2005

Variation in Isoprene Emission from Quercus rubra: Sources, Causes, and Consequences for Estimating Fluxes

Jennifer L. Funk
Chapman University, jlfunk@chapman.edu

Clive G. Jones
Institute of Ecosystem Studies

Dennis W. Gray
State University of New York

Heather L. Throop
State University of New York

Laura A. Hyatt
State University of New York

See next page for additional authors

Recommended Citation

Funk, Jennifer L., et al. "Variation in isoprene emission from Quercus rubra: sources, causes, and consequences for estimating fluxes." *Journal of Geophysical Research: Atmospheres* (1984–2012) 110.D4 (2005). DOI: 10.1029/2004JD005229

Variation in isoprene emission from *Quercus rubra*: Sources, causes, and consequences for estimating fluxes

Jennifer L. Funk[1]
Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, New York, USA

Institute of Ecosystem Studies, Millbrook, New York, USA

Clive G. Jones
Institute of Ecosystem Studies, Millbrook, New York, USA

Dennis W. Gray,[2] Heather L. Throop,[3] Laura A. Hyatt,[4] and Manuel T. Lerdau
Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, New York, USA

Received 10 July 2004; revised 15 October 2004; accepted 8 November 2004; published 17 February 2005.

[1] Isoprene is the dominant volatile organic compound produced in many forest systems. Uncertainty in estimates of leaf level isoprene emission rate stems from an insufficient understanding of the patterns and processes controlling isoprene emission capacity in plant leaves. Previous studies suggest that variation in isoprene emission capacity is substantial; however, it is not known at what scale emission capacity is the most variable. Identifying the sources of variation in emission capacity has implications for conducting measurements and for model development, which will ultimately improve emission estimates and models of tropospheric chemistry. In addition, understanding the sources of variation will help to develop a comprehensive understanding of the physiological controls over isoprene emission. This study applied a variance partitioning approach to identify the major sources of variation in isoprene emission capacity from two populations of northern red oak (*Quercus rubra*) over three growing seasons. Specifically, we evaluated variation due to climate, populations, trees, branches, leaves, seasons, and years. Overall, the dominant source of variation was the effect of a moderate drought event. In the years without drought events, variation among individual trees (intraspecific) explained approximately 60% of the total variance. Within the midseason, isoprene emission capacity of sun leaves varied by a factor of 2 among trees. During the third year a moderate 20-day drought event caused isoprene emission capacity to decrease fourfold, and the relative importance of intraspecific variation was reduced to 24% of total variance. Overall, ambient temperature, light, and a drought index were poor predictors of isoprene emission capacity over a 0 to 14-day period across growing seasons. The drought event captured in this study emphasizes the need to incorporate environmental influences into leaf level emission models.

Citation: Funk, J. L., C. G. Jones, D. W. Gray, H. L. Throop, L. A. Hyatt, and M. T. Lerdau (2005), Variation in isoprene emission from *Quercus rubra*: Sources, causes, and consequences for estimating fluxes, *J. Geophys. Res.*, *110*, D04301, doi:10.1029/2004JD005229.

1. Introduction

[2] Isoprene is one of many volatile organic compounds (VOCs) produced by plants that substantially influence atmospheric chemistry. The rapid oxidation of isoprene in the atmosphere contributes to the formation of carbon monoxide, ozone, organic nitrates, organic acids, and secondary aerosols [*Fuentes et al.*, 2000; *Atkinson and Arey*, 2003; *Claeys et al.*, 2004]. The production or depletion of ozone during oxidation depends on the relative concentration of VOCs and nitric oxides [e.g., *Roselle*, 1994]. Globally, the estimated annual production of isoprene from biogenic sources is 350 to 500 Tg C [*Guenther et al.*, 1995; *Poisson et al.*, 2000; *Levis et al.*, 2003]. Uncertainty in these

[1]Now at Department of Biological Sciences, Stanford University, Stanford, California, USA.
[2]Now at Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA.
[3]Now at School of Renewable Natural Resources, University of Arizona, Tucson, Arizona, USA.
[4]Now at Department of Biology, Rider University, Lawrenceville, New Jersey, USA.

0148-0227/05/2004JD005229

projections stems in part from an insufficient understanding of the patterns and processes controlling the isoprene emission capacity in plant leaves.

[3] Empirical leaf level isoprene emission models are often used in conjunction with canopy radiation models and land cover data to generate flux estimates over large spatial and temporal scales [*Lamb et al.*, 1993; *Baldocchi et al.*, 1999; *Guenther et al.*, 2000]. The most widely used leaf level model [*Guenther et al.*, 1993] adjusts a static, species-specific standardized emission rate (isoprene SER, isoprene emission at a standard light and temperature) to ambient light and temperature conditions using correction factors that are based on the short-term response of isoprene emission to light and temperature. While the short-term response of isoprene emission to light and temperature is relatively well understood, we have a limited understanding of the variance associated with isoprene SER and how this translates into overall model variance. Examining the major sources of variation in isoprene SER can improve isoprene SER estimates by identifying the most important level for measurement replication (e.g., intraspecific, intraplant, population) and important processes to incorporate into models (e.g., seasonal temperature patterns, drought).

