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DIURNAL VARIATION IN THE BASAL EMISSION RATE OF ISOPRENE

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Abstract. Isoprene is emitted from numerous plant species and profoundly influences tropospheric chemistry. Due to the short lifetime of isoprene in the atmosphere, developing an understanding of emission patterns at small time scales is essential for modeling regional atmospheric chemistry processes. Previous studies suggest that diurnal fluctuations in isoprene emission may be substantial, leading to inaccuracies in emission estimates at larger scales. We examined diurnal patterns in the basal emission rate of isoprene in red oak (*Quercus rubra*), eastern cottonwood (*Populus deltoides*), and eucalyptus (*Eucalyptus saligna*) and the influence of light and temperature on the magnitude of these diurnal patterns. Maximum diurnal increases in isoprene emission were large in cottonwood (45%) and oak (25%), with increases exceeding 100% in 1-yr-old cottonwoods. Eucalyptus showed no diurnal variation in emission. All species showed diurnal declines in photosynthesis. Across species, there was a positive correlation between maximum diurnal change in both isoprene emission and photosynthesis. The magnitude of diurnal increase in isoprene emission varied when individual cottonwoods were sampled repeatedly over three days. Temperature and light history, integrated from 1 to 48 hours prior to measurement, did not explain these variations in diurnal emission. Diurnal increases in emission were present when plants were shaded to $<7\%$ ambient light. Our results indicate that diurnal fluctuations in emission are large and species specific, and must be considered when estimating emission rates for use in short-term regional atmospheric-chemistry models.

Key words: ambient light and temperature; atmospheric chemistry, modeling; basal emission rate; diurnal patterns; Eucalyptus; *hydrocarbon emission, biogenic; isoprene;* Populus*;* Quercus.

INTRODUCTION

Isoprene (2-methyl 1,3-butadiene) is the most abundant biogenic volatile organic compound (VOC) emitted from many temperate and tropical forests. In North America, isoprene alone accounts for 35% of the total nonmethane hydrocarbon input to the atmosphere (Guenther et al. 2000). Isoprene is quickly oxidized in the lower atmosphere primarily by hydroxyl radical (OH•), leading to the formation of carbon monoxide (Granier et al. 2000) and, in the presence of NO_x, substantial quantities of ozone (Chameides et al. 1988, Jacob and Wofsy 1988). By reducing the oxidative capacity of the troposphere, the oxidation of isoprene by OH• increases the lifetime of other VOCs, including methane, an important greenhouse gas (for recent reviews see Atkinson [2000] and Fuentes et al. [2000]). Constructing accurate estimates of isoprene emission is therefore essential in modeling tropospheric chemistry dynamics.

Because the lifetime of isoprene in the atmosphere is short, modeling the dynamics of atmospheric chemistry at regional scales requires an understanding of the variation in isoprene emission over small time intervals. Specifically, the lifetime of isoprene depends on the composition of oxidizing species and is on the order of minutes to hours when oxidized by OH• or NO_3 and one day when oxidized by O_3 (Fuentes et al. 2000). Current models construct emission estimates by adjusting a measured or modeled static basal emission rate (BER) of isoprene, standardized to a light level of 1000 μmol photon·m^{-2}·s^{-1} and leaf temperature of 30°C, to ambient light and temperature conditions (Guenther et al. 1993). However, isoprene BER of individual leaves can vary by up to 50% over a few days (Guenther et al. 1991, Monson et al. 1994, Fuentes and Wang 1999, Geron et al. 2000) and data from two *Quercus* individuals indicate that isoprene BER may vary up to 100% within a single day (Sharkey et al. 1999, Geron et al. 2000). Consequently, diurnal emission patterns of isoprene BER could lead to under- or overestimation of isoprene emission if sampling is not stratified by time of day.

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At larger spatial scales, these uncertainties restrict efforts to accurately predict the dynamics of gases that result from isoprene oxidation, such as methylvinyl ketone, methacrolein, and carbon monoxide (Fehsenfeld et al. 1992, Paulson and Seinfeld 1992), and the ability of tropospheric photochemical models to predict the severity and duration of air-pollution episodes (Roselle 1994). On longer time scales, these inaccuracies can lead to errors in the estimation of the tropospheric sink for methane and source for ozone, which, in turn, causes errors in the estimation of source and sink strengths of these compounds on a global scale (Poisson et al. 2000). By understanding the diurnal variation in isoprene BER, we will be able to explain the discrepancy between modeled and measured diurnal leaf-level fluxes of isoprene (Guenther et al. 1993, 1996, Harley et al. 1997, Steinbrecher et al. 1997) and thus reduce the uncertainty surrounding our models of tropospheric chemical processes.

