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Isoprene Emission from Aspen Leaves[1]

Influence of Environment and Relation to Photosynthesis and Photorespiration

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ABSTRACT

Isoprene emission rates from quaking aspen (*Populus tremuloides* Michx.) leaves were measured simultaneously with photosynthesis rate, stomatal conductance, and intercellular CO_2 partial pressure. Isoprene emission required the presence of CO_2 or O_2, but not both. The light response of isoprene emission rate paralleled that of photosynthesis. Isoprene emission was inhibited by decreasing ambient O_2 from 21% to 2%, only when there was oxygen insensitive photosynthesis. Mannose (10 millimolar) fed through cut stems resulted in strong inhibition of isoprene emission rate and is interpreted as evidence that isoprene biosynthesis requires either the export of triose phosphates from the chloroplast, or the continued synthesis of ATP. Light response experiments suggest that photosynthetically generated reductant or ATP is required for isoprene biosynthesis. Isoprene biosynthesis and emission are not directly linked to glycolate production through photorespiration, contrary to previous reports. Isoprene emission rate was inhibited by above-ambient CO_2 partial pressures (640 microbar outside and 425 microbar inside the leaf). The inhibition was not due to stomatal closure. This was established by varying ambient humidity at normal and elevated CO_2 partial pressures to measure isoprene emission rates over a range of stomatal conductances. Isoprene emission rates were inhibited at elevated CO_2 despite no change in stomatal conductance. Addition of abscisic acid to the transpiration stream dramatically inhibited stomatal conductance and photosynthesis rate, with a slight increase in isoprene emission rate. Thus, isoprene emission is independent of stomatal conductance, and may occur through the cuticle. Temperature had an influence on isoprene emission rate, with the Q_{10} being 1.8 to 2.4 between 35 and 45°C. At these high temperatures the amount of carbon lost through isoprene emission was between 2.5 and 8% of that assimilated through photosynthesis. This represents a significant carbon cost that should be taken into account in determining midsummer carbon budgets for plants that are isoprene emitters.

Isoprene (2-methyl-1,3-butadiene) is one of the primary nonmethane hydrocarbons emitted from some plant leaves (2, 13–15, 18, 34). Isoprene emission occurs from a number of apparently unrelated plant species, and tends to be most common among woody, tree species (2, 14, 33). Several past studies have monitored daily patterns of isoprene emission from plants in an effort to assess the relative contribution of vegetation to chemical processes in the atmosphere (13, 32, 34). Other studies have analyzed metabolic links between isoprene emission and the photosynthetic and photorespiratory pathways (5, 16, 19) or the influence of environmental parameters on isoprene emission rates (28–30). Some previous studies have concluded that isoprene emission is related to photorespiratory production of glycolate (5), and accordingly is highly dependent on light and temperature (30).

However, most of the studies conducted to date have used excised leaf tissue for inhibitor studies of the relationships between isoprene emission and photosynthesis or photorespiration (5, 16) or whole plant canopies for gas exchange studies of the relationships between isoprene emission and varying environmental factors (2, 13, 28–30). Such studies have had limitations in relating the rate of isoprene emission to the component processes of photorespiration and photosynthesis, either because of the uncertain specificity of the inhibitors, or because of the lack of precise gas exchange responses to environmental factors that exist in whole-plant canopies. Sanadze *et al.* (20, 21) have used individual leaves to examine the O_2 and light dependence of isoprene emission in poplar leaves. It was concluded that mitochondrial respiration is involved in isoprene biosynthesis and that some aspect of isoprene biosynthesis is tightly coupled to the photosynthetic electron transport system.

In this study, experiments were conducted with a leaf gas exchange system that was coupled to a gas chromatograph which was modified to quickly and accurately analyze nonmethane hydrocarbons from individual, attached leaves enclosed in the gas exchange cuvette. This system was used to examine the relationships between isoprene emission and photorespiration or photosynthesis, and the influence of light, temperature, CO_2 partial pressure, O_2 partial pressure, and humidity on the rate of isoprene emission.

MATERIALS AND METHODS

Plant Material

Two experimental trees (*Populus tremuloides* Michx.) were used for all the studies. The trees were 2 years old, approxi-

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mately 1.5 m tall, and were grown in 15 L plastic pots. They were obtained approximately 2 weeks before initiating the experiments from a local nursery in Boulder, CO. After they were obtained, the trees were kept in a partially shaded greenhouse with midday light intensities of 700 to 800 μmol m^{-2} s^{-1} and midday temperatures of 26 to 32°C. The trees were watered daily with tap water and fertilized weekly with quarter-strength Hoagland solution. All measurements were conducted between June 15 and September 3, 1988.