[4] Substantial intraspecific variation in isoprene SER has been well documented [*Kempf et al.*, 1996; *Owen et al.*, 1998; *Funk et al.*, 1999; *Isebrands et al.*, 1999; *Geron et al.*, 2001], and measured emission rates from individuals of the same species can vary by more than threefold. Intraplant variability in isoprene SER can also be substantial, with the most extreme differences occurring between sun and shade leaves [*Guenther et al.*, 1991; *Harley et al.*, 1997; *Lerdau and Throop*, 2000; *Kesselmeier and Staudt*, 1999]. Studies have found up to fourfold differences in isoprene SER between sun and shade leaves in both temperate and tropical forests [*Harley et al.*, 1996; *Lerdau and Throop*, 2000]. However, the importance of intraspecific and intraplant variability in isoprene SER relative to variation at other levels of organization (e.g., population, annual) has never been directly examined. Likewise, temporal variation in isoprene SER has been examined in individual plants within a day [*Sharkey et al.*, 1999; *Geron et al.*, 2000; *Funk et al.*, 2003] and within a season [*Monson et al.*, 1994; *Pier and McDuffie*, 1997; *Fuentes and Wang*, 1999; *Geron et al.*, 2000] but never across years. Diurnal, weekly, and seasonal trends in isoprene SER are thought to be controlled by longer-term patterns of light and temperature [*Sharkey et al.*, 1999; *Geron et al.*, 2000; *Hanson and Sharkey*, 2001; *Petron et al.*, 2001], possibly through the influence of light and temperature on substrate availability [e.g., *Rosenstiel et al.*, 2002] and isoprene synthase activity [e.g., *Wolfertz et al.*, 2003].

[5] This study applied a variance partitioning approach to isoprene SER data collected across multiple spatial and temporal scales. This approach allows for more comprehensive analysis than is possible when examining isoprene SER at a single scale (e.g., population, single season). Single-scale studies cannot compare the observed variation to other sources and, thus, are unable to assess the relative importance of sources of variation in isoprene SER. We monitored isoprene emission rates from two local populations of northern red oak (*Quercus rubra*) over three growing seasons to evaluate variation in isoprene SER due to populations, trees, branches, leaves, seasons, and years. Descriptive studies of patterns play an essential role in identifying hypotheses for experimental studies and in understanding processes that occur across large spatial and temporal scales [*Underwood et al.*, 2000]. Thus a secondary goal of the project was to correlate observed isoprene SER patterns with potential regulatory factors, such as climatic variables, in order to identify the principal causes of variation in isoprene SER. Last, we explored the consequences of variation in isoprene SER for flux estimation, including field methodology and constraints imposed on current leaf level models.

2. Methods

2.1. Overview

[6] This study was conducted at the Institute of Ecosystem Studies (IES) in Millbrook, New York, United States (41°51′N, 73°45′W, elevation 130 m), where the mean annual temperature is 9.5°C and mean annual rainfall is 1 m. During the summers of 1997 to 1999, we measured isoprene emission rates and photosynthetic rates from naturally occurring red oak trees growing in two locations on the IES property, approximately 6.5 km apart. We defined the individuals at the two locations to be in two different populations based solely on spatial segregation. The first population of oaks, at Fowler Pond (FP), was sampled in 1997 and consisted of mature trees measuring 5 to 12 m in height. FP trees were located on a flat plateau with moderately deep, well-drained rocky soils. The second oak population was located uphill from the IES Greenhouse (GH) and included smaller, mature oaks ranging in height from 0.5 to 3 m. GH trees were located on slopes with deep, excessively drained gravelly soils. Differences in plant size between the GH and FP populations likely result from inferior soils and high levels of deer browsing at the GH site. Isoprene emission rates from GH oaks were measured every summer during the 1997–1999 study period. Because red oak readily hybridizes with black oak (*Q. velutina*), we chose trees that most closely resembled red oak based on leaf morphology. However, at the GH site, it is likely that the selected trees varied with respect to the degree of red-black oak hybridization. All trees at the GH site were caged to prevent herbivory by deer. FP trees were tall enough for shoots to be above the browsing height of deer.

[7] To examine spatial and temporal variability in isoprene SER, we compiled two data sets from multiple oak individuals measured over the 3-year study period (Table 1). Data set 1 was collected in 1997 and examined spatial variability in isoprene SER at the population, tree, branch, and leaf level. Data from both FP and GH populations were included. Leaf and branch level variation were examined by sampling four leaves from two different branches on each of the trees measured. Data set 2 was used to examine both spatial and temporal variability in isoprene SER. As in data set 1, variability in isoprene SER was examined at the tree and branch level. To examine seasonal and annual variability in isoprene SER, we tagged two leaves on each of 10 trees (leaves were located on separate branches) and monitored isoprene SER over two growing seasons. The same 10 trees were used in 1998 and 1999. Because red oaks are deciduous, different leaves were used in each year, although we sampled leaves from the same branch.

Table 1. Summary of Isoprene Standardized Emission Rate (SER) Data Sets Compiled From Two Red Oak Populations at the Institute of Ecosystem Studies in Millbrook, New York

Population	Trees	Branches/Tree	Leaves/Branch	Year	Measures/Season
			Data Set 1: Spatial Variability		
Fowler Pond (FP)	30	2	2	1997	1
Greenhouse (GH)	10	2	2	1997	1
			Data Set 2: Spatial and Temporal Variability		
Greenhouse (GH)	10	2	1	1998	9
Greenhouse (GH)	10	2	1	1999	8 or 9

[8] Gas exchange measurements were made nine times throughout the summer of 1998 and eight (n = 3 trees) or nine (n = 7 trees) times during the summer of 1999. Early season (spring) increases and late season (autumn) decreases in isoprene SER resulting from changes in leaf ontogeny have been well studied [*Monson et al.*, 1994; *Pier and McDuffie*, 1997; *Fuentes and Wang*, 1999; *Geron et al.*, 2000]. Mid season fluctuations in isoprene SER can be large but are less well understood. They are likely driven by changes in ambient light, temperature, and water availability rather than ontogenetic changes in leaves. We sampled individuals multiple times during the mid season to assess how day-to-day fluctuations in isoprene SER compare to other sources of variation in isoprene SER. Trees were measured in the same order each sampling day to reduce the potential for diurnal increases in isoprene SER to affect estimates of other measured sources of variation [e.g., *Funk et al.*, 2003].

