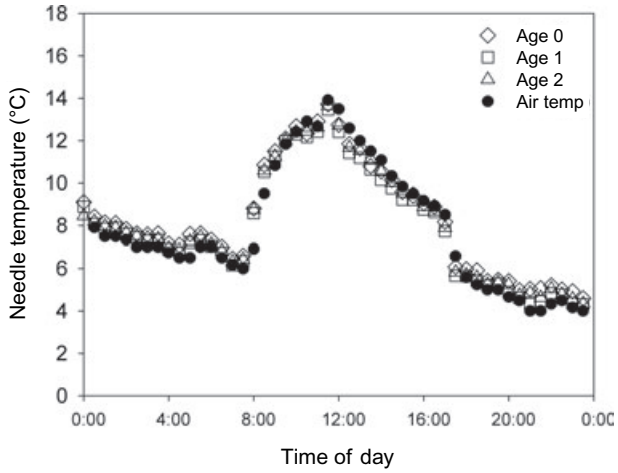

Figure 7. Daily pattern of *Pinus ponderosa* needle temperatures measured on Age 0 (diamonds), Age 1 (squares), Age 2 (triangles) needles growing under conditions of full sun on 9 October 2000. Temperatures were measured using thermocouple junctions pressed against the needle surface, and plotted measurements represent 30-min averages of temperature readings collected once per minute.

under full sun. Similarly, it has been shown that artificial shading reduced isoprene basal emission rates (Hanson & Sharkey 2001a; Funk *et al*. 2003), and that leaves developing in more shaded regions of the canopy exhibit reduced isoprene emission capacity (Harley *et al*. 1997).

These reductions in isoprene emission observed in shaded foliage are usually paralleled by changes indicating that shade altered photosynthetic physiology and leaf morphology. Leaves adapted to more shaded canopy locations are thinner, reach photosynthetic saturation at lower light intensities, and have lower maximal photosynthetic rates than leaves developing in full sun (Harley *et al*. 1997). Hanson and Sharkey (Hanson & Sharkey 2001b) offer an exception in which basal isoprene emission declined with shade but photosynthetic capacity did not change. However, in response to shading, *P. ponderosa* needles did not show any changes that would suggest physiological acclimation to a shaded environment, such as changes in the pattern of photosynthetic light response (Fig. 1) or maximal photosynthetic capacity for any needle age class (Table 1, Fig. 2). This absence of a response to shading was observed in needles that developed entirely in shade (Age 0) as well as in needles that developed in full sun and were shaded following one year (Age 1 needles) or two years (Age 2 needles) of full sun exposure. This observation that photosynthetic physiology did not change in response to shade is interesting considering that total chlorophyll content increased (although only slightly) in response to the shade treatments (Fig. 4), and illustrates that, at least in *P. ponderosa*, biochemical differences (total chlorophyll content) may not translate into observable physiological differences (photosynthetic rates). Unlike broadleaf deciduous trees, which readily alter their leaf morphology and physiology in response to canopy position/shading (Ellsworth & Reich 1993; Harley *et al*. 1996), *P. ponderosa* foliage appears to show only a limited response to shade, increasing chlorophyll content (Fig. 4, Table 2) but not altering chl *a*/*b* ratios (Table 2), and exhibiting no changes in photosynthetic rates (Table 1, Fig. 2). Similar patterns were observed by Bond *et al*. (1999) who found significant increases in chlorophyll content and only slight (but significant) changes in photosynthesis per unit biomass in more shaded regions of a *P. ponderosa* canopy. The apparent inability of photosynthesis to respond to shading in *P. ponderosa* may explain why MBO emission also failed to respond to the shade treatment. It remains an intriguing question whether isoprene emitting conifers (Spruces and some Firs) will respond to shade like isoprene emitting angiosperms or the MBO-emitting conifer *P. ponderosa*.

Temperature, needle age, and seasonal pattern of MBO emission

Although the ambient light environment in which a needle resides did not influence basal MBO emission rates, the ambient temperature history experienced by a needle had a dramatic effect on basal MBO emission rates. MBO basal emission correlated linearly with ambient temperature (Fig. 6); and followed a seasonal pattern (Fig. 3) that was remarkably similar to that observed by Gray *et al*. (2003). These responses relating MBO basal emission and ambient temperature are consistent with observations of a strong relationship between temperature and isoprene basal emission rates shown for many taxa (reviewed in Sharkey & Yeh 2001; Lerdau & Gray 2003).

