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Isoprene Increases Thermotolerance of Isoprene-Emitting Species[1]

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Isoprene-emitting plants lose a large portion of their assimilated C as isoprene. Because isoprene synthesis can be regulated, it has been assumed that isoprene benefits the plant. Since the rate of isoprene emission from leaves is highly responsive to temperature, we hypothesized that isoprene benefits plants by increasing their thermotolerance. We used three methods to measure isoprene-induced thermotolerance in leaves. Each technique assayed thermotolerance under conditions that suppressed endogenous isoprene synthesis. When measured by chlorophyll fluorescence, thermotolerance of kudzu (*Pueraria lobata* [Willd.] Ohwi.) leaves increased as much as 4°C in very low light. With higher light, isoprene increased thermotolerance of kudzu leaves by as much as 10°C. When measured as the temperature at which photosynthesis declined to zero, thermotolerance increased with added isoprene by 2.5°C. All three measures of thermotolerance were dose dependent. Both fluorescence techniques also showed isoprene-induced thermotolerancé in white oak (*Quercus alba* L.). Thermotolerance was not observed in bean (*Phaseolus vulgaris* var Linden), a species that does not emit isoprene. None of the experiments was designed to determine the mechanism of thermotolerance, but we theorize that isoprene functions by enhancing hydrophobic interactions in membranes.

Isoprene is the most abundant biogenic nonmethane hydrocarbon entering the atmosphere (Feshenfeld et al., 1992). Although small amounts of isoprene are emitted by bacteria (Kuzma et al., 1995), algae (Bonsang et al., 1992), and animals (Jones et al., 1995), vascular plants are the largest source (Guenther et al., 1995). Strong isoprene emitters such as oak trees (*Quercus* spp.) emit isoprene at 0.1 to 3% of the rate of C assimilation (Tingey et al., 1979; Monson and Fall, 1989; Loreto and Sharkey, 1990). Isoprene reacts with the OH radical in the presence of NO_x to form tropospheric ozone (Trainer et al., 1987; Thompson, 1992), which is toxic to humans and reduces crop yields (Reich and Amundson, 1985; Runeckles and Chevone, 1992). Therefore, isoprene can aggravate air pollution problems in areas where NO_x pollution is high, such as highly populated industrial areas (Chameides et al., 1988). Despite isoprene's importance in atmospheric chemistry and as a C loss from plants, little is known of its function in plants.

Isoprene emission is strongly temperature dependent in higher plants (Sanadze and Kursanov, 1966; Rasmussen and Jones, 1973; Tingey et al., 1979; Monson and Fall, 1989; Loreto and Sharkey, 1990). As leaf temperature increases from 30 to 40°C, isoprene emission increases by up to 8-fold in kudzu (*Pueraria lobata* [Willd.] Ohwi.) (Sharkey and Loreto, 1993). Kudzu plants grown at 19°C did not emit detectable levels of isoprene, but when they were moved to 26°C, they began making isoprene within 1 d (Sharkey and Loreto, 1993).

We have hypothesized that isoprene inside chloroplasts protects them from heat-induced damage (Sharkey and Singsaas, 1995). To test this, we used three methods for assaying the effect of isoprene on thermotolerance. Each method was used under conditions that prevented isoprene synthesis to allow controlled isoprene fumigation of the leaves. The three measures of thermotolerance were: (a) Chlorophyll fluorescence without actinic (causing photochemistry) light (Schreiber and Berry, 1977; Seemann et al., 1984; Havaux, 1993). Leaves do not produce isoprene without actinic light (Loreto and Sharkey, 1990; Monson et al., 1991b). (b) Chlorophyll fluorescence with actinic light. Isoprene synthesis was prevented by holding the leaf in an N_2 atmosphere (Loreto and Sharkey, 1990). (c) Gas-exchange measurements of photosynthetic CO_2 assimilation at temperatures between 30 and 50°C. These were done using leaves that had developed under low temperatures, so they did not synthesize isoprene (Loreto and Sharkey, 1990).

We report isoprene concentrations inside leaves in the field and show that isoprene at these concentrations increases thermotolerance in isoprene-emitting species up to 10°C. Finally, we show that there is a direct correlation between isoprene concentration and thermotolerance with all three methods of evaluation.