[9] Fully expanded sun leaves were chosen for gas exchange measurements. By not measuring shade leaves, we limited our examination of isoprene SER variation to sun leaves, which contribute the most to canopy level emissions [e.g., *Harley et al.*, 1996]. Leaves from FP and GH oaks measured in 1997 were removed immediately following gas exchange measurements and leaf area was measured with a LI-3100 leaf area meter (LI-COR, Lincoln, Nebraska, United States). Leaves were then dried at 60° to 70°C for 48 hours and weighed for specific leaf weight (SLW) (g cm^{-2}). The same leaves were monitored within a single growing season during 1998 and 1999, thus, SLW was not determined for measurements taken during these years. Consequently, gas exchange parameters are expressed on a leaf area and leaf mass basis for 1997 measurements and on a leaf area basis only for all 1998 and 1999 measurements.

2.2. Gas Exchange

[10] Isoprene emission rate and photosynthetic rate were measured with an open system LI-6400 portable infrared gas analyzer (IRGA) with a temperature- and light-controlled cuvette (LI-COR, Lincoln, NE, United States), coupled to a Photovac voyager gas chromatograph (GC) with a photoionization detector (Perkin-Elmer, Norwalk, Connecticut, United States) as described by *Funk et al.* [2003]. The CO_2 concentration of air in the leaf cuvette was maintained between 360 and 400 ppm with a CO_2 cartridge injector system. Humidity was maintained between 40 and 80% by removing excess water vapor from the entering air stream. Leaves were allowed to equilibrate inside the leaf chamber for 15 to 30 min prior to measurement; this time was sufficient for the leaves to be at steady state for at least 10 min prior to the measurement.

[11] Sample air was routed through the cuvette and into a Teflon ballast line at 0.4 L min^{-1}. Air was then drawn from this line into the 1-mL sample loop within the GC at 0.1 L min^{-1} for 10 s. Samples were injected onto a SupelcoWax 10 wax phase capillary column (20 m length, 0.32 mm interior diameter, 1.0 μm film thickness; Sigma-Aldrich, Bellefonte, Pennsylvania, United States), which was maintained at 40°C. We used ultrahigh purity hydrocarbon-free air as a carrier gas. Single-point calibrations were made daily by serial dilution of a 97.9-ppm mix of isoprene in air (Scott-Marrin, Irvine, California, United States).

[12] Light and temperature conditions inside the leaf cuvette were controlled during all isoprene measurements in order to obtain isoprene SER. Cuvette photosynthetic photon flux density (PPFD) was controlled at 1000 μmol m^{-2} s^{-1} with variable intensity red/blue light emitting diodes (peak irradiance at 665 nm and 470 nm). Leaf temperature was held at 30°C with dual thermoelectric devices. 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], as described by *Funk et al.* [2003].

2.3. Meteorological Data

[13] Ambient light, temperature, and precipitation data were obtained from a nearby weather station in an open field at IES (IES Environmental Monitoring Program, see http://www.ecostudies.org/emp_purp.html). The mean hourly air temperature (°C) was collected with a HMP45C temperature probe (Campbell Scientific, Logan, Utah, United States). Instantaneous global PPFD (μmol m^{-2} s^{-1}) and net radiation (W m^{-2}) data were collected with a LI-190SB light meter (Licor, Inc., Lincoln, Nebraska, United States) and REBS Model Q*7.1 (Radiation and Energy Balance Systems Inc., Seattle, Washington, United States), respectively. Precipitation was measured with a Series 5-780 universal recording rain gauge (Belfort Instrument, Baltimore, Maryland, United States). Rain collector openings were located approximately 3 m above a mowed grass surface.

[14] For the 1998 and 1999 growing seasons, we calculated a drought index (D), which is based on the ratio of cumulative precipitation (P, mm) and cumulative potential evaporation (λE, W m^{-2}) as given by *Baldocchi* [1997]:

$$D = \sum \frac{P}{\lambda E}. \quad (1)$$

Table 2. Results of Nested ANOVA With Variance Partitioning for Area-Based and Mass-Based Isoprene Emission During the 1997 Experiment (Data Set 1)[a]

Source	df_S	MS_S	F_S	P	Variance, %
		Area-Based Isoprene Emission Rate			
Population	1	85.55	0.22	0.64	0
Tree (Population)	37.8	392.14	4.74	<0.001	59.88
Branch (Tree)	38.4	82.66	2.82	<0.001	20.54
Error (Leaf)	57	29.27			19.58
		Mass-Based Isoprene Emission Rate			
Population	1	1240.31	0.48	0.49	0
Tree (Population)	37.7	2585.41	3.34	<0.001	50.19
Branch (Tree)	38.6	764.33	3.97	<0.001	31.26
Error (Leaf)	57				18.55

[a]Data set 1 examined variation between two populations (Greenhouse field site, N = 30, and Fowler Pond, N = 10) and variation among and within trees. Satterthwaite's approximation (S) was used to calculate degrees of freedom (df) because of an unbalanced design. ANOVA stands for analysis of variance; MS is mean square. P values reflect the level of F test significance.