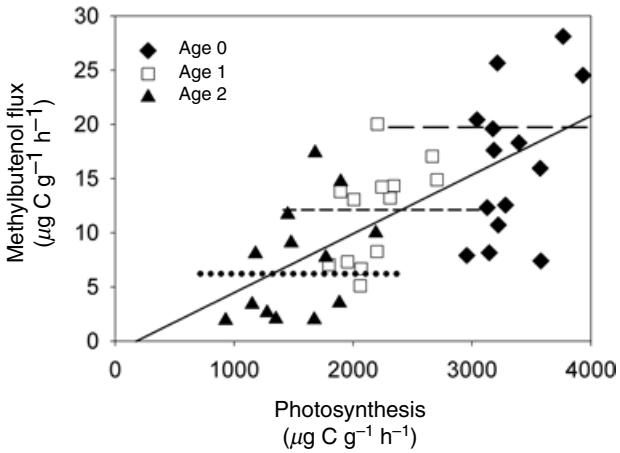

Figure 8. Relationship between basal methylbutenol emission rate and basal photosynthetic rate for Age 0 (closed diamonds), Age 1 (open squares), and Age 2 (closed triangles) needles measured between 10 June 2000 and 22 October 2000. Slopes of the response of methylbutenol emission to photosynthesis were not significantly different from zero within each age class. Broken lines indicate mean MBO emission rate for each needle age class (long dashed line = Age 0, short dashed line = Age 1, dotted line = Age 2). Solid line represents the least squares regression for data pooled across all needle age classes ($r^2 = 0.438$, $P = 0.0001$).

MBO basal emission rates and photosynthetic rates declined with needle age; however, the basis for these declines remains unclear. The results of this study clearly indicate that the position of older needle age classes in more shaded micro-environments cannot explain their lower basal emission rates since even an 80% reduction of light intensity via shade cloth failed to cause a change in basal MBO emission rate (Table 1, Fig. 2). Indirect effects of shading, mediated through lower needle temperatures of needles residing in more shaded locations, also cannot explain the observed differences in basal MBO emission in older needles, since needle temperatures measured on unshaded branches were identical for all needle age classes (Fig. 7).

A direct link between MBO emission and photosynthetic rate also cannot explain the declines in MBO emission in older needles despite the fact that older needles also experience declining photosynthetic rates. Although there is a striking pattern in which both photosynthetic rates and MBO emission rates decline with needle age (Fig. 8), when each needle age is examined individually there is no relationship between basal photosynthetic rate and MBO basal emission rate (Fig. 8, slopes within age classes not significantly different from 0). Thus, extrapolating the within-age relationship between photosynthesis and MBO emission in Age 0 needles to photosynthetic rates typical for Age 2 needles does not predict correspondingly lower MBO emission rates. This indicates that the lower MBO basal emission rates seen in older needles are not due to the lower photosynthetic rates also found in those needles. Instead, the positive correlation observed between MBO basal emission rate and photosynthetic capacity *across* ages (Fig. 8) seems to be the result of parallel declines in both processes as needles age.

CONCLUSIONS

Methylbutenol emission from *P. ponderosa* represents an important contribution to atmospheric trace gas composition of western North America and may contribute to ozone formation in this region. In many ways our understanding of isoprene emission provides a good model for understanding MBO emission. Both isoprene and MBO emission share nearly identical responses to short-term changes in the light and temperature environment experienced by foliage. Both isoprene and MBO emission exhibit longer term patterns of seasonal change in basal emission rates. For both isoprene and MBO emission these seasonal patterns appear to be driven by changes in ambient temperature. However, while basal isoprene emission exhibits longer term changes in the response to the ambient light environment, basal MBO emission appears to be unaffected by even extreme shading. The basis for this difference in emission behaviour is unclear, but might reflect a difference in the capacity of pines and angiosperms to respond to shading in general. Alternatively, the different response to shading may be the first indication of substantive differences in the manner in which isoprene and MBO are regulated. Examining the response of isoprene emitting conifers to long-term shading would be a useful first step towards determining if the shade-induced isoprene/MBO emission differences are due to peculiarities of MBO biosynthesis or broader differences in photosynthetic acclimation of angiosperms and gymnosperms to shading.

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REFERENCES

Andreae M.O. & Crutzen P.J. (1997) Atmospheric aerosols: Biogeochemical sources and role in atmospheric chemistry. *Science* **276,** 1052–1058.

Bond B.J., Farnsworth B.T., Coulombe R.A. & Winner W.E. (1999) Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. *Oecologia* **120,** 183–192.

Brasseur G.P. & Chatfield R.B. (1991) The fate of biogenic trace gases in the atmosphere. In *Trace Gas Emissions by Plants* (eds T.D. Sharkey, E.A. Holland & H.A. Mooney), pp. 1–27. Academic Press, Inc., San Diego, California, USA.