Obtaining accurate estimates of isoprene BER on short time scales requires an understanding of the physiological and abiotic factors that regulate diurnal patterns. On short time scales, light and temperature influence isoprene production by regulating substrate availability and enzyme kinetics. Across all species of plants, isoprene emission shows a hyperbolic response to light intensity (e.g., Guenther et al. 1995), although responses do not always saturate (Sharkey et al. 1996, Keller and Lerdau 1999). Emission responds exponentially to temperature, but declines at high temperatures due to enzymatic denaturation (Guenther et al. 1991, Fall and Wildermuth 1998) or possibly enzymatic regulation (Singsaas and Sharkey 2000). Several studies have examined the long-term effects of light on emission capacity (e.g., Sharkey et al. 1991, Harley et al. 1996, Litvak et al. 1996, Lerdau and Throop 1999), however the effects of daily fluctuations in light intensity, such as those resulting from changing cloud cover, on isoprene BER are not well understood. Following the induction of isoprene emission at the beginning of the growing season, prevailing light and temperature conditions are thought to control isoprene BER, possibly through the regulation of isoprene synthase activity (Monson et al. 1994, Logan et al. 2000, Lehning et al. 2001). Recent field (Sharkey et al. 1999, Geron et al. 2000) and growth-chamber (Hanson and Sharkey 2001, Petron et al. 2001) studies suggest that light and temperature history on the scale of hours to days regulate day-to-day variation in isoprene BER.

We examined diurnal patterns in isoprene BER from eastern cottonwood (*Populus deltoides*), northern red oak (*Quercus rubra*), and eucalyptus (*Eucalyptus saligna*) individuals differing in nutrient availability, genotype, and age. The objectives of our study were (1) to determine the generality of diurnal patterns in isoprene BER within and across species and (2) to explore the short-term influence of light and temperature on the magnitude of these patterns in isoprene BER using both observational and experimental approaches.

Methods

Species and site description

We conducted this study on eastern cottonwood and red oak individuals in New York (USA) and eucalyptus individuals growing in a plantation on the island of Hawaii (USA). In 1998–2000 we measured cottonwood and oak individuals from both a fertilized nursery and naturally established populations of individuals from unmanipulated field sites, all located within 10 miles of the Institute of Ecosystem Studies (IES) in Millbrook, New York, USA (41°51′ N, 73°45′ W). The mean annual temperature of the area is 9.5°C, mean annual rainfall is 1 m and elevation is ~130 m. The soils in this area are mesic, Typic Dystrudepts. In the nursery we measured 10 cottonwood and 12 oak individuals. Sapling ages ranged from 3 to 5 yr, with heights ranging from 2 to 4 m. Cottonwood individuals were propagated vegetatively one year before planting from a single clone (ST109, Stoneville, Mississippi, USA). Red oaks were obtained by transplanting 1-yr-old seedlings from Musser Forests (Indiana, Pennsylvania, USA). The plants were spaced ~2 m apart, received full sunlight for most of the day, and were irrigated by drip irrigation every 2 to 4 d. Each growing season, individual plants received a total of 3.5 g N, 3.7 g P, and 3.7 g K spread over six applications. In addition, we measured 14 cottonwood and 15 oak individuals from six unmanipulated field sites. Field plants ranged in size from 4–12 m in height.

In June of 2000 we sampled 2- and 6-yr-old individuals from a pure *Eucalyptus saligna* plantation located 13 km north of Hilo, Hawaii, USA (19°50′ N, 155°7′ W). The mean annual temperature of the site is 21°C, mean annual rainfall is 4 m, and elevation is 150 m. The soils are moderately acidic, isothermic, Typic Hydrandepts. The seedlings used to establish the 2- and 6-yr-old plantations were from a single, open-pollinated seed stock. We examined 6-yr-old individuals in a randomly selected control plot (old control), a randomly selected continuous-fertilization plot (old fertilized), and 2-yr-old individuals in a randomly selected continuous-fertilization plot (young fertilized). A total of seven individual eucalyptus trees were measured from these plots. All plants were spaced 1 m apart. Young fertilized, old fertilized, and old control individuals were 7–9 m, 20–25 m, and 15–22 m tall, respectively. All plots received 31 g N/m^2, 13 g P/m^2, 26 g K/m^2, 12.5 g Ca/m^2, 1.2 g Mg/m^2, and 10 g/m^2 balanced micronutrients in holes adjacent to the seedlings at the time of planting (6-yr-old plantings) or applied as a side dress (2-yr-old plantings) as described in Binkley and Resh (1999). The young and old fertilized plots also received quarterly applications of 5.6 g N/m^2, 2.4 g P/m^2, 4.6 g K/m^2, and annual additions of

12.5 g Ca/m^2, 5.8 g S/m^2, 2.3 g Mg/m^2, and 10 g/m^2 micronutrients. The old and young fertilized plots were fertilized one month prior to taking measurements.

Experimental design

To examine diurnal patterns in isoprene basal emission rate (BER), one leaf per tree was measured from 0800 to 1800 hours at 3–5 evenly spaced time points, depending on the number of individuals measured per day. We chose recently mature, fully expanded sun leaves for all measurements. Because of limited access to all cottonwood and oak field individuals, we selected leaves that were located on a branch near the ground rather than at the top of the canopy. Scaffold towers allowed access to upper-canopy leaves in the eucalyptus plots. For all comparisons, the leaves used for the diurnal measurements were clipped after the last measurement, transported to the laboratory in sealed polyethylene bags, refrigerated until leaf area determination, dried at 60–70°C for 48 h, and weighed for specific leaf area.