λE is approximated by the Priestly-Taylor equation:

$$\lambda E = \frac{1.26 * \varepsilon * \Theta_n}{\varepsilon + 1}, \qquad (2)$$

where Θ_n is net radiation (W m^{-2}) and ε is the increase of latent heat content per increase of sensible heat content of saturated air at ambient temperature (dimensionless [*Jones*, 1992]).

2.4. Statistical Analysis

[15] Data set 1 was analyzed by nested analysis of variance (ANOVA). Table 2 outlines the nesting structure of the ANOVA (leaf < branch < tree < population). Because of an unbalanced design, Satterthwaite's approximation was used to calculate degrees of freedom and, consequently, variance components. Nested ANOVA was conducted with Biomstat 3.30j (Applied Biostatistics, Port Jefferson, New York, United States). Data set 2 was analyzed with 1- and 2-factor repeated measures univariate ANOVA using SAS procedure GLM (SAS Institute 1996) as given by *Lively et al.* [1993] and *Funk et al.* [2003]. Because the data met the sphericity assumptions required of the repeated measures ANOVA, univariate results were used. In these analyses, TREE is treated as a random variable, while SEASON and YEAR were treated as fixed variables. Variance components from all analyses were calculated using the approach of *Winer* [1971].

[16] A forward/backward stepwise selection approach (RSQUARE method, SAS procedure REG) was used to find the best combination of light, temperature, and drought variables to explain isoprene emission patterns, as given by *Funk et al.* [2003]. Isoprene SER was averaged from all 10 oaks in a given day and then tested for correlations with various environmental variables (n = 19 time points over 1998 and 1999). Mean daily temperature, total PPFD, and drought index were integrated prior to each measurement over the previous day, previous two days, and so on up to previous 14 days. All linear regressions and Pearson product-moment correlations were performed in Statistica (Statsoft, Tulsa, Oklahoma, United States).

3. Results

3.1. Spatial Variation

[17] Spatial variation in isoprene SER was examined in both data sets. Isoprene SER did not differ between the two oak populations despite dissimilarities in tree size (Table 2). Overall, the dominant source of variation was among trees (intraspecific), which explained approximately 60% of the total variance in 1997 and 1998 (Tables 2 and 3). This corresponded to a twofold difference in isoprene SER among trees on any given day. Intraspecific variation explained a smaller portion of the total variance in 1999 as emission rates responded to drought (Table 3), as reported below. Both area- and mass-based isoprene emission rates were calculated in 1997 and showed similar patterns with respect to variance partitioning, indicating limited SLW influence. Mean seasonal emission rates were 43.6 (σ = 12.0), 37.7 (σ = 8.8), and 35.6 (σ = 11.9) nmol m^{-2} s^{-1} in 1997, 1998, and 1999, respectively.

3.2. Seasonal Variation

[18] Repeated sampling of 10 oak trees across the 1998 and 1999 growing seasons allowed us to examine temporal patterns of isoprene SER. Emission patterns were markedly different between the two years. Collectively, mean isoprene SER from the 10 oaks showed minor variation (~30%) over the 1998 growing season (Table 3 and Figure 1a). Intraspecific variation was the dominant source of variation (57% of total variance, TREE $P < 0.01$), although there was also seasonal variation in isoprene SER within individual trees (17% of total variance, SEASON by TREE interaction $P < 0.01$). In contrast, seasonal variation in isoprene SER was high during the 1999 growing season (56% of total variance, SEASON $P < 0.01$) as emission rates responded to drought (Table 3 and Figure 1b). While total precipitation over the growing season (May to September) was comparable between years (~500 mm), no precipitation occurred for a 20-day period in 1999 (Julian days 190 to 210, Figure 2). Isoprene SER was markedly depressed only during the last week of the drought (2 August 1999 measurement, Julian day 214). At this time point, the absolute variance of isoprene SER among individuals was lower (σ^2 = 8.3) than that observed on all other measurement days (mean σ^2 = 72.6) as emission rates decreased to similar, very low values across trees (Figure 1a). Emission rates recovered the following week after two rain events. Trees with higher than average isoprene SER did not show a stronger response to drought than those with lower isoprene SER (r = 0.32, P = 0.36).

[19] To determine whether climatic variables regulated isoprene emission rates over multiple days, we used step-

Table 3. Results of Univariate Repeated Measures ANOVA With Variance Partitioning for Area-Based Isoprene Emission During 1998 and 1999 (Greenhouse Field Site Population, Data Set 2)[a]

Source	df	MS	*P*	Variance, %
		1998		
Between subjects				
Tree	9	1027.99	<0.01	57.35
Error (Leaf)	10	52.47		6.17
Within subjects				
Season	8	225.11	<0.01	11.52
Season-Tree interaction	72	39.78	<0.01	17.14
Error (Season)	80	7.38		7.81
		1999		
Between subjects				
Tree	8	736.98	0.001	23.83
Error (Leaf)	9	78.09		5.65
Within subjects				
Season	7	1768.01	<0.01	56.39
Season-Tree interaction	56	35.11	<0.01	6.19
Error (Season)	63	13.74		7.95

[a]Two leaves from 10 (1998) and 9 (1999) red oaks were measured 9 (1998) or 8 (1999) times over the growing season.

wise regression to test for correlations among isoprene SER and mean daily temperature, light, and drought index, which we integrated over the previous day, previous two days, and so on up to previous 14 days. Overall, all three climatic variables were poor predictors of isoprene SER over all time periods examined across 1998 and 1999 growing seasons (all comparisons $P > 0.05$, stepwise regression). Photosynthetic rate was positively correlated with same-day drought index ($r = 0.62$, $P < 0.01$) and stomatal conductance ($r = 0.87$, $P < 0.0001$), which can be used as an indicator of drought stress [e.g., *Funk et al.*, 2004]. Isoprene SER was only weakly correlated with stomatal conductance ($r = 0.42$, $P = 0.08$).

