Ecology, 84(3), 2003, pp. 765–776

INFLUENCES OF TEMPERATURE HISTORY, WATER STRESS, AND NEEDLE AGE ON METHYLBUTENOL EMISSIONS

DENNIS W. GRAY,[1,3] MANUEL T. LERDAU,[1] AND ALLEN H. GOLDSTEIN[2]

[1]*Department of Ecology and Evolution, State University of New York, Stony Brook, New York 11794-5245 USA*
[2]*Department of Environmental Science, Policy and Management (ESPM), University of California, 151 Hilgard Hall, Berkeley, California 94720 USA*

Abstract. Volatile organic compounds emitted by plants have long been recognized as having important influences on tropospheric chemistry, most notably in contributing to the production of tropospheric ozone and aerosols. One such compound, methylbutenol (MBO), was recently identified as a major component of the volatiles emitted by ponderosa pine and several other species of pine in western North America. On short time scales, MBO emissions increase with light intensity in parallel with photosynthetic responses, but emissions and photosynthesis show opposite responses to increases in temperature. We investigate the response of MBO emission to water stress, ambient temperature, and the aging of needles in field grown *Pinus ponderosa* (ponderosa pine). Photosynthetic rates and MBO production capacities of *P. ponderosa* were measured over the course of a season on saplings at a site that experienced summer drought and at a site that received supplementary water. In addition, a water-stress alleviation experiment was performed in which drought-stressed *P. ponderosa* seedlings were re-watered at the end of the season and pre-/post-alleviation photosynthetic rates and MBO production capacities were compared. Over the season photosynthesis declined while MBO production capacity tracked changes in ambient temperature linearly both over the entire season and on a day-to-day basis. Although severe water stress reduced photosynthetic rates, there was no difference in the response of MBO production capacity to ambient temperature under either drought-stressed or well-watered conditions, and there was no change in MBO production capacity following water stress alleviation. MBO emission declined with needle age. The correlation between MBO production capacity and ambient temperature is consistent with the view that MBO may provide a protection against high temperature stress similar to that suggested for isoprene.

Key words: drought stress; 2-methyl-3-buten-2-ol; methylbutenol; needle age; photosynthesis; Pinus ponderosa; *ponderosa pine; temperature; volatile organic compounds; water stress.*

INTRODUCTION

Emission of volatile organic compounds from plants represents the single most important source of photochemically reactive compounds into the troposphere and is an important mechanism whereby terrestrial plants directly alter the oxidative capacity of the lower atmosphere (Fehsenfeld et al. 1992). Various modeling studies have implicated biogenic volatile organic compounds (BVOCs) in the formation of tropospheric ozone (e.g., Brasseur and Chatfield 1991, Chameides et al. 1992, Fehsenfeld et al. 1992). VOCs may also augment the greenhouse effects of methane by increasing the atmospheric lifetime of this greenhouse gas (Jacob and Wofsy 1988, Wuebbles et al. 1989). In light of these important regional and global scale effects, the physiological and ecological processes regulating BVOC emission into the atmosphere have received considerable attention (Tingey et al. 1979, 1981, Monson and Fall 1989, Harley et al. 1994, 1996, Monson et al. 1994), and numerous efforts have been undertaken to develop predictive models of BVOC emission based on the processes regulating emissions (Guenther et al. 1993, 1995, Fall and Wildermuth 1998). The most commonly studied BVOCs are monoterpenes ($C_{10}H_{16}$) and isoprene (C_5H_8); however, in 1991 a previously unobserved alcohol, 2-methyl-3-buten-2-ol (methylbutenol or MBO), was detected at high concentrations in air samples over the Colorado Rockies (Goldan et al. 1993). Subsequent work has shown MBO to be phytogenically produced, photochemically reactive, and important in tropospheric chemistry (Rudich et al. 1995, Fantechi et al. 1998*a*, *b*, Harley et al. 1998).

Methylbutenol production/emission affects both the atmospheric chemistry of the western United States and has implications for carbon budgets in these ecosystems. MBO is often one of the most important reducing compounds found in the troposphere of the intermountain western United States, and its importance in regulating atmospheric oxidative capacity in the lower atmosphere of the Sierra Nevada mountains of California has been demonstrated by Lamanna and Goldstein (1999), who estimate that MBO accounts for 21% of the OH radical loss rate over a mixed conifer forest

Manuscript received 27 August 2001; revised 14 June 2002; accepted 24 June 2002; final version received 21 August 2002. Corresponding Editor: K. F. Raffa.

[3] E-mail: denwgray@life.bio.sunysb.edu

in this region. Methylbutenol oxidation in the atmosphere also provides the largest regional source of acetone, a compound that plays a critical role in OH production in the upper troposphere (Goldstein and Schade 2000). In addition, MBO emission represents a sizable source of carbon lost from the emitting plant that is not currently factored into ecosystem carbon models. Annual MBO production amounts to ~0.5% of net assimilation in ponderosa pine (*Pinus ponderosa*) (Goldstein et al. 2000, Schade et al. 2000), an amount equivalent to estimates of net ecosystem productivity from many forests (Crutzen et al. 1999). Despite the importance of MBO at ecosystem and tropospheric scales, little attention has been focused on the ecological regulation of production and emission. Such mechanistic understanding is important for estimating emissions at large spatial and temporal scales and for evaluating the potential functional significance of MBO production (Lerdau et al. 1997).

Several studies have examined the short-term biochemical and physiological regulation of MBO production/emission. It is produced enzymatically in the chloroplast from dimethylallyl diphosphate that is formed through the standard plastidic pathway for isoprenoid biosynthesis, the deoxyxylulose phosphate pathway (DOXP; Rohmer et al. 1993, Lichtenthaler et al. 1997, Schwender et al. 1997, Lichtenthaler 1999, Fisher et al. 2000). MBO production is a light-dependent process that parallels the response of photosynthesis to light (Harley et al. 1998), suggesting a close association between MBO production and photosynthetic carbon assimilation. Since MBO is a polar compound and unlikely to diffuse through the leaf cuticle, emission most likely occurs through the stomata in a fashion similar to that shown for isoprene emission (Fall and Monson 1992). Once produced, MBO is released immediately into the atmosphere, with only small storage pools (probably dissolved in aqueous solution) present within the leaf. MBO emission from needles is absent in darkness (Harley et al. 1998). In response to temperature, MBO emission follows an Arrhenious temperature response, increasing exponentially to a temperature optimum of ~37°C in *Pinus ponderosa* (ponderosa pine) and subsequently declining precipitously (Harley et al. 1998). These short-term responses to light and temperature (response times of seconds to minutes) are similar to those observed for isoprene (Loreto and Sharkey 1990, Monson et al. 1991, 1992, Harley et al. 1997).

Given the similarity between the responses of MBO emission and isoprene emission at short time scales, it is reasonable to expect that factors shown to influence isoprene production at longer time scales might also affect MBO emission. Additionally, since MBO production is linked biochemically to photosynthesis through dimethylallyl diphosphate (DMADP) production via the DOXP pathway, any factors that alter photosynthetic capacity might also influence MBO production.

Two such factors are needle age and water stress. Photosynthetic rates in pines have been shown to decline with needle age after full expansion (Porté and Loustau 1998, Jach and Ceulemans 2000), and a previous study by Harley et al. (1998) has shown similar declines in MBO production capacity in older needles. However, it is unknown whether the instantaneous responses of MBO emission to variations in light and temperature also change with needle age.