One-year-old ST109 *Populus deltoides* clones, raised in the IES nursery under the same irrigation and fertilization protocols described above for 3–5 yr old saplings, were subjected to two shading experiments. We conducted the experiments on different days within a two-week period. In both experiments, wooden-frame shade boxes covered with several layers of cloth were used to shade five saplings from dusk until measurements were completed the following day. Light levels were reduced to $<$7% of ambient light in Experiment 1 and $<$1% of ambient light in Experiment 2. Light-level and air-temperature measurements taken inside and outside of each box using a LI-250 light meter (LI-COR, Lincoln, Nebraska, USA) and thermocouple thermometers showed that the shade treatment did not influence air temperature (Expt. 1, $P = 0.94$; Expt. 2, $P = 0.34$).

Gas exchange

Isoprene emission rate and photosynthetic rate were measured using an open system LI-6400 portable infrared gas analyzer (IRGA) with a temperature- and light-controlled cuvette (LI-COR) and a Photovac voyager gas chromatograph (GC) with a photoionization detector (Perkin-Elmer, Norwalk, Connecticut, USA). The CO_2 concentration of air entering the leaf cuvette was maintained at 400 ppm using a CO_2 cartridge injector system. Humidity was maintained between 40 and 80% by removing water vapor from the entering air stream. Flow rates through the cuvette and GC ballast line were held constant at 250 μmol/s. Gas exchange of abaxial and adaxial leaf surfaces (total leaf area of 6 cm^2) was measured by the cuvette.

The sample air was routed from the cuvette into a 1.75-L Teflon ballast line, which was flushed 6–10 times before measurement. Air was then drawn from this line into the 1-mL sample loop within the GC at 100 mL/min for 10 s. During the summers of 1998 and 1999 we used a SupelcoWax 10 wax phase capillary column (20-m length, 0.32-mm interior diameter, 1.0-μm film thickness [Sigma-Aldrich, Bellefonte, Pennsylvania, USA]), which was maintained at 40°C. In 2000, samples were injected onto a Quadrex 007-1 methyl silicone phase capillary column (15-m length, 0.32-mm interior diameter, 12.0-μm film thickness [Quadrex Corporation, Woodbridge, Connecticut, USA]), which was maintained at 50°C. In all years we used hydrocarbon-free air as a carrier gas. The detector output was linear across the range of isoprene concentrations used in this study, with an intercept through zero. The minimum detection was 3 ppb and detector reproducibility was within 10%. In New York, one- (1998, 1999) and two-point (2000) calibrations were made daily using dilutions from a 97.9 ppm mix of isoprene in air (Scott-Marrin, Irvine, California, USA). In Hawaii, single-point calibrations were made by vaporizing and diluting pure liquid isoprene (Sigma-Aldrich, Saint Louis, Missouri, USA).

All measurements were taken with cuvette light levels of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using a variable-intensity red and blue light-emitting diode with peak irradiance at 665 and 470nm, respectively. When ambient conditions did not permit a constant leaf temperature of 30°C, measured rates were standardized to 30°C using the algorithm developed by Guenther et al. (1993). We tested these temperature-correction equations by generating temperature curves on several red oak field individuals. During this test, both isoprene emission and photosynthetic rate were monitored at a constant light level of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ while leaf temperature levels were varied from 20° to 34°C. The actual and predicted values were similar in this temperature range. Photosynthetic rates did not vary substantially between 26° and 32°C, therefore we did not temperature correct any photosynthetic data presented in this study.

Meteorological data

Ambient light and temperature data during the study period were obtained from a nearby weather station in an open field at IES (IES Environmental Monitoring Program)[7] and from a weather station located in an open field ~100 m from the Hawaiian eucalyptus plantations. For IES comparisons, the mean hourly air temperature (in degrees Celsius) and instantaneous global photosynthetically active radiation (PAR, in micromoles per square meter per second) were collected using a HMP45C temperature probe (Campbell Scientific, Logan, Utah, USA) and LI-190SB light meter (LI-COR). For the Hawaii comparisons, instantaneous measures of air temperature and PAR were collected every 15 s by a CS500 air temperature/relative humidity probe (Campbell Scientific) and an LI-190SB quantum sensor (LI-COR), and stored on CR10X data logger.

[7] URL: ⟨http://www.ecostudies.org/research⟩

The instantaneous measures were used to calculate hourly air temperature means and total hourly PAR (in millimoles per square meter per hour).

Statistical analysis

Repeated diurnal measurements on single leaves were analyzed by multivariate repeated-measures analysis of variance (MANOVAR) using SAS procedure GLM (SAS Institute 1996). Three measurement points during the day (T1: 0800–1000 hours, T2: 1100–1300 hours, T3: 1500–1700 hours) were used as the dependent variables. Diurnal patterns of isoprene BER and photosynthesis were assessed using time as the within-subject factor (repeated measure). Differences between field and nursery sites (cottonwood and oak) were expressed as time × site interactions. A Box *M* test, performed in Statistica (Statsoft, Tulsa, Oklahoma, USA), was used to test for homogeneity of variances and covariances (data not shown). Univariate tests of normality, used to approximate multivariate normality (Manly 1994), were conducted on data within each sampling time using the Shapiro-Wilk (*W*) statistic (SAS procedure UNIVARIATE [SAS Institute 1996]). Profile transformations (contrasts) of the data were generated (SAS procedure GLM [SAS Institute 1996]) to indicate which time interval was the most important in determining diurnal patterns.