Gas Exchange System and Gas Chromatograph

The gas exchange system, including the leaf cuvette, is described in a previous report (10). Gas chromatographic analysis was carried out essentially as described elsewhere for the measurement of sulfur gas emissions from plants (3) with the following modifications. A gas stream was withdrawn from the outlet of the leaf cuvette at a constant flow of approximately 80 cm^3 min^{-1}. This outlet air was pulled through Teflon tubing into a glass drying-tube that was maintained at −80°C in a dry ice bath. With most of the water so removed, 186 standard cm^3 samples were drawn by opening a stainless steel valve, allowing the sample to flow through a Teflon capillary loop submerged in liquid nitrogen and into a previously evacuated 7.7 L calibrated stainless steel flask. Removal of most of the water from the sample prevented freeze-up of the sample loop which was used for cryogenic preconcentration. The resulting preconcentrated sample was flash heated at 80°C and automatically injected onto a 30 m fused silica capillary column with a 0.25 mm i.d. and a 1 μm thick methyl silicone stationary phase (J & W Scientific, Folsom, CA). Analysis was performed with a model 5790A gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Palo Alto, CA), using hydrogen as a carrier gas at 1 cm^3 min^{-1}. Under isothermal conditions at 30°C the retention time for isoprene was 1.96 ± 0.06 min. Calibration of the entire dryer/cryoenrichment/analysis system was carried out with an isoprene gas calibration source passed into the leaf chamber. The calibration source contained a weighed amount of isoprene (greater than 99% pure grade, Aldrich Chemical Co., Milwaukee, WI) that was quantitatively transferred into an evacuated, passivated aluminum cylinder and filled to 2000 p.s.i. with purified nitrogen. This source provided a stable gaseous source of isoprene (22.4 ppm). Using this source, an isoprene recovery of 71 ± 4% was determined for samples collected from the gas sampling stream.

Verification of isoprene emissions from aspen leaves was achieved by GC-MS. Aspen leaf discs were incubated in 1 mL H_2O in a 3.5 mL vial sealed with a Teflon septum under light (1000 μmol m^{-2} s^{-1}) for 60 to 90 min at 25°C. Samples of the vial airspace, which contained the putative isoprene peak, were analyzed on a Hewlett-Packard 5988A GC-MS system, equipped with a 25 m fused silica capillary (0.31 mm i.d., 0.52 μm stationary phase of phenylmethyl silicone). Analyses were carried out with temperature programming from −50°C to 120°C. Authentic isoprene eluted at 4.05 min under these conditions.

Experimental Protocol

For those experiments in which environmental conditions surrounding the leaf were varied, we first obtained steady state gas exchange rates (including the isoprene emission rate) under a 'standard environment.' The standard environment was a leaf temperature of 25°C, incident photon flux density of 1000 μmol m^{-2} s^{-1}, CO_2 partial pressure of 325 to 350 μbar, O_2 concentration of 21%, and a leaf-to-air water vapor concentration gradient of 1.0 to 1.3 mmol H_2O/mol air. In those studies in which inhibitors were fed to the leaves, stems with 20 to 25 leaves were severed from one of the trees while being held under water. The severed stem was then inserted through a stopper such that the cut end could be submerged in a flask containing water or a solution of the inhibitor. The inhibitors used were D-mannose and (±) *cis-trans*-abscisic acid (99% grade) both obtained from Sigma Chemical Co. (St. Louis, MO). To provide 10 μM (+) *cis-trans*-abscisic acid, a 25 mM solution of the (±) isomers was prepared in ethanol and diluted in water to 20 μM.

Experiments were conducted in duplicate or triplicate. Since replicated measurements were similar, we have presented results from one of the experiments.

RESULTS

Verification of the Isoprene Signal

Only one major hydrocarbon peak was observed when samples of gas were drawn from the leaf chamber in the presence of an illuminated leaf and analyzed by gas chromatography. This hydrocarbon was shown to be isoprene in two ways. First, an isoprene standard added in the absence of a leaf had the same retention time as the suspected isoprene peak at column temperatures of 30 and 0°C. Second, gas samples collected from the airspace of sealed vials containing air and illuminated aspen leaf disks were analyzed using mass spectrometry/gas chromatography as described in the "Materials and Methods" section. Isoprene (2-methyl-1,3-butadiene) was the principal component of this gas mixture, eluting with the same retention time, producing an identical molecular ion (m/e = 68) and fragmentation pattern as authentic isoprene.

Atmospheric Composition, Light Intensity, and Isoprene Emission Rate

To identify conditions at which isoprene emission is maximized, a leaf was exposed to the presence or absence of CO_2 and O_2 (Fig. 1). Isoprene was emitted at relatively high rates only when CO_2 or O_2 were present. Isoprene was emitted at near-maximum rates even in the absence of net photosynthetic carbon assimilation.