MATERIALS AND METHODS

Kudzu (*Pueraria lobata* [Willd.] Ohwi.) plants were grown from stem cuttings in 10-L pots filled with vermiculite/peat moss-based growing medium (Metro-Mix 360, Grace Sierra Co., Milpitas, CA), and bean (*Phaseolus vulgaris* var Linden) plants were grown in 4-L pots. Plants were grown in a reach-in environmental chamber (model E15, Conviron, Winnipeg, Manitoba, Canada). All plants received 700

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Abbreviation: T_0, temperature at which photosynthesis decreases to zero.

μmol photons m^{-2} s^{-1} at canopy height with a 16-h daylength. Plants were watered twice daily with 500 mL of one-half-strength Hoagland solution (Hoagland and Arnon, 1938), and were grown with day/night temperatures of 26/18°C. Kudzu plants grown at low temperature to prevent isoprene synthesis were grown under the same conditions as above, but day/night temperatures were 19/16°C.

White oak (*Quercus alba* L.) trees were obtained as 2-year-old dormant saplings (Musser Forests, Inc., Indiana, PA). They were planted in 8-L pots filled with peatlite. Trees were transferred into an environmental chamber at the University of Wisconsin Biotron (Madison) with a 12-h daylength to break dormancy. Incident light was 60 μmol photons m^{-2} s^{-1} at the top of the trees and day/night temperatures were 22/16°C. After 1 month in low light, the trees were transferred to a temperature-controlled greenhouse in which day/night temperatures were 23/15°C. Trees were fertilized once with 9 g of Osmocote (Grace Sierra) and watered to saturation every other day with deionized water. Daylength was extended to 14 h with 400-W metal halide lamps during February, March, and April 1995. The lamps added 100 μmol photons m^{-2} s^{-1} during daylight hours.

Field gas-exchange measurements were made on a white oak tree growing in the Duke University Forest (Chapel Hill, NC) (35°58′25″ N latitude, 79°06′05″ W longitude). A 40-m walk-up tower allowed access to leaves at the top of the canopy, approximately 30 m above ground level. The forest was a medium-aged, mixed, second-growth forest dominated by *Quercus* spp.

Isoprene Measurement

In the laboratory isoprene was quantified with an analytical gas chromatograph (Shimadzu Corp., Kyoto, Japan). Details of this system are described elsewhere (Loreto and Sharkey, 1993). A 256-nL L^{-1} standard was made daily by diluting 99.5% pure liquid isoprene (Fluka, Buchs, Switzerland) in N_2. Peak area had previously been shown to be linear from 1 nL L^{-1} to 20 μL L^{-1} (P.J. Vanderveer, unpublished data).

Isoprene analysis in the field was done with an automated, portable gas chromatograph (Scentoscreen, Sentex Systems, Inc., Ridgefield, NJ). Details of this system have been published elsewhere (Sharkey et al., 1996). A five-point standard was made for concentrations between 2 and 512 nL L^{-1} each day by diluting liquid isoprene in two stages in 2-L Tedlar bags (Supelco Inc., Bellefonte, PA), each filled with 1 L of air. Ten five-point calibrations had previously been run and compared with calibrations on the analytical gas chromatograph to check for agreement between the two systems (P.J. Vanderveer, unpublished data).

Controlling Isoprene Dose

Experiments were designed to suppress endogenous isoprene synthesis so that isoprene could be added in a controlled manner. Three methods were used to prevent endogenous isoprene synthesis: (a) fluorescence in darkness, in which leaves were assayed with a light level sufficient to cause a fluorescence signal but insufficient to cause isoprene synthesis; (b) fluorescence in N_2, in which leaves were assayed in an N_2 atmosphere; and (c) photosynthesis measurements to determine T_0, in which leaves that had developed at <20°C ambient temperature were assayed.

In all experiments leaves were placed in a cuvette with an airstream flowing over them. Air was sampled from the cuvette before experiments were started to confirm that leaves were not making isoprene. Isoprene was added to the airstream entering the chamber by flowing through an isoprene addition device. In this device air passed through a piece of polyvinyl chloride tubing (Tygon, Norton Performance Plastics, Akron, OH), which was looped through a covered Erlenmeyer flask. Ten microliters of liquid isoprene was added to the Erlenmeyer flask to yield a concentration > 500 μL L^{-1} outside the tubing. Isoprene diffused through the tubing into the airstream at a constant rate. The final concentration of isoprene in the airstream depended on air flow rate.