[20] Photosynthetic rates generally followed seasonal patterns of isoprene SER (Figure 1). In the absence of drought, photosynthetic rate ranged threefold among trees and was thus more variable than isoprene SER (twofold). Photosynthesis also responded to the 1999 drought event; however, the decline started 2 to 3 weeks earlier than the decline in isoprene SER (Figure 1b). Across years, photo-

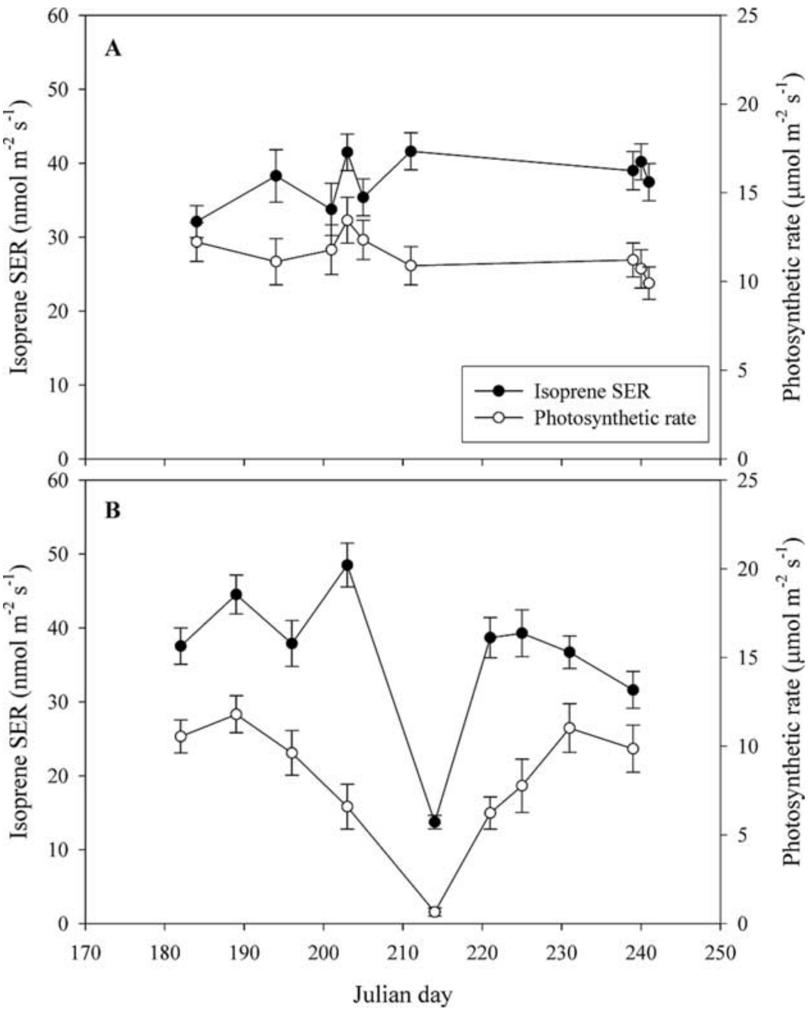

Figure 1. Seasonal patterns of standardized emission rate (SER) of isoprene (solid circles) and photosynthetic rate (open circles) for 10 red oaks in the Institute of Ecosystem Studies (IES) Greenhouse field site population during (a) 1998 and (b) 1999. Data points are means ±1 SE (n = 10 oaks per time point). Julian day is shown for both 1998 and 1999 growing seasons.