Chameides W.L., Fehsenfeld F., Rodgers M.O., *et al.* (1992) Ozone precursor relationships in the ambient atmosphere. *Journal of Geophysical Research* **97,** 6037–6055.

Ehhalt D.H., Dorn H.-P. & Poppe D. (1991) The chemistry of the hydroxyl radical in the troposphere. *Proceedings of the Royal Society of Edinburgh* **97B,** 17–34.

Ellsworth D.S. & Reich P.B. (1993) Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* **96,** 169–178.

Fehsenfeld F., Calvert J., Fall R., *et al.* (1992) Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochemical Cycles* **6,** 389–430.

Funk J.L., Jones C.G., Baker C.J., Fuller H.M., Giardina C.P. & Lerdau M.T. (2003) Diurnal variation in the basal emission rate of isoprene. *Ecological Applications* **13,** 269–278.

Geron C., Guenther A., Sharkey T. & Arnts R.R. (2000) Temporal variability in basal isoprene emission factor. *Tree Physiology* **20,** 799–805.

Goldan P.D., Kuster W.C. & Fehsenfeld F.C. (1993) The observation of a C_5 alcohol emission in a North American pine forest. *Geophysical Research Letters* **20,** 1039–1042.

Goldstein A.H., Goulden M.L., Munger J.W., Wofsy S., C. & Geron C.D. (1998) Seasonal course of isoprene emissions from a midlatitude deciduous forest. *Journal of Geophysical Research* **103,** 31045–31056.

Goldstein A.H. & Schade G.W. (2000) Quantifying biogenic and anthropogenic contributions to acetone mixing ratios in a rural environment. *Atmospheric Environment* **34,** 4997–5006.

Gray D.W. Lerdau M.T. & Goldstein A.H. (2003) Influences of temperature history, water stress, and needle age on methylbutenol emissions. *Ecology* **84,** 765–776.

Hanson D.T. & Sharkey T.D. (2001a) Effect of growth conditions on isoprene emission and other thermotolerance-enhancing compounds. *Plant, Cell and Environment* **24,** 929–936.

Hanson D.T. & Sharkey T.D. (2001b) Rate of acclimation of the capacity for isoprene emission in response to light and temperature. *Plant, Cell and Environment* **24,** 937–946.

Harley P. Fridd-Stroud V. Greenberg J. Guenther A. & Vasconcellos P. (1998) Emission of 2-methyl-3-buten-2-ol by pines: a potentially large natural source of reactive carbon to the atmosphere. *Journal of Geophysical Research* **103,** 25479–25486.

Harley P. Guenther A. & Zimmerman P. (1996) Effects of light, temperature, and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. *Tree Physiology* **16,** 25–32.

Harley P. Guenther A. & Zimmerman P. (1997) Environmental controls over isoprene emission in deciduous oak canopies. *Tree Physiology* **17,** 705–714.

Harley P.C. Litvak M.E. Sharkey T.D. & Monson R.K. (1994) Isoprene emission from velvet bean leaves: Interactions among nitrogen availability, growth photon flux density, and leaf development. *Plant Physiology* **105,** 279–285.

Hiscox J.D. & Israelstam G.F. (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57,** 1332–1334.

Jacob D.J. & Wofsy S.C. (1988) Photochemistry of biogenic emissions over the amazon forest. *Journal of Geophysical Research* **93,** 1477–1486.

Lamanna M.S. & Goldstein A.H. (1999) In situ measurements of C_2-C_{10} volatile organic compounds above a Sierra Nevada ponderosa pine plantation. *Journal of Geophysical Research* **104,** 21247–21262.

Lerdau M. & Gray D. (2003) Ecology and evolution of light-dependent and light-independent phytogenic volatile organic carbon. *New Phytologist* **157,** 199–211.

Lerdau M. & Throop H.L. (2000) Sources of variability in isoprene emission and photosynthesis in two species of tropical wet forest trees. *Biotropica* **32,** 670–676.

LI-COR (1995) *Technical Note 5: Energy Balance*. LI-COR Inc., Lincoln, NB, USA.

Loreto F. & Sharkey T.D. (1990) A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L. *Planta* **182,** 523–531.

Monson R.K. & Fall R. (1989) Isoprene emission from aspen leaves: Influence of environment and relation to photosynthesis and photorespiration. *Plant Physiology* **90,** 267–274.

Monson R.K., Harley P.C., Litvak M.E., Wildermuth M., Guenther A.B., Zimmerman P.R. & Fall R. (1994) Environmental and developmental controls over the seasonal pattern of isoprene emission from Aspen leaves. *Oecologia* **99,** 260–270.

