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Relationships among Isoprene Emission Rate, Photosynthesis, and Isoprene Synthase Activity as Influenced by Temperature[1]

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ABSTRACT

Isoprene emissions from the leaves of velvet bean (*Mucuna pruriens* L. var *utilis*) plants exhibited temperature response patterns that were dependent on the plant's growth temperature. Plants grown in a warm regimen (34/28°C, day/night) exhibited a temperature optimum for emissions of 45°C, whereas those grown in a cooler regimen (26/20°C, day/night) exhibited an optimum of 40°C. Several previous studies have provided evidence of a linkage between isoprene emissions and photosynthesis, and more recent studies have demonstrated that isoprene emissions are linked to the activity of isoprene synthase in plant leaves. To further explore this linkage within the context of the temperature dependence of isoprene emissions, we determined the relative temperature dependencies of photosynthetic electron transport, CO_2 assimilation, and isoprene synthase activity. When measured over a broad range of temperatures, the temperature dependence of isoprene emission rate was not closely correlated with either the electron transport rate or the CO_2 assimilation rate. The temperature optima for electron transport rate and CO_2 assimilation rate were 5 to 10°C lower than that for the isoprene emission rate. The dependence of isoprene emissions on photon flux density was also affected by measurement temperature in a pattern independent of those exhibited for electron transport rate and CO_2 assimilation rate. Thus, despite no change in the electron transport rate or CO_2 assimilation rate at 26 and 34°C, the isoprene emission rate changed markedly. The quantum yield of isoprene emissions was stimulated by a temperature increase from 26 to 34°C, whereas the quantum yield for CO_2 assimilation was inhibited. In greenhouse-grown aspen leaves (*Populus tremuloides* Michaux.), the high temperature threshold for inhibition of isoprene emissions was closely correlated with the high temperature-induced decrease in the *in vitro* activity of isoprene synthase. When taken together, the results indicate that although there may be a linkage between isoprene emission rate and photosynthesis, the temperature dependence of isoprene emission is not determined solely by the rates of CO_2 assimilation or electron transport. Rather, we propose that regulation is accomplished primarily through the enzyme isoprene synthase.

Isoprene (2-methyl-1,3-butadiene) is emitted from the leaves of numerous plant species in quantities that have an important impact on the oxidation potential of the troposphere (3, 8, 11, 20). Past studies have identified a linkage between isoprene emission rate and photosynthesis rate (9, 10, 13, 14). Although several details of the mechanism(s) underlying this linkage are unknown, there is good evidence that isoprene emission rate is determined, at least to some extent, by the availability of reduced carbon to support the synthesis of isoprenoid skeletons and the availability of chloroplastic ATP to support the energetic needs of the mevalonic acid pathway (16). There has been additional speculation that the rate of photosynthetic electron transport has a role in determining the isoprene emission rate (11), a view supported by the NADPH requirement of the mevalonic acid pathway. Evidence against a link between isoprene emission rate and photosynthetic electron transport rate was provided in a recent analysis of the responses of CO_2 assimilation, isoprene emissions, and Chl fluorescence to incident photon flux density and intercellular CO_2 partial pressure (9). However, to date there are such scant data describing the potential relationship of isoprene emission rate to electron transport rate that any broad conclusions must remain tentative. An additional factor to consider in the regulation of isoprene emissions and its linkage to photosynthesis is the role of the enzyme isoprene synthase, which was recently reported in plant tissues and appears to underlie the conversion of DMAPP[2] to isoprene (17).

With some knowledge of the linkages between photosynthesis and isoprene emission, it has become easier to explain the responses of isoprene emission rate to changes in environmental variables such as photon flux density and CO_2/O_2 concentrations (11). One environmental variable that is known to have a profound influence on isoprene emission rate, but has not yet been studied in terms of the mechanistic linkages, is leaf temperature. Isoprene emission rate exhibits a Q_{10} of 2 or higher at temperatures between 30 and 40°C, and decreases at temperatures above 40 to 45°C (6, 10, 19). The increase in isoprene emission rate at temperatures be-

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[2] Abbreviations: DMAPP, dimethylallyl diphosphate; F_o, initial fluorescence; q_N, nonphotochemical fluorescence quenching; Γ^*, CO_2 compensation point in absence of mitochondrial respiration.

tween 30 and 40°C occurs despite decreases in the instantaneous rate of CO_2 assimilation, suggesting that any linkage to the rate of carbon assimilation is loose, or nonexistent, as leaf temperatures are increased. A better candidate for the linkage between the temperature dependence of isoprene emission rate and photosynthesis might be found in the photosynthetic electron transport rate. The photosynthetic electron transport rate is known to also increase at temperatures between 30 and 40°C and to decrease in an irreversible manner at temperatures above 40 to 45°C (1, 18).