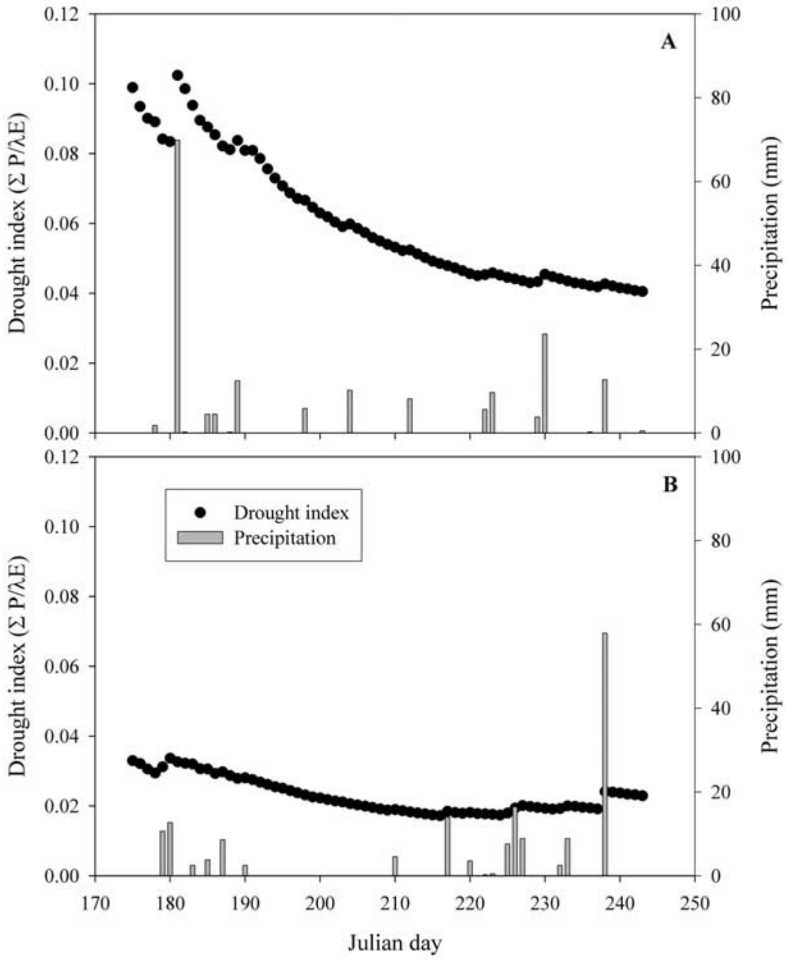

Figure 2. Seasonal patterns of daily precipitation (bars) and drought index (circles) during (a) 1998 and (b) 1999. Precipitation data were collected in an open field at IES. Julian day is shown for both 1998 and 1999 growing seasons. A lower drought index corresponds to more severe drought.

synthesis was positively correlated with isoprene SER ($r = 0.25$, $P = 0.02$); however, there was no correlation between the two variables within a single growing season. This difference is likely explained by the greater variance in both photosynthesis and isoprene SER across years than within years.

3.3. Annual Variation

[21] Mean annual isoprene emission rates for the 10 GH oaks were similar between 1998 and 1999 (Table 4, YEAR $P = 0.49$). Comparing across years, the two main sources of variation were SEASON and the interaction between SEASON and YEAR (both 21% of total variance, $P < 0.001$, Table 4). The significant interaction term was a consequence of drought in 1999 and more favorable soil moisture conditions in 1998 (Figure 2). When the 2 August 1999 measurement was excluded from the analysis, the two major sources of variation were intraspecific variation (19% of total variance, TREE $P < 0.001$), which was the primary source of variation in 1997 (Table 2) and 1998 (Table 3), and the interaction between YEAR and TREE (21% of total variance, $P < 0.01$, Table 4). The YEAR by TREE interaction is illustrated in Figure 3. While some trees maintained a relatively constant isoprene SER across years, others altered emission rates markedly.

4. Discussion

[22] Using a variance partitioning approach, this study is the first to identify major sources of variation in isoprene SER across broad spatial and temporal scales. Of the sources examined, drought on a timescale of weeks made the single largest contribution to the variance in isoprene SER, with differences in emission rate among trees making the second largest contribution. When these two sources of variation are considered in explicit statistical terms, isoprene SER is not correlated with longer-term patterns of light or temperature, a finding that has important implications for canopy-scale modeling efforts (see below). In addition, this study highlights the importance of sampling across broad temporal scales. For example, if this study had been conducted in 1997 or 1998 only, we would have concluded that intraspecific variation generally represents the largest source of variation in isoprene SER. Repeated sampling of 10 oaks in 1998 (a normal precipitation year) and 1999 (a moderate drought year) suggests that mild fluctuations in precipitation

Table 4. Results of Two-Factor, Univariate Repeated Measures ANOVA With Variance Partitioning for 1998 and 1999 Isoprene Emission Measures With and Without the Julian Day 214 Drought Point (Greenhouse Field Site Population, Data Set 2)[a]

Source	df	MS	*P*	Variance, %
All Data				
Between subjects				
Tree	7	911.68	<0.001	13.83
Error (Leaf)	8	69.94		2.3
Within subjects				
Year	1	40.96	0.49	0
Year-Tree interaction	7	549.59	<0.01	15.51
Error (Year)	8	77.57		5.1
Season	8	1129.53	<0.001	20.68
Season-Tree interaction	56	56.6	<0.001	6.71
Error (Season)	64	11.24		3.33
Year-Season interaction	8	573.42	<0.001	20.79
Year-Season-Tree interaction	56	28.48	0.01	2.11
Error (Year-Season interaction)	64	11.21		6.63
Drought Point Removed				
Between subjects				
Tree	7	881.39	<0.001	19.3
Error (Leaf)	8	65.71		3.1
Within subjects				
Year	1	671.55	0.02	4.4
Year-Tree interaction	7	515.91	<0.01	21.0
Error (Year)	8	73.00		6.9
Season	7	419.18	<0.001	9.7
Season-Tree interaction	49	59.56	<0.001	9.1
Error (Season)	56	11.77	<0.001	4.5
Year-Season interaction	7	147.18	<0.001	6.4
Year-Season-Tree interaction	49	29.64	<0.001	7.0
Error (Year-Season interaction)	56	11.25		8.5

[a]Two leaves from eight red oaks were measured 9 times during the growing season in 1998 and 1999.

can have large impacts on isoprene SER, thereby reducing the relative importance of intraspecific variation. Notably, if the 2 August 1999 isoprene SER point is removed from the analysis, intraspecific variation becomes the dominant source of variation in the 1999 data set.

