Kinetics of leaf temperature fluctuation affect isoprene emission from red oak (Quercus rubra) leaves

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Summary  Because the rate of isoprene (2-methyl-1,3-buta diene) emission from plants is highly temperature-dependent, we investigated natural fluctuations in leaf temperature and effects of rapid temperature change on isoprene emission of red oak (Quercus rubra L.) leaves at the top of the canopy at Harvard Forest. Throughout the day, leaves often reached temperatures as much as 15 °C above air temperature. The highest temperatures were reached for only a few seconds at a time. We compared isoprene emission rates measured when leaf temperature was changed rapidly with those measured when temperature was changed slowly. In all cases, isoprene emission rate increased with increasing leaf temperature up to about 32 °C and then decreased with higher temperatures. The temperature at which isoprene emission rates began to decrease depended on how quickly measurements were made. Isoprene emission rates peaked at 32.5 °C when measured hourly, whereas rates peaked at 39 °C when measurements were made every four minutes. This behavior reflected the rapid increase in isoprene emission rate that occurred immediately after an increase in leaf temperature, and the subsequent decrease in isoprene emission rate when leaf temperature was held steady for longer than 20 minutes. We concluded that the observed temperature response of isoprene emission rate is a function of measurement protocol. Omitting this parameter from isoprene emission models will not affect simulated isoprene emission rates at mild temperatures, but can increase isoprene emission rates at high temperatures.

Keywords: air pollution, biogenic emissions, forest trees, modeling, temperature response.

Introduction

Isoprene (2-methyl-1,3-butadiene) production by plants increases the thermo-tolerance of photosynthesis (Sharkey and Singsaas 1995, Singsaas et al. 1997). The increase is proportional to the concentration of isoprene inside the leaf air spaces, which is controlled by the rate of synthesis in the chloroplasts and the resistance to its efflux through the stomata. Isoprene synthesis increases in response to increasing light and leaf temperature (Sanadze and Kalandadze 1966, Loreto and Sharkey 1990, Monson et al. 1991, Litvak et al. 1996). Because isoprene is volatile, isoprene concentration inside the leaf, and hence thermo-tolerance, changes rapidly with changes in light and leaf temperature. For this reason, isoprene-induced thermo-tolerance is most important for leaves that experience rapid temperature fluctuations (Singsaas et al. 1997) as a result of sunflecks (Pearcy et al. 1996). These conditions often occur in forest canopies, which explains why a high proportion of isoprene-emitting species are found in tropical and subtropical forests (Guenther et al. 1995, Lerdau and Keller 1997).

If isoprene is to be effective in changing thermo-tolerance of leaves, isoprene synthesis rates should be greatest when leaf temperatures are highest. This is not always the case. Isoprene emission rate increases with temperature at low temperatures before reaching a maximum at some temperature T_M. Thereafter isoprene emission rate decreases with further increases in leaf temperatures. There is considerable variability in published values of T_M. In Eucalypts, T_M occurs between 35 and 40 °C, but is highly variable (Guenther et al. 1993). Several studies have reported T_M values as high as 44 °C for sun-exposed leaves of Populus, but estimates vary by as much as 10 °C (Sanadze and Kalandadze 1966, Monson et al. 1992). If T_M occurs at 44 °C or higher, then canopy isoprene emission rate will be highest during the hottest weather, supporting the theory that isoprene functions by increasing the thermo-tolerance of leaves. A low T_M (i.e., < 35 °C) indicates that canopy isoprene emission rate will be less than maximal during the warmest weather when thermo-tolerance is most needed. Determining the T_M of leaves in a forest canopy would provide an indication of the general applicability of the thermo-tolerance theory.
A correct assessment of isoprene synthesis behavior at high temperatures is also necessary for accurately modeling isoprene emission rates from forest canopies. These models are necessary to predict the influence of phytogenic emissions on atmospheric chemistry, particularly on the formation of tropospheric ozone (Traina et al. 1987, Chameides et al. 1988, Feshenfeld et al. 1992, Paulson et al. 1992). Isoprene emission rate from forest canopies is typically simulated using the equations developed by Guenther et al. (1991). This approach is based on a basal emission factor, defined as the rate of isoprene emission at 30 °C and 1000 μmol photons m⁻² s⁻¹, scaled with equations that describe the response of isoprene emission to leaf temperature and incident light. The basal emission factor and the empirical parameters of the light and temperature response equations are determined experimentally by gas-exchange measurements (Guenther et al. 1991, Guenther et al. 1993, Monson et al. 1994, Harley et al. 1996, Sharkey et al. 1996).