An additional unknown in the case of temperature effects on isoprene emission rate is whether growth temperature influences the temperature dependence of isoprene emissions, as is often observed for the temperature dependence of photosynthesis (1). The issue of temperature acclimation is especially important to atmospheric modelers who have an interest in developing emission rate algorithms to predict biospheric hydrocarbon emissions across temporal changes in temperature, be it due to seasonal change or global climate change (11).

The current study was conducted with the aim of uncovering more details about the temperature dependence of isoprene emission rate. Specifically, we wished to determine whether the temperature response of isoprene emissions could be better explained by linkage to the carbon assimilation rate, the photosynthetic electron transport rate, or the activity of isoprene synthase. A second aim was to study the influence of growth temperature on the temperature dependence of the isoprene emission rate. The studies were conducted with two species, velvet bean, an herbaceous legume that has been shown to emit relatively large amounts of isoprene (5), and aspen. Velvet bean plants were used in the studies of growth temperature and its influence on isoprene emission characteristics because of the rapid growth rates expressed by this species. For the studies of isoprene synthase and its relationship to the temperature dependence of isoprene emission, we used aspen because, to date, we have not been able to successfully stabilize isoprene synthase activity from velvet bean leaves.

MATERIALS AND METHODS

Plant Material

Velvet bean (*Mucuna pruriens* L. var *utilis*) plants were grown from seed in 2 L pots containing a potting mix of peat:vermiculite:perlite in a 2:2:1 ratio. The seeds were obtained from Glendale Enterprises (DeFuniak Springs, FL). Plants were grown in controlled environment chambers (Conviron, model E-15) programmed for one of two temperature regimens (34/28°C or 26/20°C, day/night). Photon flux densities at midplant height were 500 to 600 μmol m^{-2} s^{-1}. The photoperiod was set at 12 h. Plants were watered every other day and fertilized once a week with Hoagland solution modified to contain 1 mol m^{-3} NH_4^+ in place of an equivalent decrease in NO_3^-. To minimize leaf-to-leaf variation in photosynthesis and isoprene emission rate (5), fully expanded primary leaves (10–15 d postemergence) were used in all measurements.

In the experiment involving the temperature dependence of isoprene emissions and isoprene synthase activity, greenhouse-grown aspen trees (*Populus tremuloides* Michaux) were used. The growth conditions for these trees have been described in a previous report (10).

Gas Exchange System and Isoprene Analysis

Both the gas exchange system and isoprene analyzer have been described in detail in previous reports (7, 10, 12). The leaf chamber was commercially constructed by Bingham Interspace Co. (narrow-leaf model; Logan, UT). Temperature control was accomplished with Peltier modules mounted to the top of a perforated heat exchanger built into the top of the chamber.

Fluorescence Analysis

Chl fluorescence patterns were obtained from a pulse-modulated fluorometer (PAM 101, Walz Insts.). At the beginning of each experiment, F_o was determined in the same leaf to be used in the gas exchange measurements after dark adaptation (room light for 30 min, then darkness for 10 min). Maximal fluorescence, was determined after three saturating flashes each with intensities of 6000 μmol m^{-2} s^{-1} delivered from two parallel Schott KL 1500 lamps. Leaves were then illuminated with an experimental light intensity, during which steady-state measurements of gas exchange and fluorescence were made. After each recording of steady-state fluorescence, F_o' was measured by rapidly reducing the incident photon flux density to 20 μmol m^{-2} s^{-1} and simultaneously filtering the light through a far-red filter (Schott RG-9). The Chl fluorescence nomenclature, and the equations used for the calculation of photochemical fluorescence quenching and q_N, were adopted at the Workshop on the Use of Chlorophyll Fluorescence and Other Non-Invasive Spectroscopic Techniques in Plant Stress Physiology (21).