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured with a pulse-amplitude modulated chlorophyll fluorometer (PAM-101, Walz, Effeltrich, Germany). Modulated excitation energy and the fluorescence signal were carried through a bifurcated fiber-optic cable to the leaf. A single leaf was held in a cuvette and leaf temperature was increased from 30 to 60°C at a rate of 1°C min^{-1} during measurement. Leaf temperature was monitored with a thermocouple (Type T, Omega Engineering, Inc., Stamford, CT) pressed against the bottom of the leaf. Fluorescence and temperature signals were digitized and recorded on a computer.

For fluorescence measurements without actinic light, leaves were held in a 2.5-cm^2 cuvette. The top and bottom windows of the cuvette were covered with aluminum foil to exclude light. The tip of the fiber-optic cable was pushed through the aluminum foil against the cuvette window. Leaf temperature was controlled by circulating water through the cuvette housing from a temperature-controlled water bath. Compressed air was flowed through the cuvette at 2 L min^{-1}. A 10-mL sample of air exiting the chamber was taken every 5 min for isoprene measurement.

For fluorescence measurements with actinic light, leaves were held in an aluminum cuvette with a 10- × 14-cm window. Actinic light of 1000 μmol m^{-2} s^{-1} was provided by a xenon-arc lamp. Cuvette temperature was controlled with Peltier blocks. The tip of the fiber-optic cable was placed against the top window of the chamber at a 45° angle to avoid shading the leaf. A 2-L min^{-1} N_2 airstream was flowed through the cuvette during measurements. For isoprene measurement, 10 mL of air exiting the chamber was sampled.

Gas-Exchange Measurements

Laboratory-based gas-exchange measurements were made with a system described previously (Tennessen et al., 1994). Field-based measurements were made with a field-

portable, open-gas-exchange system (model 6400, Li-Cor, Lincoln, NE). Measurements were made as described previously (Sharkey et al., 1996).

Calculations

Gas-exchange calculations of assimilation rate and stomatal conductance to water vapor (g_s) were made using the equations of von Caemmerer and Farquhar (1981). Isoprene emission rate was calculated using the equation:

$$J_i = I_a \times FR$$

where J_i is isoprene emission rate, I_a is the isoprene concentration exiting the cuvette, and FR is the air flow rate through the cuvette.

Isoprene inside the leaf airspaces was calculated by:

$$I_i = I_a + 2.83 \times \frac{J_i}{g_s}$$

where I_i is isoprene concentration inside the leaf. We estimated the diffusion coefficient of isoprene through air based on the method of Lyman et al. (1990). The factor 2.83 is the ratio of the estimated diffusion coefficient of water vapor through air to that of isoprene through air.

Linear regressions were done by the least-squares method in a data-analysis package (Origin, Micro-Cal, Inc., Northampton, MA). Paired Student's t tests were performed using a statistical package (SYSTAT 5.2 for Windows, SPSS, Inc., Evanston, IL). All data are presented as means ± SE.

RESULTS

Isoprene Concentration Inside Leaves

To establish what levels of isoprene inside leaves occur under natural conditions, field measurements of isoprene emission were made on six occasions during three successive summers on a white oak growing in Duke University forest. During each field trip at least 18 temperature-response curves of isoprene emission were measured. These data are detailed elsewhere (Sharkey et al., 1996).

A typical example of one of these measurements is shown in Figure 1. A leaf was heated from 27 to 45°C in a gas-exchange cuvette. Isoprene emission peaked at 42°C, whereas isoprene inside the leaf continued to increase up to 18 μL L^{-1} at 44°C, at which point measurement was stopped. From these measurements, we found that isoprene concentration in airspaces of fully expanded oak leaves routinely reached 10 to 20 μL L^{-1}. We have also estimated isoprene concentrations of greater than 30 μL L^{-1} in fully expanded kudzu leaves (E.L. Singsaas, unpublished data).