Despite the lack of a requirement for net photosynthetic CO_2 assimilation in driving isoprene emission, the light dependence of isoprene emission paralleled that for photosynthesis (Fig. 2). The rate of isoprene emission was less than 13 nmol C m^{-2} s^{-1} in the dark, although some low background emission rate in the dark always occurred (data not shown). The quantum yield for CO_2 uptake (on an incident light basis) was 0.050 mol CO_2/mol quanta. The quantum yield for

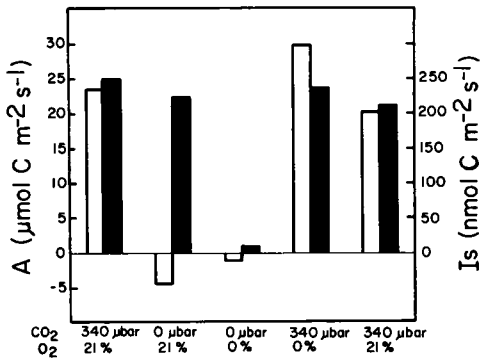

Figure 1. Rates of photosynthetic assimilation rate (A) and isoprene emission rate (Is) as a function of various combinations of atmospheric CO_2 partial pressure and O_2 concentration. A, Open bars; Is, solid bars.

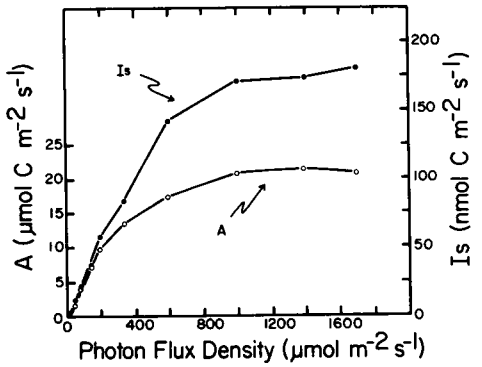

Figure 2. Dependence of photosynthetic assimilation rate (A) and isoprene emission rate (Is) on photon flux density incident on the leaf.

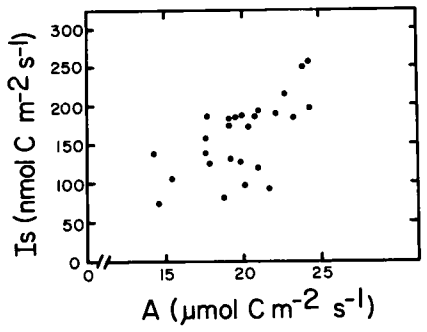

Figure 3. Relationship between photosynthetic assimilation rate (A) and isoprene emission rate (Is) determined from 26 different leaves. The two variables were significantly correlated with a Spearman correlation coefficient of $r^2 = 0.64$ ($p = 0.001$).

isoprene emission rate was 0.0003 mol isoprene C/mol quanta, only 0.6% the value for CO_2 uptake.

After analyzing the gas-exchange characteristics of over 26 aspen leaves under a standard set of environmental conditions, a positive correlation was observed between the rate of photosynthetic CO_2 assimilation and isoprene emission (Fig. 3). Thus, even though isoprene emission is not dependent upon the instantaneous rate of photosynthesis, those leaves with the greatest potential for CO_2 assimilation also exhibit the greatest potential for isoprene emission. The percentage of carbon assimilation that is emitted as isoprene (on a carbon atom basis) was 0.79 ± 0.4% ($n = 26$) at a leaf temperature of 25°C. When expressed per unit of leaf dry weight, the isoprene emissions averaged 76 μg C (g dry weight) $^{-1}$ h^{-1}. This can be compared to a value of 34 μg C (g dry weight) $^{-1}$ h^{-1} reported by Evans *et al.* (2) for quaking aspen canopies under similar environmental conditions.

Response of Isoprene Emission to O_2 Concentration

Gas-exchange measurements were conducted on two different leaves to examine whether a decrease in the O_2 concentration (with a presumed effect of decreasing the rate) resulted in a change in the rate of isoprene emission (Fig. 4). In one leaf, the photosynthesis rate was stimulated following the switch from 21% to 2% O_2 (Fig. 4a). With time, however, the photosynthesis rate began to decline slowly in the low O_2 atmosphere. The rate of isoprene emission paralleled that of photosynthesis, increasing sharply following the switch to low O_2, with a slower decline during the hour after the maximum rate was reached. In the second leaf, there was no increase in photosynthesis rate following the switch to low O_2 (Fig. 4b). Oscillations in the photosynthesis rate were observed for approximately 90 min following the switch, becoming damped with time. After 70 min in 2% O_2 the photosynthesis rate was lower than in 21% O_2. This pattern is typical of O_2-insensitive photosynthesis, as previously described (25). Oxygen-insensitive photosynthesis occurs as a result of phosphate limitations to photosynthesis, thus uncoupling the rate of CO_2 assimilation from the competitive influence of O_2 (26). In this case, however, it occurred under normal environmental and