4.1. Effects of Drought on Isoprene Emission Patterns

[23] The prominent environmental factor regulating isoprene SER was drought, which reduced emission by a factor of four during the last week of a month-long drought period in 1999. This result agrees with those from greenhouse studies, which have found isoprene SER to generally decrease with water stress [*Tingey et al.*, 1981; *Sharkey and Loreto*, 1993; *Fang et al.*, 1996; *Lerdau et al.*, 1997; *Bruggemann and Schnitzler*, 2002]. In this study, the drop in isoprene SER lagged 2 weeks behind the photosynthesis response, suggesting that photosynthesis is more sensitive to drought than is isoprene production. Other studies that have monitored isoprene SER and photosynthetic responses to water stress over time found isoprene SER to lag behind photosynthesis by 0 to 14 days [*Tingey et al.*, 1981; *Sharkey and Loreto*, 1993; *Fang et al.*, 1996; *Bruggemann and Schnitzler*, 2002]. The variation in lag time likely reflects different stress regimes, soil volume, and physiological differences among species. Isoprene emission rates are independent of stomatal conductance [*Fall and Monson*, 1992] and lagged decreases in response to drought presumably result from decreased leaf carbon availability following prolonged reductions in photosynthesis. *Bruggemann and Schnitzler* [2002] attributed decreased isoprene SER during drought to substrate limitation rather than the down regulation of isoprene synthase, an enzyme used during isoprene production, which did not differ between control and water stressed plants.

[24] The lack of a correlation between isoprene SER and the drought index was surprising given the strong influence of drought on isoprene SER (Figure 1). This result may stem from the cumulative nature of the drought index, which was not sensitive to precipitation events late in the season and did not increase sharply following the 4 August 1999 rain event. The tempered response of the drought index to precipitation events corresponded to patterns of photosynthesis, which displayed gradual decreases and increases leading up to and following the 4 August 1999 rain event. The lack of a relationship between isoprene SER and photosynthesis or the drought index suggests that modeling the response of isoprene to drought requires a different approach, perhaps involving a more comprehensive function describing substrate availability [*Bruggemann and Schnitzler*, 2002].

4.2. Intraspecific Variation

[25] The twofold difference in isoprene SER observed among trees is similar to ranges found in other species [e.g., *Kempf et al.*, 1996; *Funk et al.*, 1999; *Isebrands et al.*, 1999; *Geron et al.*, 2001]. However, many of these studies sampled multiple leaves from a few plants and cannot differentiate between intraplant and interplant variation. In this study, intraspecific variation was large relative to the variation between populations and years (no differences among means) and variation across the 1998 growing season. Seasonal variation in isoprene SER during 1998 (normal precipitation year) was comparable (~30% variation across the season among trees) to patterns observed for other species during the mid growing season [*Monson et al.*, 1994; *Pier and McDuffie*, 1997; *Fuentes and Wang*, 1999; *Geron et al.*, 2000]. The current study is the first to compare the relative importance of mid season variation to other components of variation in isoprene SER (e.g., intraspecific,

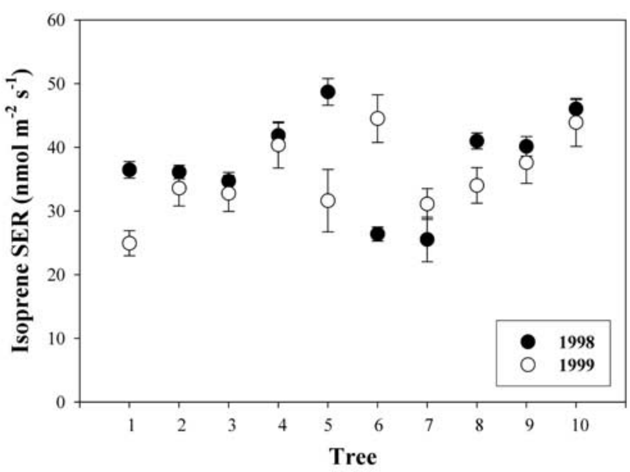

Figure 3. Annual variation in the standardized emission rate (SER) of isoprene for 10 red oaks in the Greenhouse population measured multiple times over the 1998 (n = 9, solid circles) and 1999 (n = 8, open circles) growing seasons. Data points are mean emission rates in a growing season ±1 SE.

annual) and suggests that, in a normal precipitation year, mid season variation in isoprene SER is small relative to intraspecific variation. It is important to note that SEASON may have emerged as the dominant source of variation in both 1998 and 1999 had we included early and late season measurements. Early season increases and late season decreases in isoprene SER resulting from changes in leaf ontogeny have been well characterized [*Monson et al.*, 1994; *Pier and McDuffie*, 1997; *Fuentes and Wang*, 1999; *Geron et al.*, 2000]. Our understanding of mid season fluctuations in isoprene SER is more tenuous and it was our goal to examine how mid season patterns correspond to changes in ambient light, temperature, and water availability.

[26] The twofold intraspecific variation found in this study may be attributable to genetic differences among trees, particularly if trees vary in the degree of red-black oak hybridization. However, studies using clonal *Populus* sp. found significant variation in isoprene SER within clones [*Isebrands et al.*, 1999; *Funk et al.*, 2003], suggesting that small differences in microclimate, local soil conditions, or plant history may translate into significant variation in isoprene SER. Herbivory and mechanical damage to plants may also affect isoprene SER [*Funk et al.*, 1999]. However, mammalian herbivory is unlikely to explain the observed differences because all plants at the GH site were caged and FP plants were above the reach of deer. No major insect damage was observed on the measured leaves or trees. While some trees were consistent in isoprene SER across years, others were variable (Figure 3). This result suggests that differences in isoprene SER among trees were not solely explained by static factors, such as genotype or local soil conditions.