Because of the variability in published values of $T_M$, we assessed the effects of measurement technique on the observed temperature response of isoprene emission rate. We measured isoprene emission rate from leaves in the upper layers of red oak (Quercus rubra L.) trees during July 1995. In addition, micrometeorological measurements of leaf temperature, air temperature, and incident PPFD were made. Additional experiments were carried out in the laboratory to verify and more closely investigate the results from the field experiments.

**Materials and methods**

**Site description**

The study was carried out at Harvard Forest in north-central Massachusetts 42.54° N, 72.18° W, elevation 320 m. Additional details about this site have been provided by Waring et al. (1995). Two 20-m walk-up towers were used to gain access to the top of two red oak (Quercus rubra) trees. Both trees were 20 m tall. Because there was no quantitative difference between the isoprene emission rates of the two trees, results from both trees were pooled.

**Micrometeorology**

Temperature and photosynthetic photon flux density (light, PPFD) at the leaf surface were recorded for groups of four leaves at the top of the red oak tree next to Tower II. The PPFD was measured with galium-arsenide photodiodes (Hamamatsu Corp., Bridgewater, NJ) stuck through the leaf blade. Two photodiodes were used per leaf to measure PPFD above and below the leaf simultaneously. Leaf temperature was measured with chromel-constantan thermocouples welded from 0.07-mm diameter wire (Omega Engineering, Stamford, CT). Three pieces of wire, two chromel and one constantan, were welded to make two chromel-constantan thermocouple junctions. To measure the difference between leaf and air temperature ($ΔT$), wires were threaded through the veins on the underside of the leaf and one junction was pressed against the bottom of the leaf, and the other thermocouple junction was suspended 1 cm below the leaf. Air temperature was measured in the vicinity of the leaves (not more than 0.5 m away) with a chromel-constantan thermocouple. Accuracy of the leaf temperature measurements was verified with an infrared thermometer (Everest Intercence, Tucson, AZ). Wind speed was measured in the vicinity of the leaves with needle anemometers (SoilTronics, Inc., Pullman, WA). All data were logged every 5 s with a data logger (Model CR10, Campbell Scientific, Logan, UT).

The time constant for leaf temperature change was measured on leaves of red oak trees grown in pots in a greenhouse in Madison, Wisconsin. Leaf thermocouples were attached as for the field micrometeorological measurements, and leaves were exposed to light from a 2.5 kW xenon-arc lamp attenuated to 1000 μmol photons m⁻² s⁻¹ with neutral-density screens. The light was flashed on and off every minute by means of a mechanical shutter. Leaf temperature, $ΔT$, was recorded once per second. The leaf time constant was calculated from the slope of a logarithmic plot of $ΔT$ versus time.