Linear regression was used to assess the influence of light and temperature history on isoprene emission in cottonwood and oak. Light and temperature variables (see *Meteorological data*, above) were averaged at 1, 2, 3, 4, 8, 12, 18, 24-, and 48-h time intervals. Previous work on field oak has shown that temperature and light influence isoprene BER on these short time scales (Sharkey et al. 1999, Geron et al. 2000). To avoid pseudoreplication arising from multiple measurements of the same individuals over the 48-h period, one randomly selected measure of isoprene BER per individual was used in the analysis ($n = 32$ cottonwoods; $n = 25$ oaks). A stepwise selection approach (RSQUARE method, SAS procedure REG [SAS Institute 1996]) was used to find the best combination of light and temperature variables. All other linear regressions were performed in Statistica. One-way analysis of variance was used to compare the maximum relative change in isoprene emission and photosynthesis rate between species. Planned post hoc comparisons were analyzed by a least-significant-difference approach (Statistica). In the shade experiments *t* tests were performed using SPSS (1997).

RESULTS

Diurnal patterns

Diurnal patterns of the basal emission rate (BER) of isoprene varied among the three species examined ($F = 17.15$, $P < 0.01$). Isoprene BER in cottonwood and oak were 36% and 9% higher, respectively, in the late afternoon (T3) compared to rates measured that morning (T1) (Fig. 1A, Table 1). Most of this diurnal increase occurred between T1 and noon (T2) (Table 2). While the isoprene emission of cottonwood continued to increase after T2, emissions in oak declined between T2 and T3. Therefore, we define the maximum relative change in isoprene BER as the difference in flux rate between T1 and the larger of T2 or T3. The maximum increase in isoprene BER was 45% in cottonwood and 25% in oak. Eucalyptus showed no diurnal variation in isoprene BER ($F = 1.34$, $P = 0.38$). It is important to note that a diurnal change is always relative to the morning flux measurement.

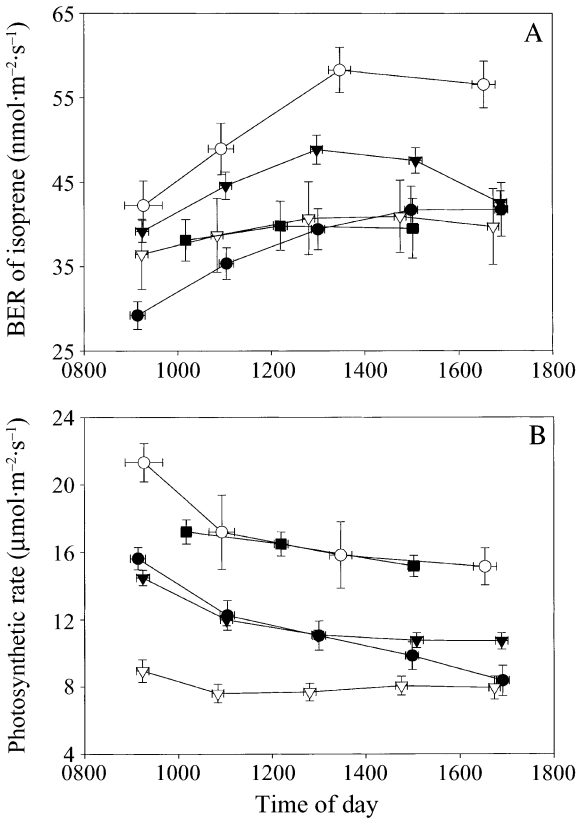

FIG. 1. Diurnal variation in (A) basal emission rate (BER) of isoprene and (B) photosynthetic rate for nursery cottonwood (open circles, $n = 10$ individuals), field cottonwood (solid circles, $n = 14$ individuals), nursery oak (open triangles, $n = 12$ individuals), field oak (solid triangles, $n = 15$ individuals) and eucalyptus (solid squares, $n = 7$ individuals). Data points are means ± 1 SE. The time scale is based on a 24-h day.

All species showed net declines in photosynthetic rate over the 8- to 10-h diurnal measurement period (Fig. 1B, Table 1). From T1 to T3, photosynthetic rate declined by 36% in cottonwood, 21% in oak, and 12% in eucalyptus. The declines for cottonwood and oak were strongest in the morning interval, while eucalyptus showed stronger declines in the afternoon (Table 2). For all species, declines in photosynthetic rate were

TABLE 1. Results of multivariate repeated-measures analysis for maximum diurnal (A) increases in basal emission rate of isoprene and (B) decreases in photosynthetic rate, in cottonwood (n = 23), red oak (n = 27), and eucalyptus (n = 7) individuals (Time).