Thermotolerance Measured by Chlorophyll Fluorescence

To study whether high concentrations of isoprene inside of the leaf increase thermotolerance, we measured chlorophyll fluorescence versus temperature curves in darkness

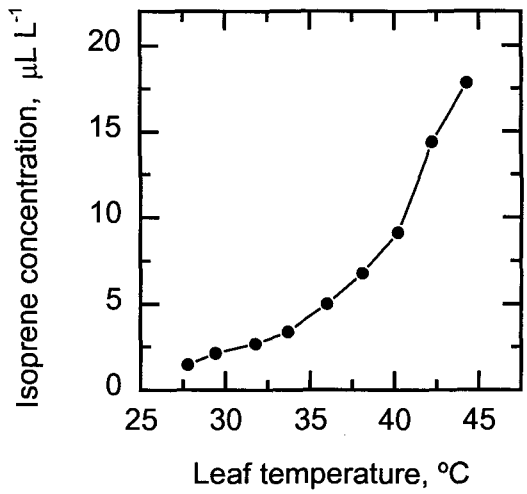

Figure 1. Isoprene concentration inside a white oak leaf at the top of the canopy. This is a typical example of 54 isoprene versus temperature-response curves done at Duke Forest over 3 years. Leaf CO_2 and water vapor exchange, flow rate, and isoprene concentration exiting the leaf chamber were measured and used to calculate isoprene flux. Internal isoprene concentration was calculated as described in "Materials and Methods."

(Fig. 2). When an excised kudzu leaflet was slowly heated at a rate of 1°C min^{-1}, chlorophyll fluorescence remained steady between 30 and 45°C. At 45.1°C, fluorescence increased dramatically (Fig. 2, solid line). Fluorescence increase correlates with irreversible thermal damage to chloroplasts (Schreiber and Berry, 1977; Seemann et al., 1984). When 20 μL L^{-1} isoprene was added to the air passed over the leaf during measurement, the fluorescence increased at 48.2°C (Fig. 2, dashed line). In this experiment isoprene increased thermotolerance of a kudzu leaflet by 3.1°C.

Experiments such as the one presented in Figure 2 were repeated seven times on kudzu leaflets, with isoprene concentrations between 0.008 and 11 μL L^{-1} (Fig. 3). To obtain accurate quantitative measurements of thermotolerance attributable to isoprene, we used paired measurements on adjacent leaflets. Individual measurements showed between a 0.5 and 3.6°C increase in thermotolerance with 0.008 and 11 μL L^{-1} isoprene added, respectively. Thermotolerance in three pairs of leaflets with no isoprene fumigation (control pairs) showed a thermotolerance of 0.08°C, not significantly different from 0. Thermotolerance was dose dependent with the log of isoprene emission (Fig. 3; r^2 = 0.85, P = 0.003, and n = 7).

To look at thermotolerance attributable to isoprene in a saturating light environment, we measured fluorescence versus temperature curves with actinic light. Examples of three runs are shown in Figure 4. Leaves were fumigated with 0, 10, or 13 μL L^{-1} isoprene during treatment. Thermal damage occurred earlier in the control leaf than in either of the other two. The increase in thermotolerance was 10°C above the control leaf when the leaf was fumigated with 13 μL L^{-1} isoprene (Fig. 4). Thirteen curves were measured, and in all cases isoprene increased thermotolerance by 4 to 15°C.

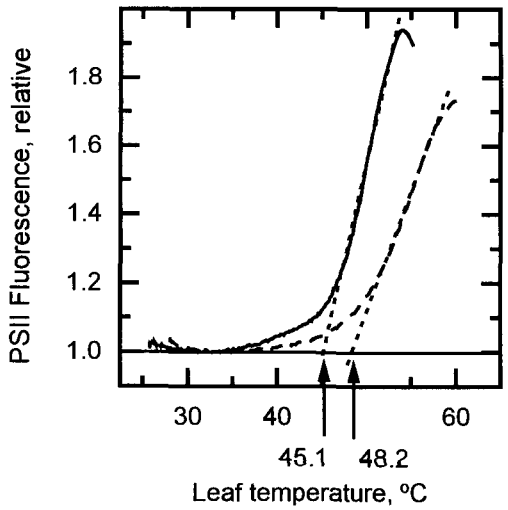

Figure 2. Typical fluorescence response of kudzu leaves in darkness. Leaves were held in a covered cuvette while the leaf was heated at 1°C min^{-1}. Compressed air (solid line) or compressed air containing 20 μL L^{-1} isoprene (dashed line) flowed over the leaf. The isoprene-fumigated leaflet showed thermal damage at 48.2°C, whereas the control leaf showed thermal damage at 45.1°C. In this pair of leaflets isoprene increased thermotolerance 3.1°C. Dotted lines are fit to the linear portion of the fluorescence curve by linear regression. This experiment was done 10 times, including 3 control pairs.