**Gas exchange**

Measurements of leaf CO₂ assimilation and isoprene emission rate were made in the field with three different systems. The first system tested was an LI-6400 Photosynthesis System (Li-Cor, Inc., Lincoln, NE). The leaf cuvette enclosed 6 cm² of leaf and was equipped with a thermostat to control leaf temperature. Light was provided by a Li-Cor light-emitting diode (LED), enabling precise control of irradiance. The LED provided light near 670 nm, which is as effective as light from a xenon-arc lamp for isoprene emission (Tennesen et al. 1994). Isoprene concentration in the leaf cuvette was measured by directing the air exiting the cuvette through a 1-m tube (Bev-A-Line IV, Cole-Parmer, Vernon Hills, IL) to a portable gas chromatograph (Scentscreen, Sentex, Inc., Ridgefield, NJ). Chromatography was performed as described by Sharkey et al. (1996). The argon ionization detector on the gas chromatograph had a similar sensitivity to isoprene as the photoionization detectors. Because the signal was not linear with respect to isoprene concentration, we calibrated the instrument against standards prepared daily by mixing liquid isoprene (Fluka Chemical Corp., Ronkonkoma, NY) with air in a Tedlar bag and making serial dilutions in additional bags. Isoprene concentrations of 32 to 512 ppb were used.

The second system comprised a Li-Cor LI-6400 Photosynthesis System coupled to a Photovac 10S70 (Photovac, Inc., Long Island, NY) portable gas chromatograph. The Photovac was equipped with a photoionization detector. Air left the Li-Cor chamber at a flow rate of 200 ml min⁻¹. The gas chromatograph utilized a pump to draw air at 100 ml min⁻¹ through a 1-ml sample loop for 10 s. Sample gas in the loop was injected onto a 0.53 mm id CP-Sil-5 column 10 m in length. The column was held at 40 °C in an isothermal oven. The carrier gas was hydrocarbon-free air. The detector output was linear across a broad range of isoprene concentrations and the calibration line had an intercept at zero. Single point calibrations were made daily by vaporizing and diluting pure liquid isoprene (Sigma/Aldrich, St. Louis, MO) according to a method recommended by Photovac (Technical Bulletin...
No. 21). The saturated head-space air of a vial containing pure isoprene at known temperature (usually 0 °C) was diluted serially in air to obtain a range of gas phase concentrations.

The third system consisted of a Campbell MPH 1000 gas-exchange system (Campbell Scientific, Logan, UT) coupled to a Photovac 10S portable gas chromatograph. Hydrocarbon-free air flowed at 0.8 to 1.2 l min⁻¹ through a nickel-plated leaf cuvette. Temperature was controlled by thermoelectric cooling and light was provided by a 300-W projector bulb mounted at a right angle to the top of the glass-topped cuvette. The light was reflected by a cold mirror (45° cold mirror, OCLI, Santa Rosa, CA) mounted at a 45° angle to the cuvette top. Humidity in the cuvette was controlled by partitioning hydrocarbon-free air between humidified and dry vessels, followed by remixing before the air entered the cuvette. An aliquot of air was taken from the leaf chamber outlet and analyzed with the Photovac gas chromatograph by the procedures described above. This gas exchange system was used to obtain emission rate data following longer-term steady state conditions achieved after slow warming or cooling of the cuvette.

Gas exchange calculations of photosynthetic carbon assimilation, stomatal conductance for water vapor, and partial pressure of CO₂ inside the leaf were made with the equations of von Caemmerer and Farquhar (1981). Isoprene emission rate was calculated as rate of air flow through the chamber times the isoprene concentration of the exiting air (Hills et al. 1992). The Campbell system used hydrocarbon-free air from pressurized cylinders, whereas the Li-Cor systems used ambient air. Isoprene concentration of ambient air is 0 to 3 ppb (Geron et al. 1997). Because the background isoprene concentration was small relative to the isoprene concentration exiting the cuvette, no correction for ambient isoprene concentration was made. The concentration of isoprene inside the leaf was calculated as described by Singsaas et al. (1997).