Species	Source of variation†	df	Pillai's trace	F	P
A) Isoprene					
Cottonwood	Time	2, 21	0.8413	55.66	<0.01
	Time × Site	2, 21	0.0068	0.07	0.93
Oak	Time	2, 24	0.7714	40.49	<0.01
	Time × Site	2, 24	0.4050	8.17	<0.01
Eucalyptus	Time	2, 3	0.4726	1.34	0.38
B) Photosynthesis					
Cottonwood	Time	2, 21	0.8561	62.46	<0.01
	Time × Site	2, 21	0.2481	3.46	0.05
Oak	Time	2, 24	0.7315	32.69	<0.01
	Time × Site	2, 24	0.4472	9.71	<0.01
Eucalyptus	Time	2, 3	0.9495	28.23	0.01

† The within-subject interaction of Time and Site refers to differences in diurnal pattern between field and nursery sites for cottonwood and oak.

matched by declines in stomatal conductance (cottonwood $F = 32.39$, $P < 0.01$; oak $F = 15.11$, $P < 0.01$, eucalyptus $F = 7.42$, $P = 0.07$). The maximum relative decrease in photosynthesis, defined as the difference in photosynthesis rate between T1 and the smaller of T2 and T3, was 39% in cottonwood, 26% in oak, and 12% in eucalyptus.

For cottonwood and oak, intraspecific diurnal patterns of isoprene BER (cottonwood $F = 2.17$, $P = 0.11$; oak $F = 1.01$, $P = 0.42$) and photosynthesis (cottonwood $F = 1.71$, $P = 0.18$; oak $F = 1.77$, $P = 0.17$) were similar across the three field sites, so data from the field sites were pooled for comparison with the nursery group. Within a species, diurnal patterns of isoprene BER and photosynthesis were more pronounced for field than for nursery individuals (Table 1, Fig. 1). While both the nursery and field cottonwoods showed a similar maximum relative increase in isoprene BER (42% and 48%, respectively), photosynthetic rates declined more steeply in the field individuals (47% vs. 28%, $F = 3.46$, $P = 0.05$). Field oaks showed a larger maximum increase in isoprene emission (30% vs. 18%, $F = 8.17$, $P < 0.01$) and a larger decline in photosynthesis (30% vs. 21%, $F = 9.71$, $P < 0.01$) than nursery oaks.

The magnitude of maximum increase in isoprene BER was positively correlated with the magnitude of maximum decrease in photosynthesis within an individual over the course of a day (n = 57 cottonwood, oak, and eucalyptus individuals, $r^2 = 0.22$, $P < 0.01$). Combining data from individual plants, species with large diurnal patterns of isoprene BER also showed large diurnal decreases in photosynthesis (Fig. 2). The relative diurnal change of both isoprene emission and photosynthetic rate was highest in cottonwood, while only the diurnal change in photosynthetic rate was significant in eucalyptus ($F = 28.23$, $P = 0.01$). The relationship between the maximum relative change in isoprene BER and the maximum relative change in photosynthesis was not significant within species (cottonwood $r^2 = 0.05$, $P = 0.30$; oak $r^2 = 0.00$, $P = 0.80$; eucalyptus $r^2 = 0.06$, $P = 0.59$), possibly due to the restricted range of isoprene emission and photosynthesis within species resulting from small sample sizes. In contrast to this diurnal pattern, isoprene BER and photosynthetic rate were positively correlated across species and sites ($r^2 = 0.11$, $P = 0.01$).

Temporal variation

Oak and cottonwood individuals measured repeatedly over three days within a 3-wk period showed con-

TABLE 2. Profile contrasts of (A) basal emission rate of isoprene and (B) photosynthetic rate for cottonwood (n = 23), red oak (n = 27), and eucalyptus (n = 7).

Source of variation	df	MS	F	P	Change (%)
A) Isoprene					
Contrast T2–T1					
Cottonwood	1	2627.57	106.89	<0.01	30.1
Oak	1	1297.82	79.53	<0.01	18.9
Eucalyptus	1	21.25	3.51	0.13	0.0
Contrast T3–T2					
Cottonwood	1	107.56	3.67	0.07	4.6
Oak	1	334.41	12.91	<0.01	−8.4
Eucalyptus	1	0.73	0.06	0.82	0.0
B) Photosynthesis					
Contrast T2–T1					
Cottonwood	1	438.59	55.79	<0.01	−25.3
Oak	1	145.20	52.23	<0.01	−20.0
Eucalyptus	1	3.67	12.10	0.02	−4.2
Contrast T3–T2					
Cottonwood	1	66.13	11.49	<0.01	−13.8
Oak	1	0.11	0.06	0.81	0.0
Eucalyptus	1	10.80	26.05	<0.01	−7.9

Notes: All sites were pooled within species for analysis. T1 (''morning'') = 0800–1100 hours, T2 (''noon'') = 1100–1300 hours, and T3 (''late afternoon'') = 1500–1700 hours.

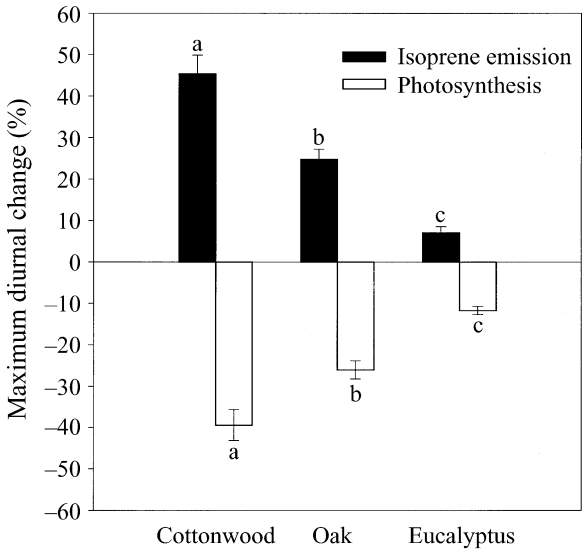

FIG. 2. The maximum relative diurnal change in basal emission rate of isoprene and photosynthesis for eastern cottonwood, red oak, and eucalyptus. All sites were pooled within species for analysis. Bars are means and 1 SE for 23 cottonwood, 27 oak, and 7 eucalyptus individuals. For a given variable, species means with the same lowercase letter are not significantly different from each other at $P < 0.05$. Diurnal patterns of isoprene emission were not significantly different from zero in eucalyptus.