Thermotolerance Measured by Gas Exchange

To determine if the protective effects of isoprene on thermotolerance of electron transport would have a noticeable effect on the rate of net CO_2 assimilation, we made gas-exchange measurements of net photosynthesis as leaf temperature was raised from 25 to 50°C. Measurements were made on kudzu leaflets that had developed at low temperature and did not synthesize isoprene. Net photosynthesis declined as leaf temperature increased from 25 to 45°C (Fig. 5, solid line). When 8 μL L^{-1} isoprene was added to the airstream over the leaf, photosynthesis declined at a higher temperature (Fig. 5, dashed line). To make a quantitative comparison between isoprene-fumigated and control leaves, we measured T_0, where the curve of photosynthesis rate versus temperature crosses zero (Fig. 5, indicated by arrows). Isoprene increased T_0 by an average of 1.8°C, with an average cuvette isoprene concentration of 9.3 μL L^{-1} (t = 4.32, P = 0.02, and n = 4).

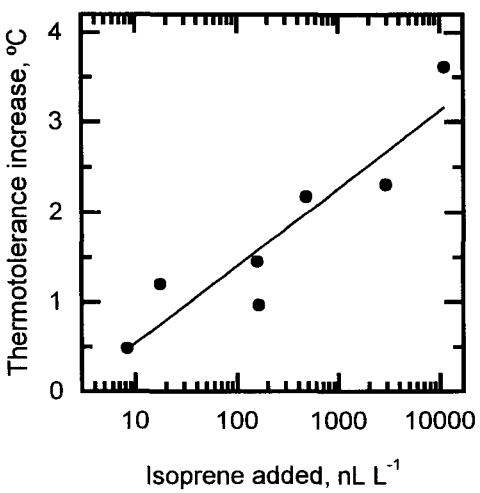

Figure 3. Dose response of thermotolerance with increasing isoprene concentration. Experiments were done as in Figure 2. Thermotolerance was measured by the difference in fluorescence rise temperature in paired leaflets for control and isoprene-added treatments. Data were fit to $y = a + b \times \log(\text{isoprene})$ (a = −0.33, b = 0.86, P = 0.003, r^2 = 0.85, and n = 7).

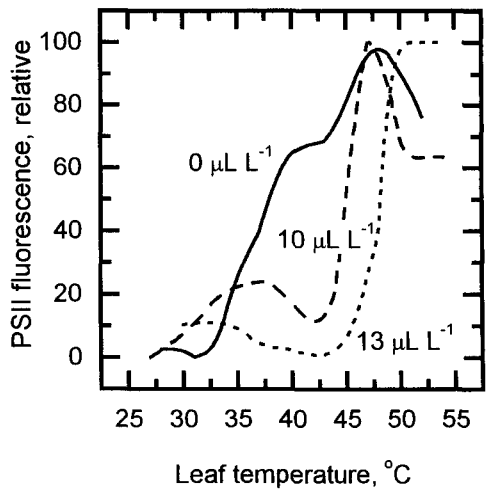

Figure 4. Fluorescence response of kudzu leaves with actinic light. Results of 3 of 14 experiments are shown. Leaves were held in a temperature-controlled cuvette with a N_2 atmosphere, a N_2 atmosphere with 10 μL L^{-1} isoprene, or a N_2 atmosphere with 13 μL L^{-1} isoprene.