Protocols

Effect of temperature on isoprene emission rate was assessed by clamping a leaf in a chamber at 20 to 30 °C and illuminating at half or full sunlight (1000 or 2000 μmol m⁻² s⁻¹). The temperature was then increased either slowly (with the Campbell system) or rapidly (with the Li-Cor LI-6400 systems). The rapid increase in temperature was effected by using heat guns to warm the cooling fins on the Li-Cor chambers. This procedure sometimes damaged adjacent leaves and on occasion damaged the leaf being measured. When this occurred, data from the damaged leaf were discarded. The speed with which the temperature response could be determined was dictated by the speed with which the gas chromatographs could operate. To investigate the effect of varying the rate of temperature increase on isoprene emission rate at Harvard Forest, two pairs of curves were obtained with the Li-Cor LI-6400/Photovac system. Another five pairs of curves were determined by using the system described by Tennesen et al. (1994) on white oak (Quercus alba L.) seedlings grown in a greenhouse in Madison, Wisconsin. Isoprene flux rates from each leaf were standardized. Equation 1 is based on the temperature correction used by Guenther et al. (1993) but with no falloff in isoprene emission rate at high temperatures.

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I_T = e^{\frac{E_a}{R} \frac{T_a - T}{T_a - T_M}},
\]

where \(T\) is leaf temperature (K), \(R\) is the molar gas constant, and \(T_M\) is the temperature (30 °C) to which measurements are standardized. Equation 1 is based on the temperature correction of Guenther et al. (1993) but with no falloff in isoprene emission rate at high temperatures.

Results

Leaf temperature dynamics

Leaf temperature was highly variable at the top of the tree. Leaf temperature varied from 20.4 to 39.3 °C during the middle eight daylight hours of July 10, 1995, whereas air temperature varied from 17.2 to 26.3 °C (Figure 1). During the measurement period, leaf temperature averaged 27 °C and air temperature averaged 21 °C. The difference between air and leaf temperatures was greatest during the morning because the leaf

Figure 1. Temperature environment of a leaf growing at the top level of a red oak tree at Harvard Forest collected on July 29, 1995. Leaf (○) and air (——) temperature were logged every 2.5 seconds between 0800 and 1600 h. Temperature was measured with a double junction chromel-constantan thermocouple held against the abaxial leaf surface to measure the difference between leaf and air temperature (ΔT). Air temperature was measured with a chromel-constantan thermocouple within 0.5 m of the leaf.
received more sunlight before noon than after noon (data not shown). The variance of leaf temperature over the whole measurement period was 11.7 °C, whereas air temperature had a variance of 1.9. Temperatures were higher and more variable in full sunlight than in shade. When incident PPFD was ≥ 500 µmol m^{-2} s^{-1}, leaf temperature had a mean of 28.3 °C and a variance of 7.8 °C, and air temperature had a mean of 21.3 °C with a variance of 2.3°C. When ambient PPFD was < 500 mol m^{-2} s^{-1}, leaf temperature had a mean of 23.0 and a variance of 2.7, and air temperature had a mean of 21.9 and a variance of 0.44.

To determine the maximum rate of leaf temperature fluctuations, we measured the time constant for temperature changes in oak leaves. Thermocouples were placed on the underside of leaves to measure the difference between leaf and air temperature (ΔT) as leaves were moved between high and low light. Data were normalized before determining the time constant with equations described by Nobel (1991). Examples of time constants recorded for a white oak leaf are shown in Figure 2. Lines were fit by linear regression to determine the leaf heating and cooling constants. Time constants were calculated as the negative inverse of the slope of the line. The heating constant averaged 14.3 ± 1.2 s and the cooling constant averaged 18.7 ± 1.2 s (mean ± standard error, n = 4).

Temperature response

The effect of temperature on isoprene emission was dependent on the measurement technique. When leaf temperature was raised from 20 to 35 °C slowly, and measurements made every 60 min, isoprene emission rate peaked at 32.5 °C (Figure 3A). In contrast, when leaf temperature was raised from 28 to 45 °C quickly, and measurements made every 4 min, isoprene emission rate peaked at 39 °C (Figure 3B). Because results from both trees were similar, data were pooled. Both sets of measurements were made on different leaves of the same red oak tree with the only difference in treatment being the rate at which leaf temperature increased.