siderable daily variation in isoprene BER (Table 3). Despite differences in flux rate, maximum daily increases in isoprene BER were similar across days in oak individuals. In contrast, the magnitude of diurnal increase varied among days in cottonwood (maximum relative increase in isoprene BER for Day 1: 19.25 ± 4.68%, Day 2: 23.99 ± 8.41%, Day 3: 117.43 ± 11.89% [mean ± 1 SE]).

Light and temperature

Overall, both average and cumulative measures of light and temperature were poor predictors of isoprene BER over a 48-h period. The average temperature 12 h prior to measurement was found to be the best predictor variable for isoprene BER in cottonwood ($r^2 = 0.34$, $P < 0.01$), although it explained a low percentage of the variance (Fig. 3). Temperature and light history explained little of the variation in isoprene emission for oak individuals ($r^2 < 0.10$ for all combinations). Including interactions terms between temperature and light did not improve the regression analysis for either species (data not shown). Cumulative temperature and light data integrated over 1–48 h prior to measurement were similarly poor predictors for both cottonwood ($r^2 < 0.34$ for all combinations) and oak ($r^2 < 0.10$ for all combinations).

Shading reduced isoprene BER in cottonwood saplings in Experiment 1 only (Table 4, Fig. 4). In both experiments, diurnal increases in emission were present in both shaded and unshaded individuals (Fig. 4A). The magnitude of maximum relative increase of isoprene BER was similar for all groups (30–60%) except unshaded plants in Experiment 2, for which emission increased more dramatically than the other groups (116%). This difference accounted for the significant time × treatment interaction term in Expt. 2. Photosynthetic rates were lower in shaded plants in Expt. 2 only (Table 5, Fig. 4B). In Expt. 1, both unshaded and shaded plants showed similar maximum relative decreases in photosynthesis (20–30%). In Expt. 2, photosynthesis rates declined sharply in shaded plants (66%) relative to unshaded plants, which showed no net change.

TABLE 3. Results of multivariate repeated-measures analysis for maximum diurnal increases in the basal emission rate of isoprene in five oak and five cottonwood individuals over three days.

Source of variation	df	MS	Pillai's trace	*F*	*P*
A) Cottonwood					
Between subjects					
Day	2	197.29		6.16	0.01
Error	12	32.03			
Within subjects					
Time	2, 11		0.8914	45.13	<0.01
Time × Day	2, 11		0.9138	5.05	<0.01
B) Red Oak					
Between subjects					
Day	2	531.95		5.83	0.02
Error	12				
Within subjects					
Time	2, 11		0.7737	18.80	<0.01
Time × Day	2, 11		0.1509	0.49	0.74

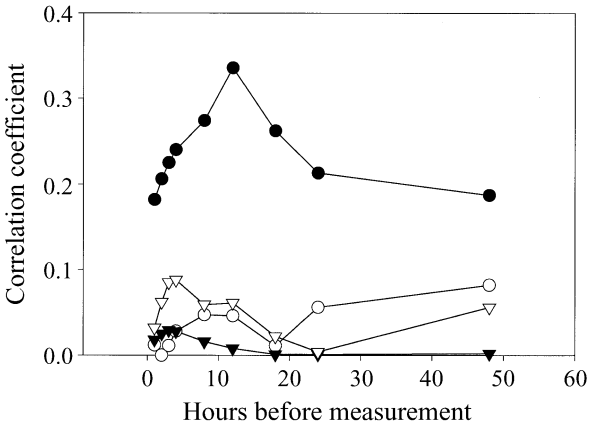

FIG. 3. Correlation coefficients for basal emission rate of isoprene vs. average ambient temperature (cottonwood, solid circles; oak, solid triangles) and light (cottonwood, open circles; oak, open triangles). Temperature and light variables were averaged at various time intervals between 1 and 48 h prior to measurement. One measure of isoprene basal emission rate from 32 cottonwood and 25 oak individuals was included in the analysis. All $r^2 > 0.016$ are significant at $P < 0.05$.

TABLE 4. Results of multivariate repeated-measures analysis for maximum diurnal increases in the basal emission rate of isoprene from shaded and unshaded cottonwood individuals ($n = 5$ trees per treatment for each experiment).