Photosynthetic electron transport rates were calculated from the fluorescence measurements using the methods of Weis and Berry (22), including the modifications of Sharkey *et al.* (15). Calibrations of quantum yield per unit of open PSII reaction centers as a function of q_N were constructed for leaves exposed to different growth and measurement temperatures. The quantum yield for electron transport was calculated from gas exchange data using the technique of Sharkey *et al.* (15). This method requires the use of mitochondrial respiration rates and the Γ^*. Mitochondrial respiration was measured as dark respiration for each leaf during the 30-min dark treatment prior to measuring the light response curves. Γ^* was estimated to be 41.6 μbar at 25°C (2). The value for Γ^* at other temperatures was calculated according to the equations provided in Brooks and Farquhar (2). The relationship between q_N and the quantum yield for electron transport corrected for open PSII reaction centers was not significantly affected by an 8°C increase in growth or measurement temperature (data not shown). This was indicated by the lack of significant change in the slope or y intercept when warm- or cool-grown plants were measured at 26 or 34°C ($P > 0.05$, Spearman correlation test). The lines are described by the following regressions: $y = -0.26\ x + 0.43$ ($r = 0.83$) for the 26°C plants, and $y = -0.23\ x + 0.34$ ($r = 0.95$) for the 34°C

plants. When using these calibration curves to calculate electron transport rates at different temperatures, we averaged the slopes and intercepts across all treatments and measurement temperatures.

Isoprene Synthase Assay

A preparation of aspen leaf isoprene synthase was partially purified by ammonium sulfate precipitation and ion exchange chromatography as described elsewhere (17). The enzyme catalyzes the Mg^{2+}-dependent conversion of DMAPP to isoprene. The assay for enzyme activity was performed in sealed, 4-mL glass vials from which headspace gas was withdrawn and analyzed by gas chromatography. Details of the assay procedure have been described elsewhere (17). The assay was conducted at different temperatures in parallel using several temperature-controlled water baths.

RESULTS

Temperature acclimation of the CO_2 assimilation rate was indicated by improved photosynthetic performance at warm leaf temperatures when plants were grown at warm temperatures and compared with cool-grown plants (Fig. 1). At cool leaf temperatures, there were no obvious differences in photosynthetic performance when comparing warm- and cool-grown plants. The isoprene emission rate exhibited a similar acclimation to growth temperature, reflected in higher emission rates at warm leaf temperatures when plants were grown in a warm regimen and compared with plants grown in a cool regimen (Fig. 1). The temperature optimum for isoprene emission rate shifted from 40°C in cool-grown plants to 45°C in warm-grown plants. The temperature dependence of electron transport rate exhibited an acclimatory response with pronounced differences between cool- and warm-grown plants at warm measurement temperatures, but not at cool measurement temperatures (Fig. 1). The temperature optimum for electron transport rate was observed to be 35°C and did not shift as a result of growth temperature. The amount of minimal F_o was observed to increase sharply at temperatures above 45°C in both warm- and cool-grown plants. Using 5°C measurement intervals, we did not detect differences in the point at which F_o increased in cool- and warm-grown plants (data not shown).

The dependence of isoprene emission rate on photon flux density was quite sensitive to temperature (Fig. 2). When both warm- and cool-grown plants were measured at 26°C, isoprene emission rates were reduced at all photon flux densities compared with emission rates at 34°C. A similar pattern was observed in the electron transport rate for warm-grown plants, but not for cool grown-plants (Fig. 2). The response of CO_2 assimilation rate to photon flux density was not significantly temperature sensitive (Fig. 2). Thus, there was no consistent correlation between the light dependence of isoprene emissions as affected by temperature and the light dependencies of electron transport rate and CO_2 assimilation rate as affected by temperature.

The quantum yields for CO_2 uptake and isoprene emission rate were determined from the initial slope of the light response curves. Quantum yields for CO_2 uptake were inhibited