Further experiments confirmed that rate of leaf heating affected the temperature at which isoprene emission rate peaked. In the field, temperature response of isoprene emission rate was measured on two red oak leaves. Leaves were held at each test temperature for 5 or 30 min before isoprene emission rate was measured (Figure 4A). Isoprene emission rate peaked at 36 °C when leaf temperature was raised slowly and peaked at 42 °C when leaf temperature was raised quickly. We also investigated the effect of rate of leaf heating on isoprene emission rate in the laboratory on greenhouse-grown white oak trees (Figure 4B). In one series, leaf temperature was increased from 30 to 45 °C in 3 °C increments every 4 min. For the other series, leaves were left for 40 min at each temperature. Isoprene emission peaked at 45 °C when leaf temperature was increased quickly and at 39 °C when leaf temperature was increased slowly.

To determine why the rate of heating changed the temperature response of isoprene emission, we imposed a continuous heat treatment on leaves while measuring isoprene at 5-min intervals (Figure 5). Leaves were held in a gas-exchange cuvette for 30 min at 30 °C to ensure isoprene emission rate was at steady state. Leaf temperature was then raised to 40 °C and held for 50 min. During the first 10 min at 40 °C, isoprene emission rate increased 2.3-fold over the rate at 30 °C. During the following 10 min, isoprene emission rate fell, eventually
achieving a new steady-state rate that was 1.8-fold higher than
at 30°C. When leaf temperature was lowered again to 30°C,
isoprene emission rate fell to 0.9 times the original rate, but
then recovered to the original rate within 25 min.

Isoprene emission simulations

Isoprene emission was simulated based on light and leaf tem-
perature measurements recorded in the field (Figure 1) as
inputs. To investigate the errors introduced by simulating iso-
prene emission on a 0.5-h timescale, isoprene emission was
simulated using the temperature response equation (Guenther
et al. 1991) for half-hour mean leaf temperature data. Rates
calculated from these simulations were compared with rates from
the same period calculated every 5 s (Figure 6). The error
in the temperature response was proportional to the tempera-
ture variation during the measurement period. Error rates were
as much as 4% when the standard deviation in leaf temperature
was 2.5°C during the 0.5-h simulation.

Because leaf temperature varied rapidly (Figures 1 and 2)
and rapid temperature variation caused $T_{MT}$ to increase (Fig-
ure 3 and 4), we used simulations of isoprene emission to
determine the effect of $T_{MT}$ on isoprene emission. Isoprene
emission rate was simulated with the Guenther et al. (1991)
model and with the Guenther et al. model in which the tem-
perature correction was replaced with Equation 2. The time
step was 5 s in this simulation. When leaf temperatures were
low, averaging 26°C, differences between the two models
were small (Figure 7A). Over the 8-h simulation period, iso-
prene emission rate was 6% higher with the modified model
than with the Guenther et al. model. As temperature increased,
the difference between the two models increased. When mean
leaf temperature was 35°C, isoprene emission rate predicted
by Equation 2 was 28% higher than the rate predicted by the
Guenther et al. (1991) algorithm (Figure 7B). The difference
between the two models increased exponentially with leaf
temperature (Figure 8).
cases. We have also observed leaf–air temperature differences of this magnitude in white oak trees in Chapel Hill, North Carolina where a mean air temperature of 30.2 °C led to leaf temperatures as high as 46 °C (J.Z. Shi, unpublished data). Incident PPFD had a considerable effect on leaf temperature, increasing both mean leaf temperature and leaf temperature variability. One possible explanation for the increase in the variability of leaf temperature is the presence of sunflecks. Roden and Pearcy (1993) found that sunflecks occurred in poplar canopies with a frequency of 3 Hz. Spectral analysis of our data did not show a dominant sunfleck frequency, but our measurement interval was too long to discern sunflecks occurring more frequently than 0.2 Hz. The fast time constants of leaf temperature changes (Figure 2) would allow changes in leaf temperature of several degrees to occur quite rapidly after a change in PPFD caused by sunflecks. Thus sunflecks could account for much of the variability in leaf temperature.