Source of variation	df	MS	Pillai's trace	F	P
A) Experiment 1					
Between subjects					
Shade	1	218.27		6.91	0.03
Error	8	31.59			
Within subjects					
Time	2, 7		0.9456	60.78	<0.01
Time × Shade	2, 7		0.1593	0.66	0.54
B) Experiment 2					
Between subjects					
Shade	1	413.44		4.18	0.08
Error	8	98.92			
Within subjects					
Time	2, 7		0.9540	72.52	<0.01
Time × Shade	2, 7		0.7809	12.47	<0.01

DISCUSSION

Generality of diurnal patterns

Our results indicate that diurnal patterns of isoprene basal emission rate (BER) can vary substantially among species; BER of oak and cottonwood were strongly diurnal while eucalyptus showed no diurnal pattern. In accordance with projections based on *Quercus* species (Sharkey et al. 1999, Geron et al. 2000), we found the maximum relative diurnal increase to be very large in *Q. rubra* and *Populus deltoides*, exceeding 100% in 1-yr-old *P. deltoides* saplings. This magnitude is greater than or comparable to documented long-term environmental influences on isoprene BER. For example, although several studies have shown no effect of water stress on isoprene BER (e.g., Guenther et al. 1999), others have documented up to 50% declines in live oak (Tingey et al. 1981) and velvet bean (Lerdau et al. 1997) and 2- to 3-fold increases following water stress in kudzu (Sharkey and Loreto 1993). Isoprene emission doubled in response to N enrichment and long-term manipulations of light environment in velvet bean (Harley et al. 1994) and potted aspen and white oak (Litvak et al. 1996). Similarly, leaf burning or wounding can result in 30 to 40% reductions in isoprene BER (Loreto and Sharkey 1993, Funk et al. 1999).

Within cottonwood and oak, diurnal patterns of isoprene BER varied both spatially and temporally. Differences between nursery and field sites may have been influenced by variation in plant age, genotype, or environmental characteristics of the sites. While the effects of N and water availability have been explored, the effects of plant age and genotype on absolute rates of isoprene emission are not well understood (Street et al. 1997, Isebrands et al. 1999). Day-to-day variation in the magnitude of diurnal increase was observed in

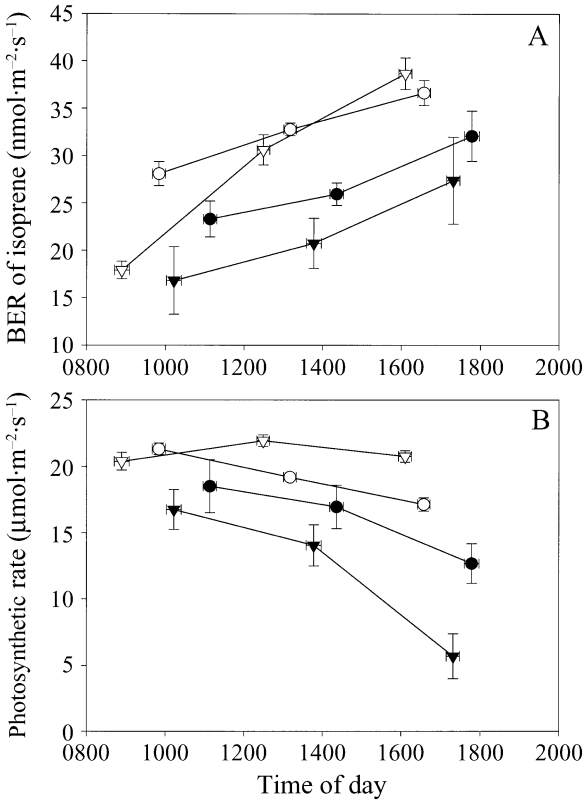

FIG. 4. Diurnal variation in (A) basal emission rate (BER) of isoprene and (B) photosynthetic rate for 1-yr-old nursery cottonwood individuals in two shading experiments. In Experiment 1, shaded individuals (solid circles) received <7% of ambient light, and unshaded individuals (open circles) received full sunlight. In Experiment 2, shaded individuals (solid triangles) received <1% of ambient light, and unshaded individuals (open triangles) received full sunlight. Data points are means ± 1 SE for five individuals. The time scale is based on a 24-h day.

TABLE 5. Multivariate repeated-measures analysis for maximum diurnal decreases in photosynthetic rate from shaded and unshaded cottonwood individuals ($n = 5$ per treatment for each experiment).

Source of variation	df	MS	Pillai's trace	F	P
A) Experiment 1					
Between subjects					
Shade	1	75.30		3.79	0.09
Error	8	19.88			
Within subjects					
Time	2, 7		0.8737	24.20	<0.01
Time × Shade	2, 7		0.2628	1.25	0.34
B) Experiment 2					
Between subjects					
Shade	1	589.28		37.40	<0.01
Error	8	15.76			
Within subjects					
Time	2, 7		0.9676	104.65	<0.01
Time × Shade	2, 7		0.9345	49.97	<0.01

cottonwood but not oak. The increased capacity for diurnal fluctuations of both isoprene BER and photosynthesis rate in cottonwood may reflect physiological differences between the two species, such as diurnal patterns of carbohydrate synthesis and export (e.g., Dickson 1991, Wullschleger et al. 1992, Tognetti et al. 1998). However, the cottonwoods and oaks were measured on different days and so it is possible that variability in daily patterns of ambient light and temperature led to differences in diurnal isoprene patterns.