Photosynthetic metabolism can also be dramatically influenced by the pronounced seasonal drought that many conifers of western North America, including pines, regularly experience. This drought reduces stomatal conductance and photosynthetic rate (Law and Waring 1994, Goldstein et al. 2000, Panek and Goldstein 2001). Whether such drought-induced reductions in carbon assimilation influence MBO emission rates over the season is unknown; however, previous studies have shown that isoprene emission responds to water stress (Sharkey and Loreto 1993, Fang et al. 1996).

In addition to the response of isoprene emission to water stress, isoprene basal emission rate has been shown to respond to ambient temperature (Monson et al. 1992, Sharkey and Loreto 1993, Hanson and Sharkey 2001, Petron et al. 2001). A similar response is suggested by Schade et al. (2000), who have shown canopy-scale MBO fluxes from a ponderosa pine plantation to correlate with ambient temperature. Photosynthesis has also been shown to acclimate to growth temperature conditions (Teskey and Will 1999, Saelim and Zwiazek 2000). However, whether MBO emission acclimates to ambient temperature or responds to seasonal changes in photosynthetic rate has not been addressed at the leaf level.

In this study we investigate the linkages between MBO production/emission and carbon metabolism at ecological time scales and test the prediction that MBO production is tightly linked to photosynthetic metabolism across these longer ecological scales. We ask whether the instantaneous response of MBO emission to light and temperature changes through the season and with needle age, investigate the influence of water stress on MBO emission, and quantify the impact of ambient temperature on MBO emission. Our measurements allow us to determine to what extent photosynthetic metabolism and MBO production are coupled. We examined water stress effects by measuring leaf gas exchange and MBO emission from ponderosa pine growing at a site that underwent the natural progression of seasonal drought and a nearby site that was watered to alleviate drought stress. We also investigated the effect of water stress alleviation, as may happen following autumn rains, by watering drought-stressed seedlings at the end of the summer. The impacts of needle age and ambient temperature history were determined by measuring light and temperature response

curves and basal MBO emission rates across a range of needle ages under a variety of environmental conditions. From these studies we can determine the extent to which MBO metabolism is independent of (or dependent upon) photosynthesis. This determination is the first step toward an ecological understanding of MBO flux regulation.

Methods

Site and species

Experiments were conducted in the central Sierra Nevada Mountains of California, USA, at the University of California at Berkeley Blodgett Forest Research Station and on adjacent property owned and operated by Sierra Pacific Industries. Blodgett Forest Research Station is located east of the town of Georgetown, California, 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 at two locations. The first location, referred to as the Tower site, was an even-aged plantation of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.; aged 7–8 yr, 3–5 m in height) owned by Sierra Pacific Industries. This site also 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.). The Tower site was established in 1997 and outfitted with a hydrocarbon sampling and micrometeorological tower that provided detailed flux and climate records for the site. Additional details of this site and the tower are reported in Lamanna and Goldstein (1999) and Goldstein et al. (2000). Trees at this site underwent a natural progression of summer drought (Panek and Goldstein 2001). The second location, referred to as the Watered site, was an even-aged stand of ponderosa pine and giant sequoia (*Sequoia giganteum* (Lindl.) Buchholz; aged 10–15 yr, 3–5 m in height) located <1 km southwest of the Tower site in compartment 620 of Blodgett Forest Research Station. A subset of trees at this site was fitted with drip irrigation lines encircling the base of each tree at 1 m distance from the bole (approximately the outer edge of the crown). Trees were watered by gravity feed from a tank that supplied sufficient water to keep the soil surface damp throughout the summer. Data on water potential measurements made at these sites, showing declines at the Tower site but not at the Watered site, can be found in Panek and Goldstein (2001).

We conducted all experiments on ponderosa pine because it produces large quantities of MBO and covers extensive areas of the forested western United States. Ponderosa pine is a fast-growing, drought-tolerant species commonly planted and harvested for timber production. It is generally viewed as shade intolerant and reaches a maximum age in excess of 300 yr. Although monoterpene emissions from ponderosa pine have been described in detail (e.g., Lerdau et al. 1994), no extensive field studies of MBO emission had been conducted prior the research at Blodgett Forest (Harley et al. 1998, Baker et al. 1999, Schade et al. 2000, Schade and Goldstein 2001).

Experimental designs

We used two treatments to assess the impacts of water stress and needle age on MBO and photosynthetic metabolism. In 1998 measurements were made at the Tower and Watered sites over the course of a season, from June through October. To determine if the slow onset of water stress that occurred at the Tower site influenced MBO production capacity, we compared MBO production capacity at both sites at the beginning of the season and again at the end of the season after sizable water stress differences (as measured by stomatal conductance) had accrued between trees at the Tower and Watered sites (Fig. 3).

In 1999 the photosynthetic parameters and MBO fluxes of a set of eight seedlings (aged 3–4 yr) were measured in late August following the natural onset of seasonal drought in the field. Half of these were then watered by saturating the soil around their base with 45 L of water each day. After 7 d both the watered and unwatered seedlings were remeasured for both photosynthetic parameters and MBO emission rate.

In 1998 measurements of photosynthetic parameters and MBO fluxes were made on age 0 (current flush), age 1 (1 yr old), and age 2 (2 yr old) needles at both the sites. Measurements were made four times between June and October and alternated between the Tower site and Watered site. Measurements at a site took ~1–2 wk to complete and were repeated at ~1-mo intervals. At the Tower site two branches on each of eight trees were measured, and at the Watered site two branches on each of six trees were measured. Needles were harvested and dried at the end of each measurement sequence. All measurements were taken at a photosynthetic photon flux density (PPFD) of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photons between 400 and 700 nm, a temperature of 30°C, and a CO_2 concentration of 350 ppm (in micromoles of CO_2 per mole of air) in the cuvette (basal emission rate, sensu Monson et al. 1995). Light and temperature response curves were made at the Tower site on all available needle age classes of two trees early in the season and again after the development of moderate water stress. In addition, between 28 July and 9 August of 1998, repeated measurements of photosynthetic parameters and MBO basal emission rates were made on the same set of needles on a single tree located at the Tower site at 1700 hours each day.

Analytical methods

Gas exchange and chromatography protocols.— Leaf level measurements of photosynthetic parameters and MBO emission rates were made using a LI-COR

6400 portable gas exchange system (LI-COR, Lincoln, Nebraska, USA) and a Voyager portable gas chromatograph (GC; PE photovac, Norwalk, Connecticut, USA). The LI-COR 6400 allows good control of the light, temperature, and CO_2 environment experienced by the foliage being measured. Light control in this system is achieved with a series of red and blue light-emitting diodes (peak irradiance 665 nm and 470 nm) mounted in the top of the leaf cuvette. Temperature is regulated using thermoelectric (Peltier) coolers (LI-COR) 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. The Voyager GC contains three columns and a 0.5-mL sample loop, operates isothermally, and uses a photionization detector for detection and quantification of volatiles.

Two three-needle fascicles (six needles) were clamped into the LI-COR cuvette, and the cuvette was flushed with 100 μmol/s of ambient air scrubbed of hydrocarbons by passage through an activated charcoal filter. Exhaust gases from the cuvette were routed via Teflon tubing past the sample inlet port on the GC. The GC sample loop was loaded via an internal sampling pump, and a 0.5-mL aliquot was injected onto a methyl silicone capillary column (15 m length, internal diameter 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 ppb (in nanomoles of MBO per mole of air), and precision was within 10%. Chamber air typically had mixing ratios between 10 and 100 ppb (in nanomoles of MBO per mole of air). Five point standard curves diluted from a 100 ppm (in micromoles of MBO per mole of air) gas standard (Scott-Marrin, Riverside, California, 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, 350 ppm CO_2 (in micromoles of CO_2 per mole of air), and a saturating PPFD of 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photons between 400 and 700 nm until steady state MBO flux was attained (30–40 min). 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photons, 350 ppm CO_2, and the lowest temperature it was possible to obtain 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 vs. basal emission rate/production capacity.—Since MBO emission is both light and temperature dependent, these factors must be held constant to allow comparison among measurements and investigation of factors regulating emission at longer time scales. We measured MBO and photosynthetic metabolism under constant conditions 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.