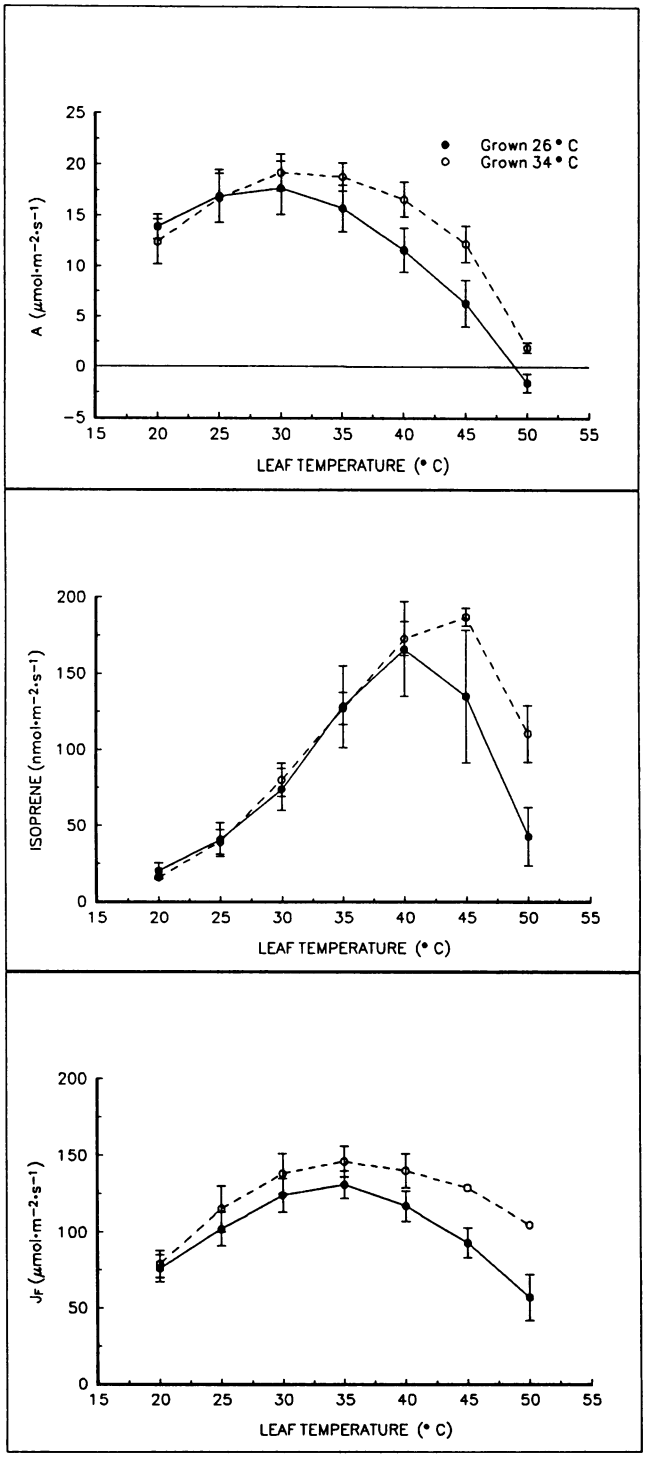

Figure 1. The CO_2 assimilation rate (A), isoprene emission rate, and photosynthetic electron transport rate (J_F) as a function of leaf temperature in warm- (○) and cool-grown (●) velvet bean plants. Symbols represent the mean ± SE (n = 2–3).