Effect of light and temperature

In the past few years, several studies have explored the potential regulation of isoprene BER by hourly and daily changes in light and temperature. Field studies have found temperature and light conditions over 6–48 h to explain diurnal and day-to-day variation in isoprene BER in oak species (Sharkey et al. 1999, Geron et al. 2000). In concordance with these field observations, studies using growth chambers to manipulate light and temperature found these variables to explain patterns in isoprene BER. Petron et al. (2001) found that isoprene BER responded to both hourly changes in temperature and the average temperature of the previous 15 d. Hanson and Sharkey (2001) found isoprene BER to acclimate quickly (hours) to changes in growth temperature, while responding on both short (minutes to hours) and long (4–6 d) time scales to changes in light environment. However, our examination of cottonwood and oak individuals suggests that short-term light and temperature variables (1–48 h) are inadequate as predictors of diurnal patterns of isoprene BER. This discrepancy may result from differences in experimental design. For example, Geron et al. (2000) used data collected from one individual plant over 100 d. While this approach eliminates sources of inter-individual variation, which can be great even within a genotype (this study), the nonindependence of data points is expected to inflate regression coefficients.

Although short-term light and temperature history did not correlate with patterns of isoprene BER within cottonwood and oak, a comparison of light and temperature patterns across forest types suggested they may be important. While day-to-day fluctuations of light intensity were similar at the Hawaii and New York sites, patterns of average daily temperature were very different between sites. In Hawaii, the average daily temperature for adjacent days differed by no greater than 0.2°C per day while average daily temperature could differ by >5°C between adjacent days in New York. The temperature range within a day was often close to 20°C in New York compared to 6°C in Hawaii. Our limited data set concurs with the idea that large fluctuations in daily light and temperature patterns could result in large diurnal patterns of isoprene BER, as observed in our New York individuals, and may also explain day-to-day variation in isoprene BER. This suggests that short-term variation in isoprene BER may be ignored when constructing inventories of isoprene for tropical regions, characterized by low daily and day-to-day variation in temperature. The contrast between diurnal patterns of isoprene emission in tropical eucalyptus and temperate oak and cottonwood has important consequences for global and regional models of isoprene emission and emphasizes the need to examine the generality of our findings.

Basal emission rates of isoprene can be reduced in response to short-term shading (Lehning et al. 1999, this study), but the effects of shading on diurnal patterns of isoprene BER remain unclear. While the majority of isoprene emitted from a canopy originates in the top, sunlit layers (Harley et al. 1996), differential effects of shading on diurnal patterns of isoprene BER with canopy depth could have implications for modeling canopy fluxes. As isoprene emission responds differently to varying light and temperature, understanding the influence of shade on short-term patterns of isoprene BER requires separating the effects of light intensity from leaf temperature (Harley et al. 1996, Singsaas et al. 1999). Although there were no differences in ambient air temperature inside and outside the shade boxes, leaf temperature was not measured and may have interacted with light intensity in influencing diurnal patterns.

Implications for modeling

Diurnal changes in isoprene BER are large enough to merit consideration in hourly emission calculations, estimations of isoprene emission from forest ecosystems, and modeled predictions of the impact of isoprene on atmospheric chemistry and air quality. From our present study, it appears that differences in the magnitude of diurnality among genera are comparable to differences in absolute isoprene BER among genera (e.g., Kesselmeier and Staudt 1999). Thus, incorporating diurnal patterns of isoprene BER into leaf-level models may be species specific, as are absolute rates of BER (e.g., Guenther et al. 1996). The large diurnal changes in isoprene BER and the variability in those changes across days within and between taxa native to mid-latitude regions suggest that it is particularly important to incorporate diurnal effects in emission estimates from these regions. In addition, different degrees of diurnality in sun vs. shade leaves suggest that these fractions should be isolated in models and may also show interspecific patterns.

In its original form, the leaf-level model of Guenther et al. (1995) assumed a static isoprene BER. As more research has demonstrated the dynamic nature of isoprene BER, this algorithm has been modified to better approximate measured fluxes. For example, to reduce variability between modeled and measured seasonal patterns of isoprene BER, Guenther (1997) added a sine function and Schnitzler et al. (1997) added a new term describing the seasonality of isoprene synthase activity. Petron et al. (2001) modified the temperature correction

term in the Guenther model to reflect the dependence of isoprene BER on both hourly temperature and temperature over the previous 15 d. Given the variable and conflicting results from studies examining light and temperature effects on patterns of isoprene BER (Sharkey et al. 1999, Geron et al. 2000, Hanson and Sharkey 2001, Petron et al. 2001, this study), we suggest that incorporating diurnality of isoprene BER into a leaf-level model requires a better understanding of the mechanisms underlying this phenomenon. The marked relationship we find between the maximum relative change in isoprene emission and in photosynthesis suggests that diurnality in isoprene BER is linked to photosynthetic processes. Specifically, the relationship between isoprene BER and light and temperature may result from changes in enzyme activity, substrate availability, or photosynthetic electron transport, mediated by light and temperature. However, Lehning et al. (1999) found no diurnal change in enzyme activity. Altering current leaf-level models without considering these regulatory mechanisms may limit the utility of the models to the species or environmental conditions used to create the model. Further investigation of the factors underlying diurnal changes in isoprene BER may also lend insight into the functional basis of isoprene emission.

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