Area- vs. 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 laboratory showed that projected leaf area is approximately one-half the calculated needle surface area. 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 mean caliper-measured radius of all three needles. The perimeter (p) of each needle was calculated according to the following equation:

$$p = 2/3\ \pi r_{mean} + 2r_n$$

where r_{mean} = the mean needle radius, $2/3\ \pi r_{mean}$ = the length of the curved surface of each needle, r_n = the radius of each individual needle, and $2r_n$ = 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.

Statistical methods

The relationship between MBO emission and ambient temperature was analyzed by linear regression using the SAS procedure GLM (SAS 1990). Differences in MBO production capacity and photosynthetic rate due to needle age were tested using ANOVA. Differences in the shape of the light and temperature response curves for photosynthesis and MBO emission were examined using the Kolmogorov-Smirnov test (SYSTAT 1997). The effect of water stress on MBO production capacity was investigated by comparing the slopes of the regression lines describing the relation-

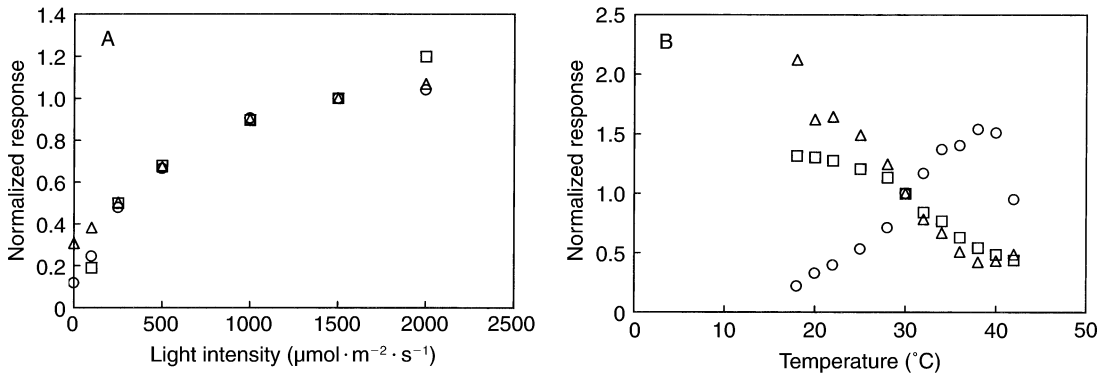

FIG. 1. Response of methylbutenol (MBO) emission (circles), photosynthesis (squares), and stomatal conductance (triangles) to (A) light intensity (in micromoles of photons per square meter per second) or (B) temperature. Temperature was held constant at 30°C for light response curves, and light was held constant at a light intensity of 1500 μmol photons·m^{-2}·s^{-1} for temperature response curves. Data were normalized by dividing each light response measurement by the value obtained at 1500 μmol photons·m^{-2}·s^{-1} light intensity and each temperature response measurement by the value obtained at 30°C.

ship between ambient temperature and MBO production capacity at the Tower and Watered sites and by ANCOVA using the GLM procedure of SAS. The effect of water stress alleviation was analyzed using ANOVA.

RESULTS

Instantaneous responses

Methylbutenol emission and photosynthetic rates increased with light intensity, and neither process showed light saturation even at PPFD 2000 μmol·m^{-2}·s^{-1} photons (Fig. 1A). Despite this similarity in instantaneous (time scale of seconds to minutes in duration) response to light, MBO emission and photosynthesis responded very differently to temperature. In response to increasing temperature, both photosynthesis and stomatal conductance declined steadily; however, MBO emission increased exponentially reaching a maximum around 40°C (Fig. 1B). Above this temperature MBO emission rates declined precipitously and failed to equilibrate at a constant emission rate. In addition, the shape of the light and temperature response curves for both MBO emission and photosynthesis in all three needle age classes were identical (Fig. 2A, B; $P > 0.05$ for all comparisons).

Seasonal patterns

Over the course of the season, stomatal conductance and photosynthesis showed progressive declines at both the Tower and Watered sites (Fig. 3A, B). The magnitude of these declines was greater at the unwatered Tower site and illustrates that the watering treatment prevented some of the water stress experienced by trees at the Tower site. In contrast to the pattern of declines seen for stomatal conductance and photosynthesis, MBO production capacity exhibited a unimodal response. MBO emissions were initially very low compared with photosynthetic rate, increased to a peak in July, and subsequently declined throughout the remainder of the season (Fig. 4A, B). Similar responses of MBO emission were seen at both the Tower site and Watered site (Fig. 5A).

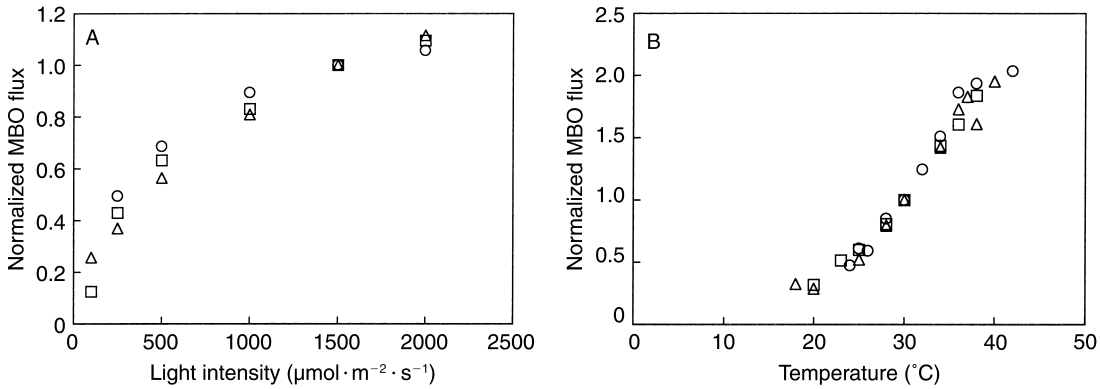

FIG. 2. Response of MBO emission to (A) light intensity and (B) temperature among current flush needles (circles), 1-yr-old needles (squares), and 2-yr-old needles (triangles). Temperature was held constant at 30°C for light response curves, and light was held constant at a light intensity of 1500 μmol photons·m^{-2}·s^{-1} for temperature response curves. Data were normalized as in Fig. 1.

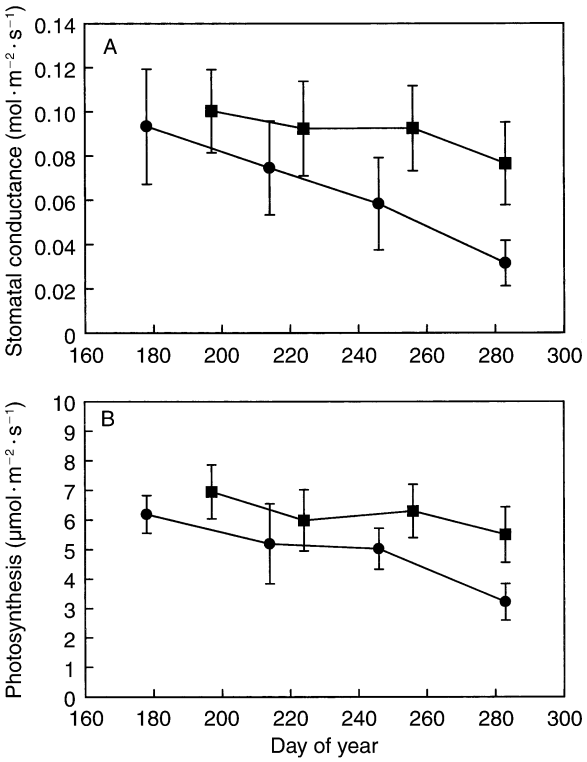

FIG. 3. Seasonal changes in (A) stomatal conductance or (B) photosynthesis for saplings at the Tower site (circles) and Watered site (squares; means ± 1 SD). Data were collected at standard conditions (photosynthetic photon flux density = 1500 μmol·m^{-2}·s^{-1}, 30°C).