Monson R.K., Jaeger C.H., Adams W.W. III, Driggers E.M., Silver G.M. & Fall R. (1992) Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. *Plant Physiology* **98,** 1175–1180.

Monson R.K., Lerdau M.T., Sharkey T.D., Schimel D.S. & Fall R. (1995) Biological aspects of constructing volatile organic compound emission inventories. *Atmospheric Environment* **29,** 2989–3002.

Oehlert G.W. (2000) *A First Course in Design and Analysis of Experiments*. W.H. Freeman, New York, USA.

Sanadze G.A. & Kursanov A.L. (1966) On certain conditions of evolution of the diene C_5H_8 from poplar leaves. *Soviet Plant Physiology* **13,** 184–189.

SAS S.I.I. (1990) *SAS/STAT User's Guide*, Vol. 2; *6 12.* SAS Institute Inc., Cary, NC, USA.

Schade G.W. & Goldstein A.H. (2001) Fluxes of oxygenated volatile organic compounds from a ponderosa pine plantation. *Journal of Geophysical Research* **106,** 3111–3123.

Schade G.W. & Goldstein A.H. (2002) Plant physiological influences on the fluxes of oxygenated volatile organic compounds from ponderosa pine trees. *Journal of Geophysical Research* **107,** ACH2.1–ACH2.6.

Schade G.W., Goldstein A.H., Gray D.W. & Lerdau M.T. (2000) Canopy and leaf level 2-methyl-3-buten-2-ol fluxes from a ponderosa pine plantation. *Atmospheric Environment* **34,** 3535–3544.

Schnitzler J.P., Lehning A. & Steinbrecher R. (1997) Seasonal pattern of isoprene synthase activity in *Quercus robur* leaves and its significance for modeling isoprene emission rates. *Botanica Acta* **110,** 240–243.

Seiler W. & Conrad R. (1987) Contribution of tropical ecosystems to the global budget of trace gasses, especially CH_4, H_2, CO, and N_2. In *The Geophysiology of Amazonia* (ed. R.E.D.J. Wiley), pp. 133–160. John Wiley, New York, USA.

Sharkey T.D. & Loreto F. (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of Kudzu leaves. *Oecologia* **95,** 328–333.

Sharkey T.D., Singsaas E.L., Lerdau M.T. & Geron C.D. (1999) Weather effects on isoprene emission capacity and applications in emission algorithms. *Ecological Applications* **9** (4), 1132–1137.

Sharkey T.D. & Yeh S.S. (2001) Isoprene emission from plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **52,** 407–436.

Singsaas E.L. (2000) Terpenes and the thermotolerance of photosynthesis. *New Phytologist* **146,** 1–4.

Singsaas E.L., Laporte M.M., Shi J.-Z., Monson R.K., Bowling D.R., Johnson K., Lerdau M., Jasentulyitana A. & Sharkey T.D. (1999) Kinetics of leaf temperature fluctuation affect isoprene emission from red oak (*Quercus rubra*) leaves. *Tree Physiology* **19,** 917–924.

Staudt M. & Bertin N. (1998) Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (*Quercus ilex* L.) leaves. *Plant, Cell and Environment* **21,** 385–395.

Staudt M. & Seufert G. (1995) Light-dependent emission of monoterpenes by Holm Oak (*Quercus ilex* L.). *Naturwissenschaften* **82,** 89–92.

SYSTAT (1997) *Systat, Version 7.0.1 for Windows.* SPSS Inc., Chicago, IL, USA.

Tingey D.T., Evans R. & Gumpertz M. (1981) Effects of environmental conditions on isoprene emission from live oak. *Planta* **152,** 565–570.

Tingey D.T., Manning M., Grothaus L.C. & Burns W.F. (1979) The influence of light and temperature on isoprene emission rates from live oak. *Physiologia Plantarum* **47,** 112–118.

Wellburn A.R. (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* **144,** 307–313.

Wuebbles D., Grant K., Connell P. & Penner J. (1989) The role of

atmospheric chemistry in climate change. *Journal of the Air Pollution Control Association* **39,** 22–28.

Zimmerman P.R. (1979) *Testing of Hydrocarbon Emissions from Vegetation, Leaf Litter, and Aquatic Surfaces and Development of a Methodology for Compiling Biogenic Emission Inventories.* Final report. United States Environmental Protection Agency, Research Triangle Park, USA.

Zimmerman P.R., Chatfield R.B., Fishman J., Crutzen P.J. & Hanst P.L. (1978) Estimates on the production of CO and H_2 from the oxidation of hydrocarbon emissions from vegetation. *Geophysical Research Letters* **5,** 679–682.

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