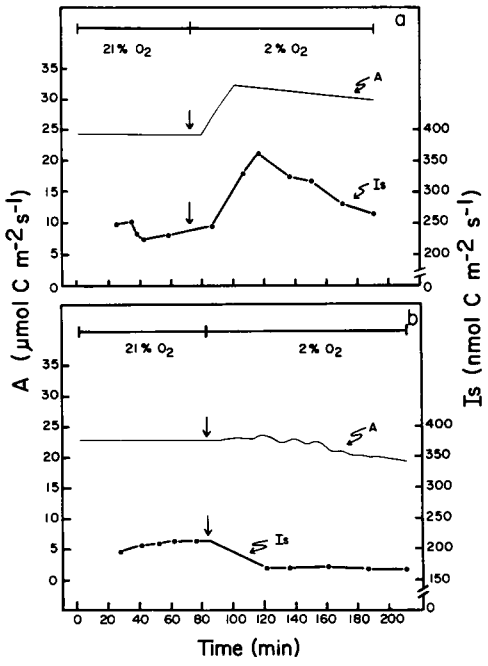

Figure 4. Responses of photosynthetic assimilation rate (A) and isoprene emission rate (Is) to a step change from an atmosphere containing 21% O_2 to one containing 2% O_2. Experiments from two different leaves are shown, one in panel a and one in panel b. The straight arrows indicate the times of the atmosphere change.

atmospheric conditions, whereas it has previously been demonstrated at low temperatures or high CO_2 partial pressures (7, 17, 25). The isoprene emission rate decreased slightly following the switch to low O_2 in this leaf.

Oxygen concentrations between 2 and 15% resulted in stimulated photosynthesis rates, compared to those observed at 2% O_2 (Fig. 5). At O_2 concentrations above 15%, photosynthesis rates were inhibited relative to those observed at 2%, but the inhibition was considerably less than that typically observed for C_3 leaves (11). Once again, this pattern is typical of O_2-insensitive photosynthesis (25). When limited by phosphate, the presence of O_2 can stimulate photosynthesis through the photorespiratory release of phosphate by phosphoglycolate phosphatase. The isoprene emission rate decreased by 25 to 35% as the O_2 concentration decreased from 28% to 2%.

The results presented in Figures 4 and 5 suggest that the isoprene emission rate increases or remains constant when the O_2 concentration is decreased in the absence of O_2-insensitive photosynthesis, and decreases in the presence of O_2-insensitive photosynthesis. Because O_2-insensitive photosynthesis has been related to phosphate limitations (7, 26), we examined the effect of mannose fed through the stem on the rate of isoprene emission. Mannose feeding is known to result in decreased availability of Pi for photosynthesis (27), and induces O_2-insensitive photosynthesis (7). In the present study, feeding with 10 mM mannose resulted in dramatic decreases in both isoprene emission rate and photosynthesis rate within 20 min (Fig. 6). These decreases were not due to stomatal closure and limitations to the intercellular CO_2 supply, since calculated intercellular CO_2 partial pressures increased from 228 to 294 μbar following the mannose treatment (data not shown).

Dependence of Isoprene Emission Rate on CO_2 Partial Pressure and Stomatal Conductance

The CO_2 dependence of photosynthesis in quaking aspen leaves was typical of previous reports for other aspen species (Fig. 7) (6). In two of the three leaves examined, isoprene emission rates remained nearly constant as the CO_2 partial pressure was decreased from normal ambient values (325–350 μbar external to the leaf) to values near the compensation point (Fig. 7). In a third leaf the rate increased by 21% over the same range of CO_2 partial pressures. All three leaves exhibited substantial reductions in isoprene emission rate as CO_2 partial pressures were increased to 640 μbar external to the leaf.

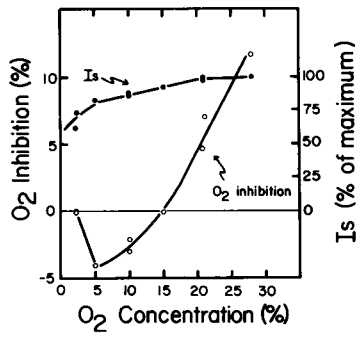

Figure 5. Responses of oxygen inhibition of photosynthesis (measured as a percentage of the rate in 2% O_2; $A_{2\%} - A_{x\%}/A_{2\%}$) and isoprene emission rate (measured as a percentage of the maximum observed rate) to atmospheric O_2 concentration. The points were derived from two experiments.

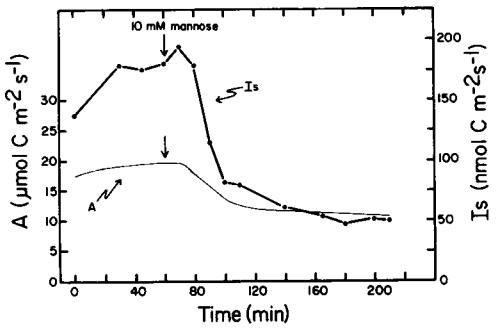

Figure 6. Responses of photosynthetic assimilation rate (A) and isoprene emission rate (Is) to the addition of 10 mM mannose to the transpiration stream. Environmental conditions were as described for the "standard environment" in "Materials and Methods."