Needle age

Needle age had pronounced effects on the physiological capacities of both MBO production and photosynthesis. Both photosynthetic capacity and MBO production capacity declined with needle age, with the strongest declines coming between the 1-yr-old and 2-yr-old needles (Fig. 4A, B; ANOVA $F_{2,14} = 39.28$, $P = 0.0001$; $F_{2,14} = 25.9$, $P = 0.0001$). These effects of needle age were independent of the ambient temperatures at which measurements were made.

Relationship between photosynthetic capacity and MBO production capacity

Examination of the seasonal patterns of photosynthetic capacity and MBO production capacity in Fig. 4A and B shows that photosynthesis and MBO production capacity have similar trajectories (declines) at the end of the season. However, the behavior of these two processes is very different at the beginning of the season, as illustrated by the low MBO production capacities coupled with high photosynthetic rates in all needle age classes present during the first sampling period at the Tower site (Fig. 4A, B).

Replotting the data contained in Fig. 4 initially showed a putatively strong positive relationship between mean photosynthetic capacity and MBO production capacity when viewed over the entire season (Fig. 6A, Table 1). However, this relationship was due to differences in photosynthetic capacity and MBO production capacity between different needle age classes (Fig. 6A, Table 1). When each age class of needles was considered separately, there was no relationship between photosynthesis and MBO production within a sampling period (Fig. 6B, all $P > 0.05$, $r^2 < 0.36$). Similarly, for a single needle age class, there was no relationship between photosynthesis and MBO production within a sampling date or across all sampling dates (Fig. 6C, all $P > 0.05$, $r^2 < 0.51$).

Ambient temperature and MBO production capacity

Although the trajectory of seasonal changes in MBO production capacity differed slightly between the Tower and Watered sites, in both cases MBO production capacity closely followed seasonal changes in ambient temperature (Fig. 5A, Table 2). Daily measurements of MBO production capacity taken on a single tree at 1700 hours over a 2-wk period also revealed a close tracking of ambient temperature by MBO production capacity (Fig. 5B, Table 2). A more detailed analysis of the relationship between MBO basal emission capacity and daily maximum temperature was performed using lin-

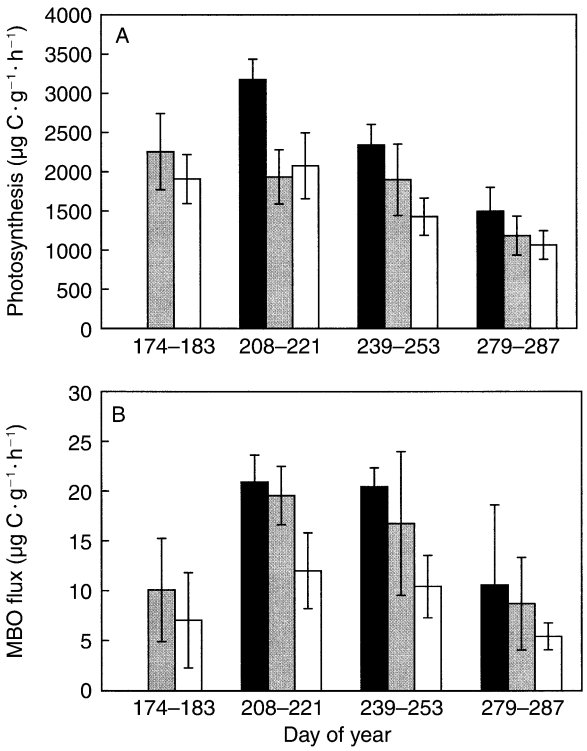

FIG. 4. Seasonal pattern of (A) photosynthetic rate and (B) MBO basal emission rate for current flush needles (solid bars), 1-yr-old needles (gray bars), or 2-yr-old needles (white bars) at the Tower site in 1998 (means ± 1 SD). Data are expressed as micrograms of carbon fixed through photosynthesis or emitted as MBO per gram of needle dry mass per hour.

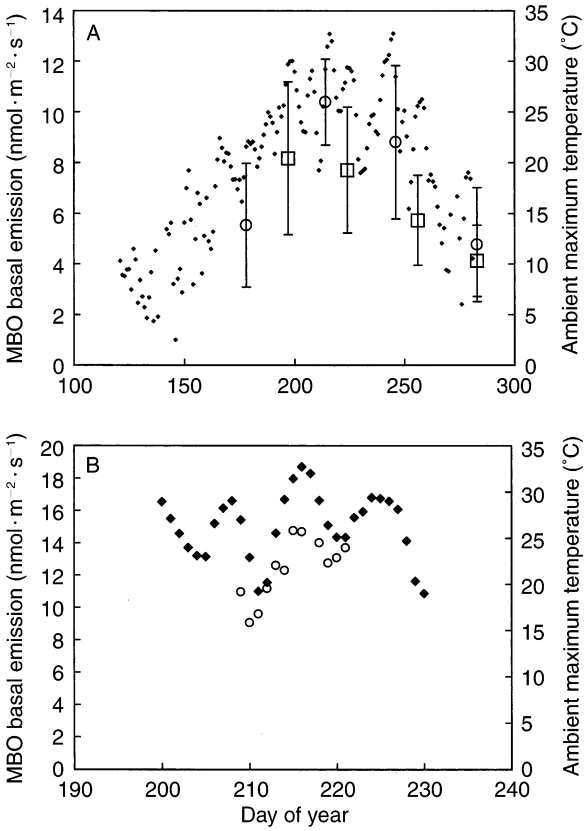

FIG. 5. (A) Pattern of daily maximum temperature (diamonds) and mean MBO basal emission rate per sampling period at the Tower site (circles) or Watered site (squares; means ± 1 SD). (B) Pattern of daily maximum temperature (diamonds) and MBO emission capacity (circles) at the Tower site. Measurements were made at 30°C and light intensity of 1500 μmol photons·m^{-2}·s^{-1}.