Isoprene emission rate increased with increasing leaf temperature until a critical “falloff” temperature was reached, beyond which isoprene emission rate declined (Figures 3–5). The falloff temperature was highly dependent on the rate of temperature change (Figure 5). Isoprene emission rate increased for the first 10 min after a change in leaf temperature. However, beyond 20 min, isoprene emission rate decreased to a new steady state that was lower than the rate during the first 10 min. Temperature-response measurements made at 4-min intervals reflect isoprene emission at the peak emission rate, whereas measurements made at 60-min intervals only reflect the lower emission rate reached after 20 min. This regulation of isoprene emission is responsible for the apparent variability in falloff temperature seen in Figure 4 and reported in earlier work (Guenther et al. 1991, Guenther et al. 1993, Harley et al. 1996).

The behavior of isoprene emission at high temperatures supports the hypothesis that isoprene protects leaves from rapid temperature fluctuations. Because isoprene emission rate falls off at much higher temperatures when leaf temperature increases rapidly than when leaf temperature increases slowly, isoprene concentration is highest in leaves subjected to rapid temperature increases. Leaf temperature increases and decreases with time constants of less than 20 s (Figures 1 and 2). The field instruments were limited to making a single isoprene measurement every 4 min. Very rapid increases in leaf temperature may result in even higher values of $T_M$ than those observed, provided that the time constant for isoprene emission changes is not much slower than that for leaf temperature changes. Because isoprene emission decreases after 20 min at high temperature, isoprene may be less effective in protecting leaves from temperature increases that last 20 min or longer. However, this may not be the case in all species, because thermo-tolerance is dependent on both rate of isoprene synthesis and stomatal conductance to isoprene. Stomatal conductance declined during a 20-min exposure to high temperature; however, the change in stomatal conductance was not sufficient to maintain a high isoprene concentration during the experimental period (data not shown).

The response of isoprene emission rate to leaf heating rate may significantly impact isoprene emission from forest cano-
plies; however, this behavior is not taken into account in current isoprene emission models. Isoprene emission rate can increase with a time constant of as little as 8.2 s in laboratory experiments (Singsaas and Sharkey 1988). Because the response of isoprene emission rate to temperature is nonlinear, error is introduced into models by averaging leaf temperature over a long period of time (Figure 6), though this error is relatively small. The error is highest when leaf temperature is most variable, which coincides with the highest leaf temperatures. Use of a temperature-response model with a low $T_M$ may introduce further errors that are potentially substantial. Although leaf temperature averaged around 30°C, leaf temperatures near 40°C were not uncommon (Figure 1). Simulating isoprene emission with a low $T_M$ would underestimate isoprene emission during these high temperature episodes. Because $T_M$ was 45°C or higher when leaf temperature was raised quickly, we dropped it from the Guenther et al. (1993) temperature algorithm and simulated isoprene emission with an Arrhenius equation (Equation 1). This resulted in little difference in isoprene emission rate when leaf temperature was low, but errors increased markedly at high temperatures (Figures 7 and 8). This difference may help explain the 50% error in isoprene emission models at high temperatures (Geron et al. 1997).

We conclude that changes in isoprene emission rate when leaves are held at steady-state result in lower $T_M$ when the temperature response of isoprene emission is measured at a time interval of 60 min compared with a time interval of 4 min. For this reason, $T_M$ is highly dependent on measurement technique. Isoprene emission was highest during short high-temperature episodes, supporting the theory that isoprene protects leaves from rapid excursions to high temperature. For modeling purposes, $T_M$ does not affect isoprene emission simulations at low temperature, but removing $T_M$ from temperature correction algorithms will increase estimated isoprene emission rates during warm weather.

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