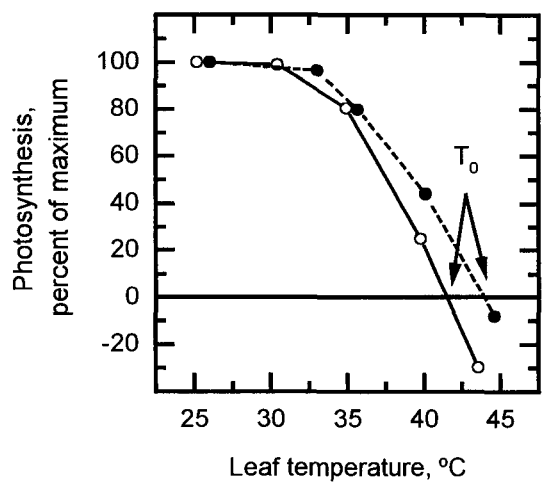

Figure 5. Photosynthesis response to temperature of two non-isoprene-emitting kudzu leaves. Photosynthesis was measured as temperature increased from 25°C until T_0 (arrows), the point at which photosynthesis was below 0 μmol CO_2 m^{-1} s^{-1}. T_0 was 43.8°C with 8 μL L^{-1} isoprene added to the airstream over the leaf (●). The control leaf (○) had a T_0 of 41.4°C. Isoprene increased T_0 by 1.8°C on average in four leaves (t = 4.32, P = 0.02). CO_2 partial pressure was held at 35.5 Pa, and PPFD was 1200 μmol photons m^{-2} s^{-1} throughout the experiments.

To investigate whether isoprene synthesized by the leaf increased thermotolerance in kudzu leaves, we made gas-exchange measurements of photosynthesis versus temperature. Isoprene emission and internal airspace isoprene concentration were calculated during the measurements. Since internal airspace isoprene concentration continuously increased during measurement because of the increased leaf temperature, the highest concentration calculated was used for a comparison. T_0 was calculated as in Figure 4. Leaves with more than 1.5 μL L^{-1} internal isoprene had a mean T_0 of 44°C (Fig. 6), whereas leaves that produced no isoprene had a T_0 of 41.4 ± 0.3°C (data not shown; t = 7.83, P = 0.004, and n = 4). In the 11 leaves measured, T_0 increased in a dose-dependent manner with isoprene concentration inside the leaf during the measurement (Fig. 6; r^2 = 0.58, P = 0.01, and n = 11).

Other Species

We wanted to test whether isoprene increased thermotolerance of species other than kudzu. We chose white oak as a representative isoprene-emitting species. Because not all species emit isoprene, we also tested a nonemitting species, *P. vulgaris*. We used chlorophyll fluorescence in darkness to measure thermotolerance in isoprene-fumigated and nonfumigated leaves.

Without added isoprene, white oak leaves showed thermal damage at 41.5°C (Table I). When 20 μL L^{-1} isoprene was added to the airstream flowing over the leaf, thermal damage occurred at 43.1°C. The isoprene increased thermotolerance of oak leaves by 1.6°C under these conditions (Table I; t = 3.63, P = 0.02, and n = 4).

Bean leaves were more thermotolerant than oak or kudzu leaves even without added isoprene. Thermal damage occurred in bean leaves at higher temperatures than in either isoprene-fumigated or control oak or kudzu leaves (Table I). Fluorescence measurements in darkness show that bean leaves were damaged at 48.8°C, whereas isoprene-fumigated leaves were damaged at 47.4°C. This difference was not significant (Table I; t = 1.84, P = 0.14, and n = 5). Fluorescence measurements with actinic light showed no difference in thermotolerance between isoprene-fumigated and unfumigated leaves (data not shown). Therefore, isoprene does not contribute to thermotolerance in bean leaves.

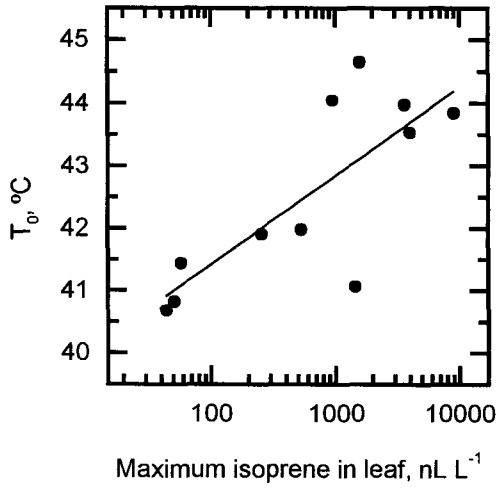

Figure 6. Dose response of T_0 with endogenous isoprene. Photosynthesis versus temperature curves were measured for kudzu leaves as in Figure 5. Maximum isoprene in the leaf during the experiment was calculated from isoprene emission rate and stomatal conductance. Data were fit to $T_0 = a + b \times \log(\text{isoprene})$ (a = 38.6, b = 1.41, P = 0.005, r^2 = 0.61, n = 11).