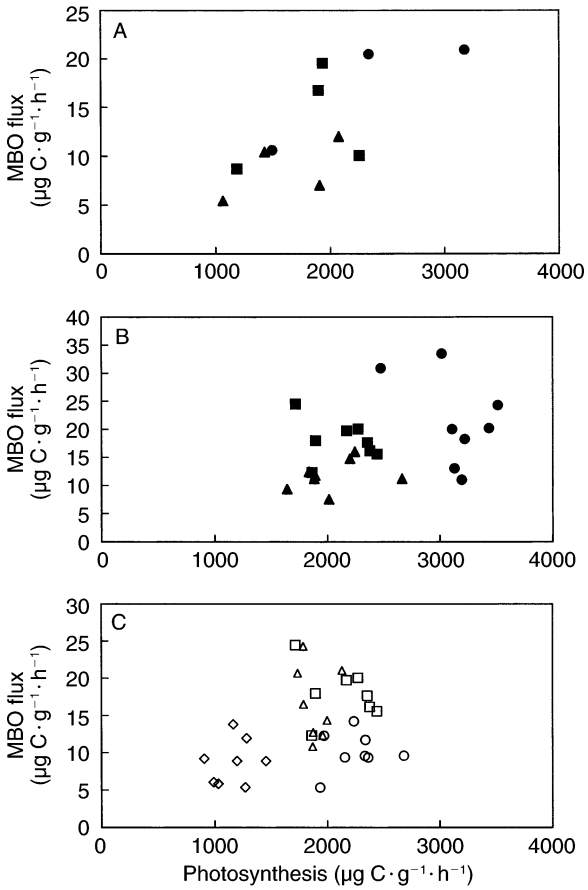

FIG. 6. Correlation between photosynthetic capacity and MBO production capacity at the Tower site. All measurements were made at a cuvette temperature of 30°C and a light intensity of 1500 μmol photons·m^{-2}·s^{-1}. (A) Each point represents the mean MBO emission of a needle age class for a sampling period. (B) Each point represents the mean MBO emission of a needle age class on a tree during the second sampling period. (C) Each point represents the mean MBO emission from age 1 needles on a tree during a sampling period. Key to symbols: solid circles = age 0, solid squares = age 1, solid triangles = age 2, open circles = days 174–183, open squares = days 208–221, open triangles = days 239–253, open diamonds = days 279–287.

ear regression and revealed that there was no difference in the slope of the MBO response to ambient temperature observed at the Tower site, Watered site, or resulting from the daily measurements (Fig. 7, Table 2; homogeneity of slopes comparison $F_{1,99} = 0.02$, $P = 0.8849$). Similar relationships between photosynthesis and ambient temperature were not observed (linear regression $P > 0.05$).

Effects of water stress

Investigation of potential effects water stress might have had on MBO production capacity was complicated by the strong influence of ambient temperature. We evaluated whether water stress influenced MBO production capacity using an ANCOVA design in which ambient temperature (daily T_{max}) was treated as a covariate when comparing the Tower site and the Watered site. Similar results were obtained when daily maximum, minimum, or mean temperature was used as the covariate. Slopes of the response of MBO to T_{max} showed no differences between sites (Fig. 7, Table 2; homogeneity of slopes comparison $F_{1,99} = 0.02$, $P =$

TABLE 1. Regression parameters obtained at the Tower site in the central Sierra Nevada Mountains, California, USA.

Needle age class	Sampling days	Slope	Intercept	r^2	P
All	174–183	0.007	0.292	0.519	0.012
0	208–221	0.006	2.939	0.786	0.306
1	239–253	0.004	6.374	0.124	0.647
2	279–287	0.004	2.320	0.364	0.396

Notes: Photosynthetic capacity and methylbutenol (MBO) production capacity were related through the linear model $y = ax + b$, where y = MBO production capacity (in micromoles of carbon per gram per hour) and x = photosynthetic rate (in micromoles of carbon per gram per hour). Regressions were run for all needle age classes separately and together using mean measurements for each of the four sampling periods. Sampling periods correspond to those shown in Fig. 4A, B.

TABLE 2. Regression parameters obtained seasonally at the Tower and Watered site and daily at the Tower site between 28 July and 9 August 1998.

Site	Slope	Intercept	r^2	P
Tower	0.443	−2.35	0.538	0.0001
Water	0.371	−2.13	0.551	0.0072
Daily	0.354	3.12	0.611	0.0027

Notes: Parameters were related through the linear model $y = ax + b$ where y = basal methylbutenol (MBO) emission rate (in nanomoles per square meter per second) and x = the daily maximum ambient temperature. Slopes differed significantly from 0, but no differences between slopes of each group could be detected. Intercepts did not differ significantly from 0.

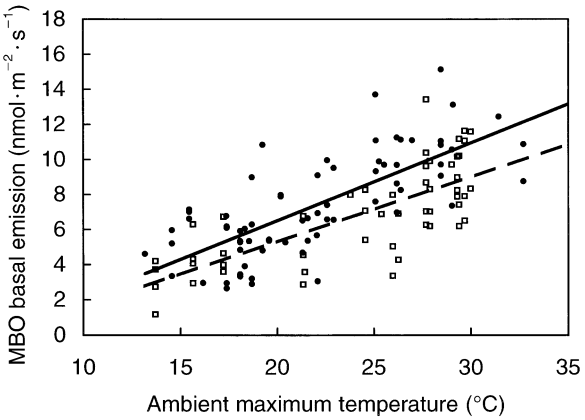

FIG. 7. Correlation between MBO emission capacity and daily maximum temperature at the Tower site (circles) and Watered site (squares). Each point represents a single measurement. Lines are best-fit linear regressions for the Tower site (solid) or Watered site (dashed). Data are expressed on a unit needle area basis.

0.8849), despite differences in the degree of plant water stress.

With the effect of temperature factored out, there were no differences in MBO production capacity at either site at the beginning of the season (ANCOVA $F_{1,27} = 0.77$, $P = 0.3873$) when stomatal conductances were comparable (Fig. 3, ANOVA $F_{1,28} = 2.59$, $P = 0.1188$), and no differences in MBO production capacity at the end of the season (ANCOVA $F_{1,25} = 0.44$, $P = 0.5145$) when stomatal conductance at the Tower site had declined by 66% (from 0.09 mol $H_2O \cdot m^{-2} \cdot s^{-1}$ to 0.03 mol $H_2O \cdot m^{-2} \cdot s^{-1}$) and was 60% lower than at the Watered site (ANOVA $F_{1,28} = 84.18$, $P = 0.0001$).

The possibility that rapid changes in water stress might cause a sudden change in MBO production capacity irrespective of the temperature response was addressed in the 1999 water-stress alleviation experiment. These data revealed that despite a doubling of photosynthesis, from 3.81 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 7.63 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($F_{1,6} = 23.11$, $P = 0.003$), and a tripling of stomatal conductance, from 0.033 $mol \cdot m^{-2} \cdot s^{-1}$ to 0.096 $mol \cdot m^{-2} \cdot s^{-1}$ ($F_{1,6} = 18.2$, $P = 0.0053$), following the re-watering of drought-stressed plants, no change in MBO production capacity was observed (Table 3; $F_{1,6} = 0.00$, $P = 0.9843$).

We also note that the stomatal conductances and photosynthetic rates of control plants were initially lower than those of the water stress alleviation treatment plants prior to watering (Table 3) and lower than those observed at the end of the season at the Tower site in 1998 (Fig. 3A, B). This suggests that the control plants may have been slightly more water stressed than either the alleviation treatment or Tower site plants. Yet, despite these low stomatal conductances and photosynthetic rates, the control plants had higher MBO emission rates (Table 3). This might possibly hint that at levels of water stress greater than those observed in 1998 at the Tower site, MBO production capacity might increase. However, from these data it is unclear whether the higher MBO pre-alleviation production capacities of the control plants are due to water stress or variability among the trees sampled.

DISCUSSION

Instantaneous responses

The instantaneous response of MBO emission to light and temperature was similar to that previously observed for isoprene in sun-adapted leaves (Sharkey et al. 1996, Harley et al. 1997, Lerdau and Throop 2000), but differed somewhat from that observed by Harley et al. (1998) for MBO. Harley et al. (1998) observed photosynthesis to saturate at a PPFD of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and MBO emission to continue to increase above a PPFD of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in ponderosa pine. However, we found that photosynthesis

TABLE 3. Stomatal conductance, photosynthesis, and methylbutenol (MBO) emission capacity of drought-stressed *Pinus ponderosa* seedlings before and after late-season re-watering.