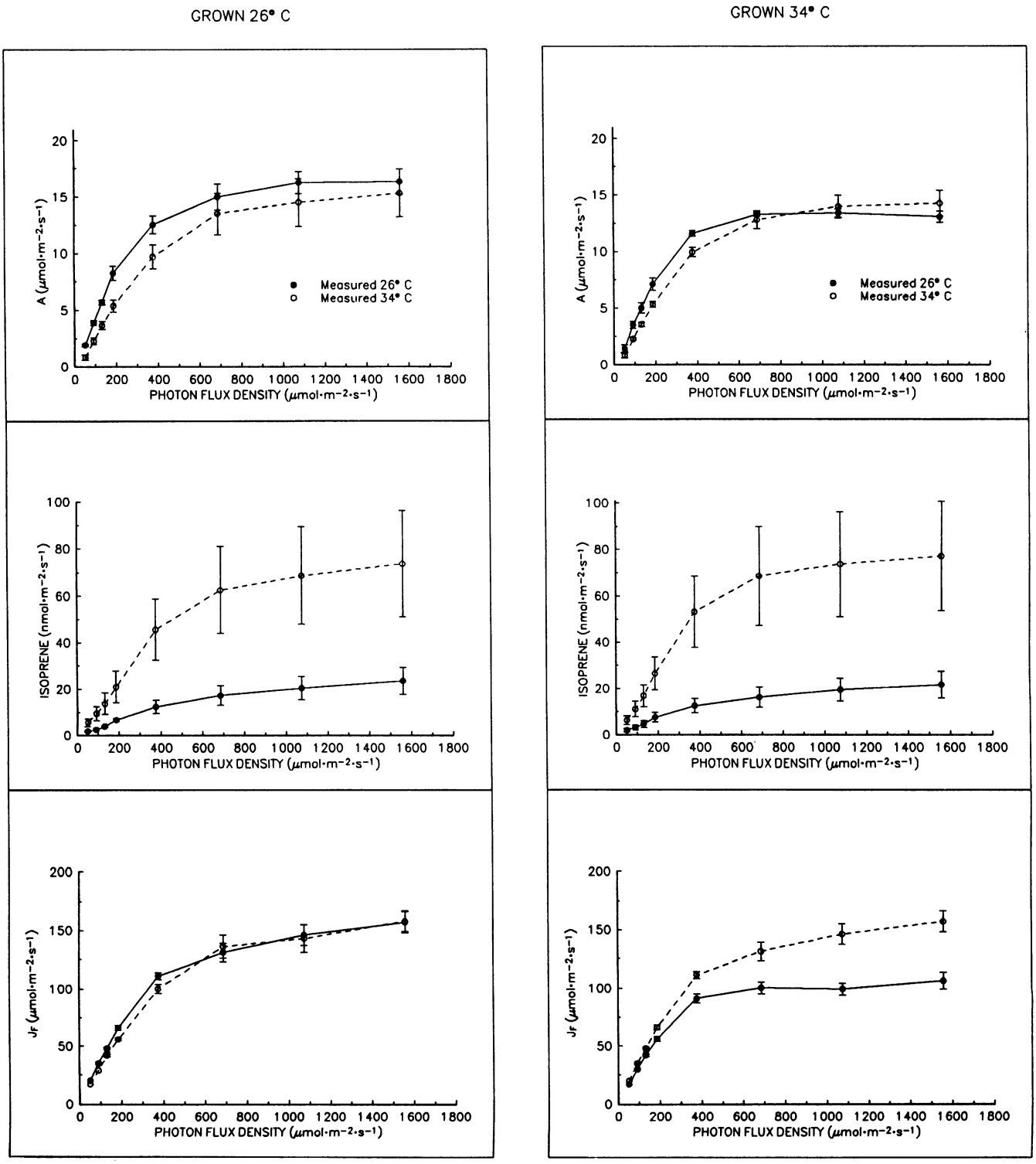

Figure 2. The CO_2 assimilation rate (A), isoprene emission rate, and photosynthetic electron transport rate (J_F) as a function of incident photon flux density in velvet bean plants grown at a daytime temperature of 26°C (left panels) or 34°C (right panels). Measurement temperature was either 26°C (●) or 34°C (○). Symbols represent the mean ± SE (n = 4–5).

by an increase in measurement temperature from 26 to 34°C (Table I). The quantum yields for isoprene emission, however, were stimulated by temperature for both warm- and cool-grown plants, exhibiting Q_{10} values between 1.7 and 2.1.

The recent discovery of isoprene synthase activity in leaves (17) provided an opportunity to test the temperature dependence of this enzyme. The enzyme has not yet been successfully extracted from velvet bean leaves, but has been obtained from leaves of several other isoprene-emitting plants, including aspen. The temperature dependence of partially-purified isoprene synthase from aspen leaves is shown in Figure 3. Isoprene synthase activity increased with a Q_{10} between 2.1 and 3.3 at temperature increases between 25 and 40°C. Above 40°C, enzyme activity was relatively constant up to 50°C, at which point a marked reduction was observed. The temperature dependence of isoprene emissions from intact aspen leaves paralleled that for *in vitro* enzyme activity (Fig. 3).

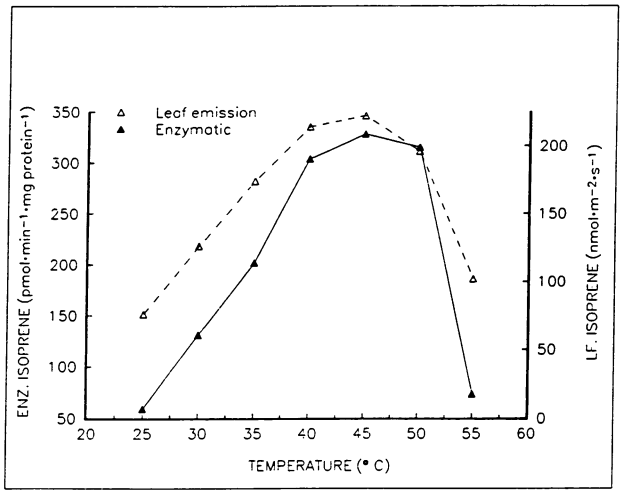

Figure 3. The temperature dependence of aspen leaf isoprene emission rate (△ [*in vivo*]) and aspen isoprene synthase activity (▲ [*in vitro*]). A partially purified preparation of isoprene synthase, which catalyzes the conversion of DMAPP to isoprene, was used for the enzymatic assay. Symbols represent the mean of two to four replicate measurements.