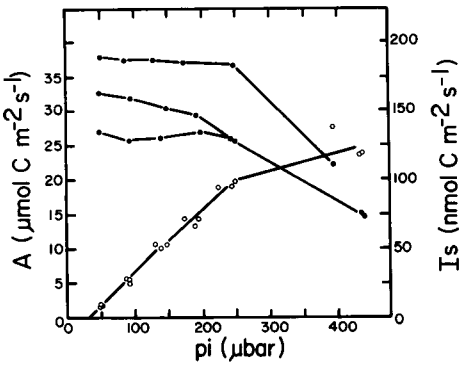

Figure 7. Responses of photosynthetic assimilation rate (A; open symbols) and isoprene emission rate (Is; closed symbols) to intercellular CO_2 partial pressure (pi). The points represent measurements from three different experiments.

In order to evaluate whether this inhibition of isoprene emission rate by high CO_2 was related to stomatal closure, experiments were conducted in which stomatal conductance was varied through adjustments to the leaf-to-air water vapor concentration gradient (Δw) in both normal and elevated CO_2 partial pressures. Increases in Δw resulted in sharp declines in stomatal conductance in two separate experiments when analyzed in normal CO_2 partial pressures (Fig. 8). The rate of isoprene emission did not decrease as sharply as stomatal conductance. Additionally, rates of isoprene emission were similar in magnitude despite a 1.5-fold difference in the absolute stomatal conductances of the two leaves. These results suggest that isoprene emission is not tightly linked to stomatal conductance. This hypothesis was tested further by examining the effects of abscisic acid (ABA) on stomatal conductance and the rate of isoprene emission (Fig. 9). Upon feeding a leaf with 10 μM ABA, both stomatal conductance and photosynthesis rate declined in parallel within 20 min. The ABA treatment resulted in an 86% reduction in stomatal conductance and a 70% reduction in photosynthesis rate. The

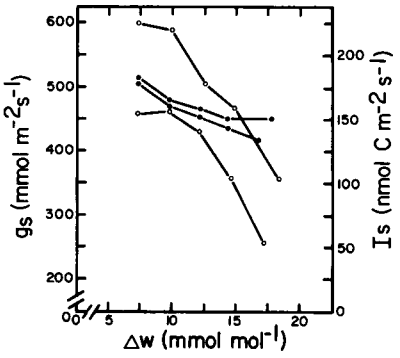

Figure 8. Responses of stomatal conductance (gs; open symbols) and isoprene emission rate (Is; closed symbols) to the leaf-to-air water vapor concentration gradient (Δw). Points represent the results from two different leaves.

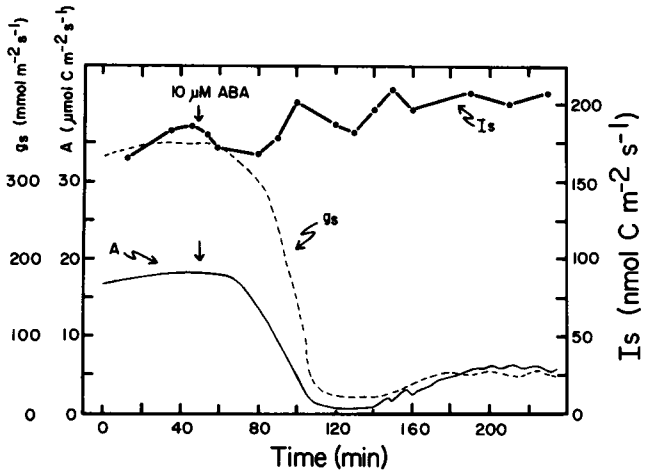

Figure 9. Responses of photosynthetic assimilation rate (A), stomatal conductance (gs), and isoprene emission rate (Is) to the addition of 10 μM abscisic acid (ABA) to the transpiration stream.

rate of isoprene emission, however, increased slightly over the same time period.

When isoprene emission rate was plotted as a function of stomatal conductance, it was evident that there was only a slight correlative relationship (Fig. 10). However, there was an obvious inhibitory effect of elevated CO_2 on isoprene emission rate that was not related to decreases in stomatal conductance. This is evident in that at equal stomatal conductances the rate of isoprene emission is lower at 640 μbar ambient CO_2 than at 340 μbar.

Temperature Dependence of Isoprene Emission Rate

The Q_{10} of isoprene emission was 2.4 between 30 and 40°C for one of the experimental leaves and 1.8 for the second leaf (Fig. 11a). The percentage of carbon assimilation that was emitted as isoprene (on carbon atom basis) increased from approximately 0.4% at 20°C to 8.4% at 42.5°C (Fig. 11b).