Watering treatment	Stomatal conductance ($mol \cdot m^{-2} \cdot s^{-1}$)		Photosynthesis ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		MBO flux ($nmol \cdot m^{-2} \cdot s^{-1}$)	
	Pre	Post	Pre	Post	Pre	Post
Control	0.013 ± 0.002	0.020 ± 0.008	1.50 ± 0.42	2.30 ± 0.55	3.57 ± 0.82	3.93 ± 1.70
Alleviation	0.033 ± 0.013*	0.096 ± 0.027*	3.81 ± 0.91*	7.63 ± 0.130*	2.70 ± 1.18	2.69 ± 1.24

Notes: Pre-alleviation measurements took place on 20 August 1999 and post-alleviation measurements took place on 27 August 1999. Alleviation treatment seedlings received 45 L of water per day for 7 d prior to remeasurement. Control seedlings remained unwatered through this time. Significant differences between measurement periods are indicated with asterisks ($P < 0.05$).

and MBO emission showed highly similar responses to PPFD and that neither photosynthesis nor MBO emission showed signs of reaching saturation even at light levels approaching full sun intensities.

The similarity between the short-term response of photosynthesis and MBO emission to light (Fig. 1A) suggests a metabolic linkage between these two processes that can be understood in the context of what is known about the biochemical pathways involved. Light provides the energy input driving the Calvin cycle to produce the triose phosphate substrates, adenosine triphosphate (ATP), and nicotinamide adenine dinucleotide phosphate (NADPH) used by the DOXP pathway to produce isopentenyl diphosphate (IDP). IDP is then converted to DMADP via an isomerase and DMADP is converted to MBO by MBO synthase in the chloroplast (Lichtenthaler 2000). In contrast, the very different responses of photosynthesis and MBO emission to temperature indicate that this apparent linkage between photosynthesis and MBO emission is not one where a set percentage of photosynthate is allocated to MBO production and suggests that these processes have different underlying controls.

The observation that MBO emission does not appear to be limited by temperature-induced limitations in photosynthesis (Fig. 1B), and hence substrate production, can be understood in the context of the relative fluxes of carbon through each pathway. The fluxes of carbon through the MBO emission pathway typically range in the low nanomolar amounts (nanomoles of carbon per square meter per second) whereas photosynthetic carbon fluxes are ~1000 times greater in micromolar amounts (micromoles of carbon per square meter per second). Thus, even when photosynthetic rates (fluxes) are very low, MBO synthase is unlikely to become substrate limited because carbon fluxes to MBO are small compared to carbon fluxes through the Calvin cycle.

Seasonal patterns

The seasonal pattern of MBO emission capacity observed in this study is consistent with recent studies on the seasonality of isoprene emission (Sharkey and Loreto 1993, Monson et al. 1994, Schnitzler et al. 1997, Goldstein et al. 1998, Sharkey et al. 1999). However, whereas the leaves of deciduous isoprene-emitting species do not begin to emit until they have fully expanded, pine needles show strong emission of MBO while they are still expanding. The time of onset for MBO emission has yet to be determined; however, MBO emission was observed in expanding needles when they were <3 cm long (2–3 wk old; <20% of full expansion). It is interesting to note that the young needles of two isoprene-emitting conifers, Norway spruce (*Picea abies*) and Colorado blue spruce (*Picea pungens glauca*), produce isoprene before they have fully expanded. This may indicate that different mechanisms regulate the developmental onset of MBO emission or isoprene emission in conifers and angiosperms.

Regulation of MBO production capacity

Temperature.—While the pattern of changes in MBO production capacity was strongly correlated with ambient temperature over the season and day to day (Table 2), measurements of photosynthesis did not correlate with ambient temperature ($F_{1,60} = 0.084$, $P = 0.7731$, $r^2 = 0.0014$). Furthermore, photosynthesis proved to be a poor predictor of MBO production capacity within an age class of needles sampled over the season (Fig. 6C, Table 1) or across needle age classes within a single sampling date (Fig. 6B, Table 1). Thus, changes in MBO emission capacity do not appear mediated directly through changes in leaf level photosynthetic rate. Rather, it appears that ambient temperature regulates MBO production capacity.

The mechanism by which changes in temperature induce changes in MBO production capacity is not currently known; however, studies of isoprene regulation have shown that seasonal changes in isoprene emission are best explained by seasonal changes in isoprene synthase content (Monson et al. 1992, Kuzma and Fall 1993, Schnitzler et al. 1997, Lehning et al. 1999). We suggest that a similar process occurs in MBO-producing pines and that temperature regulates the content of MBO synthase within the chloroplast.

Water stress.—Although the levels of water stress achieved in these experiments (Fig. 3A) affected photosynthetic metabolism (Fig. 3B), there is no evidence that MBO emission was influenced either directly by changes in water stress (Table 3) or indirectly through the changes in photosynthetic metabolism (Fig. 6A–C, Table 1). Similarly, the sudden alleviation of water stress did not result in changes in MBO production capacity (Table 3).

In contrast to these results, several workers have shown that water stress and the alleviation of stress influence the emission of isoprene (Sharkey and Loreto 1993, Fang et al. 1996, Lerdau et al. 1997). This difference may be explained by the fact that, although the degree of water stress experienced by the end of the growing season in 1998 was pronounced, even the most water-stressed control treatment trees in the 1999 study had not become sufficiently stressed to undergo midday stomatal closure. It is possible that MBO emission might change under conditions of more severe water stress.

Photosynthesis.—Although MBO emission follows photosynthesis very closely in response to light (Fig. 1A) and MBO production capacity corresponds to changes in photosynthetic capacity over most of the season (Fig. 4A, B), this does not result from photosynthesis regulating MBO production capacity. Instead this relationship arises because plant water stress (water availability) and ambient temperature changed in parallel in 1998.

The unusually wet winter and spring of 1998 resulted in the first set of measurements being taken when water availability was at its seasonal peak. Following the end of the rainy season the summer drying cycle ensued and continued through the end of the sampling periods. This seasonal drying trend is reflected in the pattern of declines in stomatal conductance seen at the Tower site (Fig. 3A) and resulted in matching declines in photosynthesis (Fig. 3B). Concurrent with the cessation of rains, ambient temperature began to rise such that by the first sampling period at the Watered site and the second sampling period at the Tower site, temperatures were at their seasonal maxima. Subsequently, temperatures declined over the rest of the season following a pattern closely matching that of water availability seen in stomatal conductance. As a result, with the exception of the measurements taken at the Tower site in the first sampling period, there was a close correspondence between photosynthetic capacity and MBO emission capacity.

Fig. 6A illustrates that any correlation seen between mean photosynthesis and MBO production capacity is due to differences among needle age classes. Trees were also sufficiently variable in their photosynthetic and MBO production capacities that within an age class there was no relationship between photosynthesis and MBO production capacity at any date or across all ages within a sampling date (Fig. 6B, C). The fact that no relationship exists between photosynthetic capacity and MBO production capacity, despite the close tracking of MBO emission and photosynthesis in response to light, can be explained in the same way as the failure of MBO emission to track photosynthesis in response to short-term (instantaneous) changes in needle temperature (previously described). Since the flux of carbon through the MBO synthesis pathway is tiny relative to the flux of carbon through the Calvin cycle, MBO synthesis is unlikely to become substrate limited unless photosynthetic rates become very small.