Table I. *Temperature of thermal damage with and without exogenous isoprene*

Thermotolerance measured by dark fluorescence in white oak and bean. Temperature of irreversible thermal damage during dark fluorescence experiments on isoprene-treated and untreated leaves. Experiments were performed as in Figure 2. Temperature is ± SE.

Species	Temperature of Thermal Damage		P	n
	Isoprene	No isoprene		
	°C			
White oak	43.1 ± 0.2	41.5 ± 0.2	0.02	4
Bean	47.4 ± 1.0	48.9 ± 1.3	0.14	5

DISCUSSION

The strong dependence of isoprene emission on temperature (Sanadze and Kursanov, 1966; Rasmussen and Jones, 1973; Tingey et al., 1979; Monson and Fall, 1989; Loreto and Sharkey, 1990) led us to hypothesize that isoprene increases thermotolerance of leaves. We tested this using three different methods. Each method blocked isoprene synthesis, so that isoprene could be added in a controlled manner. All three methods showed that isoprene increases thermotolerance of isoprene-emitting species up to 10°C. There was a dose-response relationship between isoprene concentration and thermotolerance. Isoprene fumigation did not increase thermotolerance of *P. vulgaris*, a nonisoprene-emitting bean species.

In kudzu isoprene fumigation increased thermotolerance more with actinic light than in darkness. With light present, isoprene increased the thermotolerance of kudzu leaves by as much as 10°C (Fig. 3), whereas in darkness, thermotolerance increased by 4°C. This is not surprising, because high light and high temperature often occur simultaneously (Sharkey, 1996). Another membrane-dissolved isoprenoid, zeaxanthin, has been shown to protect against high light (Demmig-Adams, 1990; Johnson et al., 1993) and high temperature (Havaux and Tardy, 1996; Havaux et al., 1996).

We also assayed thermotolerance by measuring CO_2-exchange rates as leaves were heated. T_0, the temperature at which leaves had zero net CO_2 assimilation (Fig. 4), was increased by as much as 2.5°C. Using leaves that synthesized their own isoprene, we found a dose-response relationship between isoprene and T_0 (Fig. 6). Leaves that emitted more isoprene had a higher T_0 than did those that produced little isoprene. We conclude that isoprene improves the CO_2 assimilation capacity of leaves at high temperature.

Isoprene emission is one of several known thermotolerance mechanisms. Plants may acquire thermotolerance by synthesizing zeaxanthin (Havaux and Tardy, 1996) or heat-shock proteins (Vierling, 1991). The observation that desert plants experience high tissue temperatures, even though relatively few desert species emit isoprene (Monson et al., 1991a), indicates that other thermotolerance mechanisms are relevant to these species. This may also be true of bean plants, which had higher thermotolerance than oak. With no isoprene fumigation, bean plants were damaged at 48.9°C, whereas oak leaves were damaged at 41.5°C (Table I). These plants did not benefit from isoprene fumigation. The relationship among thermotolerance caused by isoprene, zeaxanthin, and heat-shock proteins is unknown. It is unknown whether more than one of these mechanisms can occur in a single species, whether they have overlapping functions, or whether they protect against temperature stresses at different times or under different conditions.

Of these three thermotolerance mechanisms, isoprene is likely to be the most rapidly induced. Isoprene synthesis rate increases rapidly as leaves increase in temperature, and isoprene concentration builds up inside the leaf airspaces, increasing thermotolerance as leaves heat up. In contrast, acquired thermotolerance by de novo heat-shock protein synthesis or xanthophyll conversion can take several minutes to hours (Vierling, 1991; Havaux, 1993). When leaves cool down, isoprene synthesis decreases and isoprene concentration inside the leaf drops. Because isoprene emission can change rapidly as leaf temperature increases, isoprene emission may benefit most plants that experience rapidly fluctuating leaf temperatures. We have shown that these conditions occur in a white oak canopy (Sharkey, 1996).