DISCUSSION

Previous research into the relationship between isoprene emission and photosynthesis has concluded that isoprene

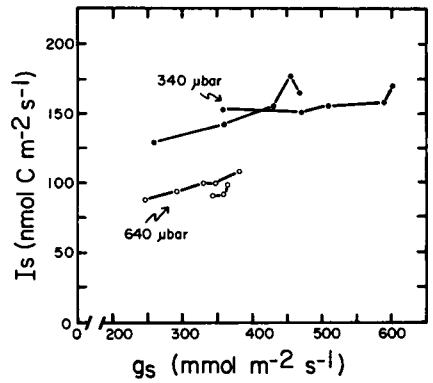

Figure 10. Relationship between isoprene emission rate (Is) and stomatal conductance (gs) at two ambient CO_2 partial pressures. The points represent results from four different experiments with those from each experiment connected by a common line.

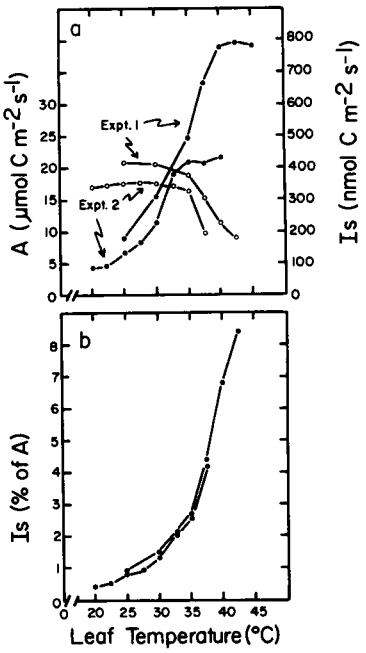

Figure 11. a, The responses of photosynthetic assimilation rate (A; open symbols) and isoprene emission rate (Is; closed symbols) to leaf temperature. The points represent results obtained from two different experiments. b, The isoprene emission rate (expressed as a percentage of the photosynthetic assimilation rate on a carbon atom basis) as a function of leaf temperature. The points are for the same experiments presented in panel a.

biosynthesis occurs with carbon derived from photosynthetic carbon reduction cycle intermediates (19). However, other studies with inhibitors of photorespiration have led to a reinterpretation of the link between carbon reduction-cycle intermediates and isoprene biosynthesis (5). These workers suggested that isoprene is synthesized from carbon that flows through the photorespiratory pathway. This suggestion was supported by evidence that chloroplastic terpenoids can be synthesized from isotopically labeled glycolate (24). Recent review articles discussing the nature of isoprene emission from leaves have relied on these previous studies to conclude that

isoprene biosynthesis is probably linked to photorespiration through the utilization of photorespiratory intermediate metabolites (8, 12).

The results of the present study are not consistent with a direct, and exclusive, link between photorespiratory metabolites and isoprene biosynthesis in quaking aspen leaves. In the presence of 2% O_2, a condition which should suppress photorespiratory metabolism to negligible levels, we observed no substantial reductions in isoprene emission, unless there was evidence of phosphate-limitations to photosynthesis. Even with no O_2 present, the isoprene emission rate was near its maximum. If isoprene emission rate were directly linked to the use of photorespiratory metabolites we would have expected greater reductions in isoprene emission when photorespiration was inhibited. Under conditions of O_2-insensitive photosynthesis, which has been related to chloroplastic phosphate-limitations (4, 26), slight reductions in isoprene emission were observed under low O_2 (Fig. 4b). Such reductions were not evident in the absence of O_2-insensitive photosynthesis (Fig. 4a). (Although in the results of Fig. 4a isoprene emission rates were reduced after reaching a peak in 2% O_2, we interpret the reductions as being coincident with the onset of slightly reduced photosynthesis rates as the leaf slowly exhibits evidence of O_2-insensitive photosynthesis. Note the slow decline in photosynthetic rate in low O_2.) The results presented in Figure 5 demonstrate that O_2-insensitive photosynthesis was common in the experimental leaves under normal environmental conditions.

The fact that isoprene emission rate was so dramatically inhibited by mannose is consistent with a link between isoprene biosynthesis and the export of photosynthetically derived carbon from the chloroplast via the phosphate translocator found in the chloroplast envelope. Such a requirement for phosphate-driven transport would explain the apparent negative correlation between isoprene emission rate and oxygen-insensitive photosynthesis. Thus, under phosphate-limited conditions photosynthesis would exhibit O_2 insensitivity and isoprene emission rate would be reduced.

There is an alternative explanation to account for the inhibition of isoprene emission rate by mannose. Since mannose ties up cytoplasmic phosphate, it could inhibit the ATP-dependent synthesis of isopentenyl pyrophosphate from mevalonic acid. If isopentenyl pyrophosphate is a direct precursor of isoprene, a decrease in its pool size could inhibit isoprene synthesis. This explanation could also be consistent with the observations that isoprene emission rate is inhibited under conditions of O_2-insensitive photosynthesis. Phosphate limitations under O_2-insensitive photosynthesis could also result in reduced rates of production of ATP (26), inhibiting the biosynthesis of isopentenyl pyrophosphate. At present, this hypothesis seems as viable as that describing a link between isoprene emission rate and carbon transport from the chloroplast.