Needle age.—Both photosynthesis and MBO emission declined with needle age; however, the basis for these declines is still unclear. In many trees, leaves adapt to their light environment, and this can result in reduced photosynthetic capacities of leaves in shaded portions of the canopy (Ellsworth and Reich 1993, Harley et al. 1996). Reduced isoprene emissions have also been reported from shade-adapted leaves (Harley et al. 1996, 1997). However, such a shade acclimation response does not seem likely for the observed declines in MBO emission, since the canopy architecture of ponderosa pine admits considerable amounts of light deep into the canopy; and 2-yr-old needles were observed to receive full sun during a portion of the day. The observation that all needle age classes showed similar light response curves for both photosynthesis and MBO emission also suggests that leaf physiology was similar across needle ages (Fig. 2A). Given the strong influence ambient temperature has on MBO production capacity, one wonders if older needles located deeper in the canopy could be in a cooler microenvironment than the younger needles. But this too seems unlikely given that needles track air temperature very closely and needle boundary layer conductances are small and unlikely to lead to much temperature increase from solar insolation.

Ecological importance.—During warm dry periods, MBO emission may constitute a sizable proportion of the carbon assimilated by the plant. This is due to the divergent responses of instantaneous MBO emission and photosynthetic rates to temperature, combined with the observation that MBO production capacities remain up-regulated despite limitations to photosynthesis resulting from water stress. Over the summer of 1998, MBO emission at the Tower site averaged 0.5% of net assimilation (NPP) but peaked at 1% from age 1 needles in July. This carbon loss measured at the leaf level is in good agreement with canopy-level estimates of non-CO_2 carbon fluxes from this site, based on work by Schade and Goldstein (2001) showing that oxygenated VOCs (of which MBO made up ~40%) made up a mean of 1.3% of net ecosystem carbon exchange (NEE) at this site. Additionally, oxidation of MBO has been shown to account for as much as 63% of the acetone budget in areas containing MBO-emitting trees (Goldstein and Schade 2000). This, as well as the potential impact MBO may have on regional ozone production (Harley et al. 1998, Lamanna and Goldstein 1999), has led to attempts to model MBO emission. An important outcome of this study is that for the purposes of predicting ecosystem MBO inputs, the impact of water stress on photosynthetic metabolism can be ignored. Ambient temperature, however, is an important factor to include.

Although MBO emission by ponderosa pine may represent a carbon cost of only a small percentage of photosynthesis, compared to other secondary compounds produced by ponderosa pine, this cost is quite large. For example, based on MBO fluxes measured in this study and fluxes reported for monoterpenes (Lerdau et al. 1994, Lerdau and Gershenzon 1997) the amount of carbon allocated to MBO is 2–10 times greater than the amount allocated to monoterpene production in ponderosa pine. This large investment of energy into MBO production, coupled with the fact that MBO production capacity is regulated independently of photosynthesis and is not a fixed percentage of carbon assimilation, suggests that MBO may serve an adaptive function in emitting pines.

The large effect of temperature upon instantaneous emission rate and the strong effect of ambient temperature on emission capacity suggest that this role is associated with plant response to high temperatures. Such a thermotolerance hypothesis has been suggested for isoprene (Sharkey and Singsaas 1995, Singsaas et al. 1997), and given the similar metabolic origin, cellular location of synthesis, and ecological regulation

between isoprene and MBO, it would be surprising if MBO did not also serve a similar function.

ACKNOWLEDGMENTS

This work was made possible by a NASA Earth System Science Fellowship to D. W. Gray, a contract with the California Air Resources Board (award 98-328), and a grant from the University of California Agricultural Experiment Station to A. H. Goldstein. M. T. Lerdau was supported during manuscript preparation by a Bullard Fellowship from Harvard University. We thank the Blodgett Forest Research Station staff for their invaluable logistical support and Sierra Pacific Industries for allowing us to carry out research on their property. We especially thank Dave Rambeau for construction and maintenance of the watering system, Robert Heald for aid in selecting field sites, Russell Seufert for the use of the carpentry shop as summer research space for D. W. Gray, and Jeanne Panek and Gunnar Schade for many stimulating discussions. D. W. Gray also thanks the Forest Technicians for their innumerable contributions. We also thank Fakhri Bazzaz, Thomas Sharkey, Kenneth Raffa, and an anonymous reviewer for constructive comments on the manuscript.

LITERATURE CITED

Baker, B., A. Guenther, J. Greenberg, A. Goldstein, and R. Fall. 1999. Canopy fluxes of 2-methyl-3-buten-2-ol over a ponderosa pine forest by relaxed eddy accumulation: field data and model comparison. Journal of Geophysical Research-Atmospheres **104**(D21):26 107–267 114.

Brasseur, G. P., and R. B. Chatfield. 1991. The fate of biogenic trace gases in the atmosphere. Pages 1–27 *in* T. D. Sharkey, E. A. Holland, and H. A. Mooney, editors. Trace gas emissions by plants. Academic Press, San Diego, California, USA.

Chameides, W. L. et al. 1992. Ozone precursor relationships in the ambient atmosphere. Journal of Geophysical Research **97**(D5):6037–6055.

Crutzen, P. J., R. Fall, I. Galbally, and W. Lindinger. 1999. Parameters for global ecosystem models. Nature **399**:535.

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

Fall, R., and R. K. Monson. 1992. Isoprene emission rate and intercellular isoprene concentration as influenced by stomatal distribution and conductance. Plant Physiology **100**:987–992.

Fall, R., and M. C. Wildermuth. 1998. Isoprene synthase: from biochemical mechanism to emission algorithm. Journal of Geophysical Research **103**(D19):25 599–25 609.

Fang, C., R. K. Monson, and E. B. Cowling. 1996. Isoprene emission, photosynthesis, and growth in sweetgum (*Liquidambar styraciflua*) seedlings exposed to short- and long-term drying cycles. Tree Physiology **16**:441–446.

Fantechi, G., N. R. Jensen, J. Hjorth, and J. Peeters. 1998*a*. Determination of the rate constants for the gas-phase reaction of methyl butenol with OH radicals, ozone, NO_3 radicals, and Cl atoms. International Journal of Chemical Kinetics **30**(8):589–594.

Fantechi, G., N. R. Jensen, J. Hjorth, and J. Peeters. 1998*b*. Mechanistic studies of the atmospheric oxidation of methyl butenol by OH radicals, ozone and NO_3 radicals. Atmospheric Environment **32**:3547–3556.

Fehsenfeld, F., J. Calvert, R. Fall, P. Goldan, A. B. Guenther, C. N. Hewitt, B. Lamb, S. Liu, M. Trainer, H. Westberg, and P. Zimmerman. 1992. Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. Global Biogeochemical Cycles **6**: 389–430.

Fisher, A. J., B. M. Baker, J. P. Greenberg, and R. Fall. 2000. Enzymatic synthesis of methylbutenol from dimethylallyl diphosphate in needles of *Pinus sabiniana*. Archives of Biochemistry and Biophysics **383**:128–134.

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

Goldstein, A. H., M. L. Goulden, J. W. Munger, S. C. Wofsy, and C. D. Geron. 1998. Seasonal course of isoprene emissions from a midlatitude deciduous forest. Journal of Geophysical Research **103**(D23):31 045–31 056.

Goldstein, A. H., N. E. Hultman, J. M. Fracheboud, M. R. Bauer, J. A. Panek, M. Xu, Y. Qi, A. B. Guenther, and W. Baugh. 2000. Effects of climate variability on the carbon dioxide, water, and sensible heat fluxes above a ponderosa pine plantation in the Sierra Nevada (CA). Agricultural and Forest Meteorology **101**:113–129.

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

Guenther, A., et al. 1995. A global model of natural volatile organic compound emissions. Journal of Geophysical Research **100**(D5):8873–8892.

Guenther, A. B., P. R. Zimmerman, and P. C. Harley. 1993. Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analyses. Journal of Geophysical Research **98**(D7):12 609–12 617.

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

Harley, P., V. Fridd-Stroud, J. Greenberg, A. Guenther, and P. Vasconcellos. 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**(D19):25 479–25 486.