4.3. Effects of Light and Temperature Environment

[27] Our examination of isoprene SER from the same 10 oaks over two growing seasons yielded no relationship between isoprene SER and light or temperature. While this result concurs with that found in an earlier field study on red oak and eastern cottonwood (*Populus deltoides*, *Funk et al.*, 2003], other field studies found that isoprene SER was correlated with light and temperature averaged over the previous 6 to 48 hours [*Sharkey et al.*, 1999; *Geron et al.*, 2000]. Similarly, growth chamber studies found that isoprene SER responds to light and temperature on both short (minutes to hours) and long (4 to 15 days) timescales [*Hanson and Sharkey*, 2001; *Petron et al.*, 2001]. It is probable that the twofold intraspecific variation in isoprene SER observed in our study masked any relationship between isoprene SER and climatic variables. Previous field studies that reported positive relationships between isoprene SER and climatic variables monitored emission rates from one or two trees, which likely obscured the potential contribution of intraspecific differences to variation in isoprene SER [*Sharkey et al.*, 1999; *Geron et al.*, 2000]. Similarly, growth chamber studies that constrain other sources of variation (e.g., herbivory, fluctuations in nutrient and water variability) are more likely to detect significant changes in isoprene emission rate in response to manipulated light and temperature regimes. Our failure to detect significant correlations among isoprene SER, light, and temperature suggests that a twofold intraspecific variation is important relative to long-term effects of light and temperature and illustrates the need to quantify plant to plant variability in order to constrain model predictions.

4.4. Consequences for Flux Estimation

[28] Of the sources examined, drought had the largest impact on variability in isoprene SER, with intraspecific differences in emission rate dominating during nondrought periods. These results suggest that estimates of isoprene flux at landscape scales must consider weather patterns during the period of interest and that measurement replication should be focused at the level of individual trees. Intraspecific and intratree variation in emission rate may influence the reproducibility of canopy flux measurements as the flux footprint (the area of forest that is measured) varies with changes in meteorological conditions and wind direction over time.

[29] However, two other sources of variation, shade leaves and interspecific variation, were excluded from the study. By focusing on sun leaves, we eliminated a large source of intraplant variation. Because isoprene SER of sun and shade leaves can vary by a factor of four [*Harley et al.*, 1996; *Lerdau and Throop*, 2000], our twofold estimate of sun leaf variation likely underestimates actual intraplant variation in isoprene SER. Isoprene emission rates from sun and shade leaves are typically treated separately in canopy emission models, so quantifying the variation in isoprene SER exclusively from shade leaves may also be useful. Isoprene SER varies by over an order of magnitude among species [e.g., *Kempf et al.*, 1996; *Isebrands et al.*, 1999; *Geron et al.*, 2001; *Serca et al.*, 2001; *Harley et al.*, 2003], which makes interspecific variation the dominant overall source of variation. If interspecific variation in isoprene SER within a stand or region is high because of a diversity of nonemitting, low-emitting, and high-emitting plant species, intraspecific variation may exert a relatively minor influence on canopy flux model output [*Geron et al.*, 1997; *Harley et al.*, 2004]. For example, the error associated with species-specific biomass partitioning may be greater than the error associated with species-specific isoprene SER estimates.

[30] Our results also demonstrate that incorporating environmental influences into leaf level emission models is important, but including lagged effects of temperature and light may not be necessary. The reduced intraspecific variance observed on 2 August 1999 suggests that uncertainty in species-specific isoprene SER estimates may be less important when modeling isoprene emission during intermediate-scale extreme climate events. Moderate drought events are common in temperate ecosystems (e.g., IES Environmental Monitoring Program, http://www.ecostudies.org/emp_purp.html), and it is likely that the fourfold decrease of isoprene SER in response to moderate drought observed in this study is common across ecosystems, but this response may not be true for all VOCs (e.g., methylbutenol [*Gray et al.*, 2003]). The mechanistic underpinning of the response of isoprene SER to water stress will likely depend on many factors, including plant productivity, soil moisture, and temperature. For estimates that can be used in atmospheric models, however, it will be sufficient to incorporate an empirical "drought response term" that modifies isoprene SER during multiweek-scale drought events. The fact that such events typically occur during the hottest times of the summer, when ozone events are most severe, highlights the importance of developing

a drought response term that can be incorporated into landscape-scale emissions models.

[31] **Acknowledgments.** The authors thank T. Schubert, D. O'Dell, C. Baker, H. Fuller, D. Bulkeley, S. Murphy, L. Surbeck, P. Townsend, H. Malcolm, and R. Winchcombe for technical assistance and V. Kelly for providing meteorological data. P. Harley, D. Padilla, J. Gurevitch, and B. Lamb provided valuable comments on the manuscript. This work was supported by NSF (DEB-9652658) and NASA (NAG 5-6223) Interagency Terrestrial Ecology and Global Change Initiatives: NSF/DOE/NASA/USDA Joint Program on Terrestrial Ecology, NSF, EPA and SUNY predoctoral fellowships to J.L.F., NSF-REU Supplement (to DEB-9652658) and NSF-REU Site grants (BIR-9322283, DBI-9605098, DBI-9988029) and the Institute of Ecosystem Studies. This work is a contribution to the program of the Institute of Ecosystem Studies and the Department of Ecology and Evolution at Stony Brook University.

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J. L. Funk, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020, USA. (funk@stanford.edu)

D. W. Gray, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269, USA.

L. A. Hyatt, Department of Biology, Rider University, 2083 Lawrenceville Road, Lawrenceville, NJ 08648, USA.

C. G. Jones, Institute of Ecosystem Studies, Box AB, Millbrook, NY 12545, USA.

M. T. Lerdau, Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY 11794-5245, USA.

H. L. Throop, School of Renewable Natural Resources, University of Arizona, Tucson, AZ 85721-0043, USA.