DISCUSSION

The results from this study demonstrate for the first time that the temperature response of the isoprene emission rate from plant leaves is capable of adjustment depending on the plant's growth temperature regimen. Such temperature adjustments have been well defined in the case of photosynthesis (1). However, the results of the current study do not provide support for the existence of a direct linkage between temperature acclimation of isoprene emissions and that of photosynthesis. This conclusion is based on three observations.

First, the results presented in Figure 2 indicate that isoprene emissions exhibit a strong dependence on photon flux density that changes markedly with temperature despite no significant change in electron transport rate or CO_2 assimilation rate. The same rate of electron transport or CO_2 assimilation results in quite different rates of isoprene emission depending on temperature. Although isoprene synthesis may require reduced carbon from the reductive pentose phosphate pathway and/or ATP and NADPH from the light-dependent reactions of photosynthesis, the results of Figure 2 indicate that the point of control for the temperature response of isoprene emissions is beyond these photosynthetic linkages.

Second, based on the poor correlation between the temperature dependence of photosynthetic processes and isoprene emission rate in warm- and cool-grown plants (Fig. 1), we conclude that the observed acclimatory adjustments of the isoprene emission rate to growth temperature are due to changes in a control point beyond the linkages to CO_2 assimilation rate or electron transport rate.

Third, the results of Table I demonstrate that even at the level of extreme light limitations, the linkage between photosynthesis and isoprene emissions is loose with respect to increases in measurement temperature. The quantum yield for CO_2 uptake in C_3 plants is known to be quite sensitive to increases in temperature (4). This is due to the increase in photorespiration that occurs at warmer temperatures, a process that draws ATP away from the carbon reduction cycle and reduces the rate of CO_2 assimilation (1). The quantum yield for isoprene emission increases as temperature increases, indicating that it is not sensitive to competition with photorespiration for available chloroplastic ATP. This despite previous evidence that isoprene emissions are, in fact, affected by reduced levels of chloroplastic ATP (9, 10).

All of the results discussed above would be better explained by hypothesizing a linkage between the temperature response of isoprene emission rate and isoprene synthase activity, rather than photosynthesis. The temperature dependence of the enzymatic production of isoprene correlates closely with the temperature dependence of isoprene emissions from intact leaves (Fig. 3). Temperature acclimation of isoprene emissions could result from adjustments in the thermal stability of isoprene synthase and/or changes in the temperature dependence of its activity brought about by changes in activation state or chloroplast environment. It has recently been demonstrated that the temperature dependence of isoprene emissions from *Eucalyptus* leaves could accurately be predicted by an algorithm based on a thermodynamic expression of enzyme temperature dependence with thresholds added to account for high temperature inactivation (6). The results of

Table I. *Quantum Yields for CO_2 Uptake and Isoprene Emission as a Function of Temperature*

Each value represents the mean ± SE of three to six measurements on different plants. Quantum yield is calculated as the linear slope of the response of CO_2 uptake or isoprene emission to increase in the incident photon flux density between 50 and 185 μmol m^{-2} s^{-1}.

Growth Temperature	Measurement Temperature	CO_2 Uptake	Isoprene Emission
°C	°C	*mol/mol*	*μmol/mol*
26/20	26	0.048 ± 0.004	0.045 ± 0.008
26/20	34	0.039 ± 0.003	0.061 ± 0.009
34/28	26	0.043 ± 0.002	0.047 ± 0.010
34/28	34	0.037 ± 0.003	0.081 ± 0.004

the current study demonstrate that the algorithm describing the temperature dependence is likely supported by a solid biochemical basis.

The existence of temperature acclimation with respect to isoprene emissions could complicate future efforts to model the emission of isoprene from forest canopies (11). In this study, we did not observe significant differences in the temperature dependence of isoprene emissions between cool- and warm-grown plants at measurement temperatures between 20 and 40°C. Thus, one algorithm describing emissions as a function of temperature would provide an accurate basis for modeling at most biologically relevant temperatures. However, the acclimation pattern could vary for other species and different growth temperature comparisons. Continuing studies in our laboratory will focus on more accurately defining this potential variability.

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