Isoprene Concentration

To assess thermotolerance at physiologically relevant isoprene concentrations, we determined the isoprene concentration inside leaves under natural conditions. Isoprene is synthesized in the chloroplast (Sharkey et al., 1991a, 1991b; Silver and Fall, 1995) and diffuses through the stomates (Sharkey, 1991; Sharkey and Loreto, 1993). Feeding ABA to an isoprene-emitting leaf does not affect the rate of isoprene synthesis even though the stomata close (Fall and Monson, 1992). This indicates that high concentrations of isoprene inside the leaf do not feed back on isoprene production (Sharkey, 1991), and the rate of isoprene emission is directly related to the rate of isoprene synthesis. When leaves increase in temperature, isoprene synthesis increases and stomatal conductance decreases, resulting in high internal isoprene concentrations.

We calculated internal isoprene concentrations of 10 to 25 μL L^{-1} in oak leaves growing in a forest canopy. A typical example of this is shown in Figure 1, where isoprene reached 18 μL L^{-1} in the leaves. Under laboratory conditions we have calculated isoprene concentrations greater than 10 μL L^{-1} in kudzu leaves (Fig. 6). The increasing rate of isoprene synthesis and the decreasing stomatal conductance contribute equally to the high isoprene concentration in the leaf airspaces.

The isoprene concentration inside the cuvettes during our experiments was between 0.008 and 20 μL L^{-1}. Because stomata never close completely, isoprene concentration in the intercellular airspaces was equal to that in the cuvette. This occurs because there was little net flux between the leaf and its surroundings in these experiments. We conclude that isoprene concentrations used in our experiments are consistent with intercellular airspace concentrations that occur in natural conditions.

We hypothesized that isoprene functions in the leaf in which it is synthesized. Therefore, high concentrations of isoprene that build up in the leaf are physiologically relevant. If we hypothesized that isoprene functioned outside of the leaves in which it is synthesized, atmospheric concentrations of isoprene would be relevant. Isoprene is quite dilute in the atmosphere; the isoprene concentration in an oak forest is typically less than 20 nL L^{-1} (Geron et al., 1997). Isoprene was shown to affect flowering in *Arabidopsis thaliana* (a nonemitting species) at concentrations greater than 100 nL L^{-1} (Terry et al., 1995). Without endogenous isoprene synthesis, it is impossible for isoprene to reach these concentrations inside nonemitting leaves. Other volatile compounds, such as methyl jasmonate and methyl salicylate, have been shown to have physiological effects on plants (Farmer and Ryan, 1990; Schweizer et al., 1997; Shulaev et al., 1997). In each case, the gas concentration was raised well above ambient concentration to show the intended physiological effect. Physiological effects of volatile compounds must be shown at physiological concentrations to be interpreted as a natural phenomenon.

Mechanism

Because these experiments do not indicate the mechanism by which isoprene increases thermotolerance, we can only speculate. There is evidence to suggest that heat-induced damage affects thylakoid membranes (Thomas et al., 1986; Smith and Low, 1989). Membranes subjected to high temperatures will fold up on themselves, creating holes (Williams et al., 1992). Modification of membrane properties by increasing the ratio of saturated to unsaturated fatty acid chains decreases fluidity and increases thermotolerance (Restall et al., 1979; Vigh et al., 1989). Isoprene is highly lipophilic and may partition into chloroplast membranes. This evidence led us to propose that isoprene increases thermotolerance by stabilizing thylakoid membranes at high temperatures (Sharkey and Singsaas, 1995). We propose that isoprene may stabilize (a) lipid-lipid interactions, (b) lipid-protein interactions, or (c) protein-protein interactions.

We have shown that isoprene concentration reaches high levels inside leaves of isoprene-emitting plants. At these concentrations, we showed that isoprene protects plants from damage at high temperatures using measurements of chlorophyll fluorescence and photosynthesis. Isoprene was beneficial to two isoprene-emitting species, kudzu and white oak, but had no effect on *P. vulgaris*, a nonemitting species.

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