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

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

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

Jach, M. E., and R. Ceulemans. 2000. Effects of season, needle age and elevated atmospheric CO_2 on photosynthesis in Scots pine (*Pinus sylvestris*). Tree Physiology **20**:145–157.

Jacob, D. J., and S. C. Wofsy. 1988. Photochemistry of biogenic emissions over the Amazon forest. Journal of Geophysical Research **93**(D2):1477–1486.

Kuzma, J., and R. Fall. 1993. Leaf isoprene emission rate is dependent on leaf development and the level of isoprene synthase. Plant Physiology **101**:435–440.

Lamanna, M. S., and A. H. Goldstein. 1999. In situ measurements of C_2-C_{10} volatile organic compounds above a Sierra Nevada ponderosa pine plantation. Journal of Geophysical Research **104**(D17):21 247–21 262.

Law, B. E., and R. H. Waring. 1994. Combining remote sensing and climatic data to estimate net primary production across Oregon. Ecological Applications **4**:717–728.

Lehning, A., I. Zimmer, R. Steinbrecher, N. Bruggemann, and J. P. Schnitzler. 1999. Isoprene synthase activity and its relation to isoprene emission in *Quercus robur* L. leaves. Plant, Cell and Environment **22**:495–504.

Lerdau, M., S. B. Dilts, H. Westberg, B. B. Lamb, and E. J. Allwine. 1994. Monoterpene emission from ponderosa

pine. Journal of Geophysical Research **99**(D8):16 609–16 615.
Lerdau, M. T., and J. Gershenzon. 1997. Allocation theory and the costs of chemical defenses in plants. Pages 265–277 *in* F. Bazzaz and J. Grace, editors. Resource allocation in plants and animals. Academic Press, San Diego, California, USA.
Lerdau, M., A. Guenther, and R. Monson. 1997. Plant production and emission of volatile organic compounds. Bioscience **47**:373–383.
Lerdau, M., and H. L. Throop. 2000. Sources of variability in isoprene emission and photosynthesis in two species of tropical wet forest trees. Biotropica **32**:670–676.
Lichtenthaler, H. K. 1999. The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annual Review of Plant Physiology and Plant Molecular Biology **50**:47–65.
Lichtenthaler, H. K. 2000. Sterols and isoprenoids. Biochemical Society Transactions **28**:785–789.
Lichtenthaler, H. K., J. Schwender, A. Disch, and M. Rohmer. 1997. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. FEBS Letters **400**:271–274.
Loreto, F., and T. D. Sharkey. 1990. A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L. Planta **182**:523–531.
Monson, R. K., and R. Fall. 1989. Isoprene emission from aspen leaves: influence of environment and relation to photosynthesis and photorespiration. Plant Physiology **90**:267–274.
Monson, R. K., P. C. Harley, M. E. Litvak, M. Wildermuth, A. B. Guenther, P. R. Zimmerman, and R. Fall. 1994. Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. Oecologia **99**:260–270.
Monson, R. K., A. J. Hills, P. R. Zimmerman, and R. R. Fall. 1991. Studies of the relationship between isoprene emission rate and CO_2 or photon-flux density using a real-time isoprene analyzer. Plant, Cell and Environment **14**:517–523.
Monson, R. K., C. H. Jaeger, W. W. Adams III, E. M. Driggers, G. M. Silver, and R. Fall. 1992. Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. Plant Physiology **98**: 1175–1180.
Monson, R. K., M. T. Lerdau, T. D. Sharkey, D. S. Schimel, and R. Fall. 1995. Biological aspects of constructing volatile organic compound emission inventories. Atmospheric Environment **29**(21):2989–3002.
Panek, J. A., and A. H. Goldstein. 2001. Response of stomatal conductance to drought in ponderosa pine: implications for carbon and ozone uptake. Tree Physiology **21**:337–344.
Petron, G., P. Harley, J. Greenberg, and A. Guenther. 2001. Seasonal temperature variations influence isoprene emission. Geophysical Research Letters **28**:1707–1710.
Porté, A., and D. Loustau. 1998. Variability of the photosynthetic characteristics of mature needles within the crown of a 25-year-old *Pinus pinaster*. Tree Physiology **18**:223–232.
Rohmer, M., M. Knani, P. Simonin, B. Sutter, and H. Sahm. 1993. Isoprenoid biosynthesis in bacteria—a novel pathway for the early steps leading to isopentenyl diphosphate. Biochemical Journal **295**:517–524.
Rudich, Y., R. Talukdar, J. B. Burkholder, and A. R. Ravishankara. 1995. Reaction of methylbutenol with hydroxyl radical: mechanism and atmospheric implications. Journal of Physical Chemistry **99**:12 188–12 194.
Saelim, S., and J. J. Zwiazek. 2000. Preservation of thermal stability of cell membranes and gas exchange in high temperature-acclimated *Xylia xylocarpa* seedlings. Journal of Plant Physiology **156**:380–385.
SAS. 1990. SAS/STAT user's guide. Volume 2. SAS Institute, Cary, North Carolina, USA.
Schade, G. W., and A. H. Goldstein. 2001. Fluxes of oxygenated volatile organic compounds from a ponderosa pine plantation. Journal of Geophysical Research **106**(D3): 3111–3123.
Schade, G. W., A. H. Goldstein, D. W. Gray, and M. T. Lerdau. 2000. Canopy and leaf level 2-methyl-3-buten-2-ol fluxes from a ponderosa pine plantation. Atmospheric Environment **34**(21):3535–3544.
Schnitzler, J. P., A. Lehning, and R. Steinbrecher. 1997. Seasonal pattern of isoprene synthase activity in *Quercus robur* leaves and its significance for modeling isoprene emission rates. Botanica Acta **110**:240–243.
Schwender, J., J. Zeidler, R. Gröner, C. Müller, M. Focke, S. Braun, F. W. Lichtenthaler, and H. K. Lichtenthaler. 1997. Incorporation of 1-deoxy-D-xylulose into isoprene and phytol by higher plants and algae. FEBS Letters **414**:129–134.
Sharkey, T. D., and F. Loreto. 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., and E. L. Singsaas. 1995. Why plants emit isoprene. Nature **374**:769.
Sharkey, T. D., E. L. Singsaas, M. T. Lerdau, and C. D. Geron. 1999. Weather effects on isoprene emission capacity and applications in emission algorithms. Ecological Applications **9**:1132–1137.
Sharkey, T. D., E. L. Singsaas, P. J. Vanderveer, and C. Geron. 1996. Field measurements of isoprene emission from trees in response to temperature and light. Tree Physiology **16**: 649–654.
Singsaas, E. L., M. Lerdau, K. Winter, and T. D. Sharkey. 1997. Isoprene increases thermotolerance of isoprene-emitting species. Plant Physiology **115**:1413–1420.
SYSTAT. 1997. SYSTAT version 7. 0.1 for Windows. SPSS, Chicago, Illinois, USA.
Teskey, R. O., and R. E. Will. 1999. Acclimation of loblolly pine (*Pinus taeda*) seedlings to high temperatures. Tree Physiology **19**:519–525.
Tingey, D. T., R. Evans, and M. Gumpertz. 1981. Effects of environmental conditions on isoprene emission from live oak. Planta **152**:565–570.
Tingey, D. T., M. Manning, L. C. Grothaus, and W. F. Burns. 1979. The influence of light and temperature on isoprene emission rates from live oak. Physiologia Plantarum **47**: 112–118.
Wuebbles, D., K. Grant, P. Connell, and J. Penner. 1989. The role of atmospheric chemistry in climate change. Journal of the Air Pollution Control Association **39**:22–28.