If isoprene biosynthesis and emission is dependent upon maintenance of carbon flow from the chloroplast, then it does not necessarily have to be linked to the instantaneous rate of CO_2 assimilation. Studies of photosynthetic metabolite concentrations as a function of intercellular CO_2 partial pressure have revealed that even near the CO_2 compensation point, when the instantaneous rate of net CO_2 assimilation is low, the concentration of triose phosphates is near maximum (1, 31). Presumably, some of the triose phosphates are derived from starch breakdown at these low photosynthesis rates. Thus, as long as triose phosphates continued to be synthesized in the chloroplast the rate of isoprene biosynthesis and emission could be uncoupled from the instantaneous rate of net photosynthesis. In the present study, the emission of isoprene required CO_2 or O_2 in the atmosphere (Fig. 1). This suggests that either photosynthesis or photorespiration is required for isoprene synthesis. Such a requirement might be explained if CO_2 or O_2 assimilation are required to maintain the photosynthetic electron-transport system in a state capable of generating the levels of ATP and/or NADPH needed for isoprene biosynthesis. Weis and Berry (32) have demonstrated that there is feedback control of the photosynthetic electron-transport system by CO_2 assimilation. Thus, if O_2 or CO_2 assimilation are absent, the rate of production of NADPH and ATP could decrease to levels that inhibit isoprene synthesis.

Recent studies by Schulze-Siebert and Schultz (23) and Lütke-Brinkhaus *et al.* (9) have suggested that isoprenoids are synthesized in the chloroplastic, mitochondrial, and cytoplasmic compartments. It is not known which site is responsible for synthesis of the isoprene that is emitted from plant leaves. The hypothesis that phosphate exchange between the chloroplast and cytoplasm is involved in supplying carbon for isoprene biosynthesis and emission would be consistent with at least part of the isoprene being derived through cytoplasmic synthesis.

The requirement of light for isoprene emission and the similarities in the light responses of photosynthesis and isoprene emission rate are consistent with involvement of the photosynthetic electron transport system in supplying reductant and/or ATP (through photophosphorylation) for isoprene synthesis. A similar conclusion was derived from experiments of the action spectrum of isoprene emission (20). The synthesis of hydrocarbon isoprene from photosynthetic products (*e.g.* triose phosphates) must require significant amounts of reductant, probably NADPH. At present, it is not known how such reductant or ATP interacts with the cytoplasmic, mitochondrial, or chloroplastic processes that synthesize isoprene.

A number of previous studies have demonstrated the effect of changes in ambient CO_2 concentrations on the rate of isoprene emission (5, 21, 29). Some of these studies have reported that the isoprene emission rate is highest when CO_2 concentrations are low (5, 21). In fact, this observation has been used to argue in support of a connection between isoprene emission and photorespiration, since low CO_2 concentrations result in elevated photorespiration rates. In quaking aspen, we observed a slight enhancement of isoprene emission rate at low intercellular CO_2 partial pressures in some measurements (Fig. 7). However, some leaves exhibited no enhancement, and in those that did exhibit enhancement the increased rates of isoprene emission seemed small relative to the presumed effect on photorespiration. More experimental work needs to be done before a complete understanding of the low CO_2 effect on isoprene emission is possible. The inhibitory effect of above-ambient CO_2 partial pressures on

isoprene emission rates was consistent for all three of the leaves we examined. Similar inhibitory effects have been reported in past studies (5, 21, 29). The high CO_2 effect is not due to stomatal closure, since it was observed at similar stomatal conductances (Fig. 10). Elevated CO_2 partial pressures could inhibit isoprene biosynthesis by (a) inducing phosphate imbalances within the chloroplast and cytoplasm, as suggested for some C_3 species (25), or (b) the elevated photosynthesis rates that result from increased CO_2-substrate availability could inhibit isoprene biosynthesis by competing with isoprene for reductant. Whatever the underlying reason for the reduced rates of isoprene emission, the ultimate result will have important implications for modeling efforts aimed at quantifying the role of isoprene emission in tropospheric chemical reactions in light of evidence for increasing atmospheric CO_2 concentrations (12).

The rate of isoprene emission was not significantly affected by low stomatal conductances in ABA-treated leaves (Fig. 9). Previous studies have interpreted the relationship between isoprene emission and stomatal opening in conflicting ways (5, 29). In the present study, the presence of high rates of isoprene emission despite low stomatal conductances suggests that isoprene diffuses readily through the cuticle of quaking aspen leaves. This is in contrast to the previous suggestion by Tingey *et al.* (29) that isoprene emission through the cuticle of the live oak represents a minor fraction of that emitted through stomata. In the present study, however we noted that increases in Δw (*i.e.* decreases in ambient humidity) resulted in decreases in stomatal conductance, as well as decreases in isoprene emission rate. The correlated decreases in these two parameters may be coincidental. Low ambient humidities have been suggested to result in decreased cuticular conductances to water vapor diffusion (22). Induction of reduced cuticular conductances to isoprene diffusion by low humidity might underlie the reduced isoprene emission rates that were observed at high Δw values. At present, it is not known how humidity might interact with cuticular structure to impede emission of nonpolar isoprene molecules.

As in previous studies with whole plant canopies (30), we observed a strong dependence of isoprene emission rate on leaf temperature. The Q_{10} values near two suggest that the temperature response reflects an interaction between temperature and the enzymic determinants of isoprene biosynthesis. At temperatures between 35 and 42.5°C isoprene emission represented a substantial fraction of photosynthetic CO_2 assimilation (2.5–8.0%). Thus, during hot midsummer days isoprene emission could represent a significant carbon cost to some plants.

The reasons underlying the evolution of isoprene emission in some plants are yet to be determined. The results of the present study, and previous studies (2), demonstrate that under certain environmental conditions isoprene emission can represent a significant cost to plants. However, the results presented in Figure 3 demonstrated that the cost of isoprene emission is, to some degree, matched to the carbon-gain capacity of leaves. At present there are no obvious benefits to isoprene emission. It may be that isoprene emission simply represents leakage of precursors for the various terpenoid compounds synthesized by leaves. Thus, the emission may represent a cost of maintaining active precursor pools which are available for rapid mobilization.

The demonstration of a probable link between isoprene emission rate and the electron transport reactions of photosynthesis may have useful consequences for future modeling efforts. Most current models of isoprene flux from vegetation to the atmosphere rely on empirically derived algorithms from a limited number of species. With a metabolic link to photosynthetic processes, progress can be made toward developing basic mechanistic models that define isoprene emission within the constraints of the photosynthetic capacity. Such models should have applicability to a larger number of species. Our current research efforts are aimed toward this latter goal.

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LITERATURE CITED

1. **Badger MR, Sharkey TD, vonCaemmerer S** (1984) The relationship between steady-state gas exchange of bean leaves and the levels of carbon-reduction-cycle intermediates. Planta **160:** 305–313
2. **Evans RC, Tingey DT, Gumpertz ML, Burns WF** (1982) Estimates of isoprene and monoterpene emission rates in plants. Bot Gaz **143:** 304–310
3. **Fall R, Albritton DL, Fehsenfeld FC, Kuster WC, Goldan PD** (1988) Laboratory studies of some environmental variables controlling sulfur emissions from plants. J Atmos Chem **6:** 341–362
4. **Harris GC, Cheesbrough JK, Walker DA** (1983) Measurement of CO_2 and H_2O vapor exchange in spinach leaf discs. Effects of orthophosphate. Plant Physiol **71:** 102–107
5. **Jones CA, Rasmussen RA** (1975) Production of isoprene by leaf tissue. Plant Physiol **55:** 982–987
6. **Jurik TW, Weber JA, Gates DM** (1984) Short-term effects of CO_2 on gas exchange of leaves of bigtooth aspen (*Populus grandidentata*) in the field. Plant Physiol **75:** 1022–1026
7. **Leegood RC, Furbank RT** (1986) Stimulation of photosynthesis by 2% oxygen at low temperatures is restored by phosphate. Planta **168:** 84–93
8. **Loomis WD, Croteau R** (1980) Biochemistry of terpenoids. *In* PK Stumpf, ed, The Biochemistry of Plants. A Comprehensive Treatise, Vol 4. Academic Press, New York, pp 363–418
9. **Lütke-Brinkhaus M, Liedvogel B, Kleinig H** (1984) On the biosynthesis of ubiquinones in plant mitochondria. Eur J Biochem **141:** 537–541
10. **Monson RK, Moore BD, Ku MSB, Edwards GE** (1986) Co-function of C_3 and C_4-photosynthetic pathways in C_3, C_4 and C_3-C_4 intermediate *Flaveria* species. Planta **168:** 493–502
11. **Monson RK, Stidham MA, Williams GJ, Edwards GE, Uribe EG** (1982) Temperature dependence of photosynthesis in *Agropyron smithii* Rydb. I. Factors affecting net CO_2 uptake in intact leaves and contribution from ribulose 1,5-bisphosphate carboxylase measured *in vivo* and *in vitro*. Plant Physiol **69:** 921–928
12. **Mooney HA, Vitousek PM, Matson PA** (1987) Exchange of materials between terrestrial ecosystems and the atmosphere. Science **238:** 926–932

13. **Ohta K** (1986) Diurnal and seasonal variations in isoprene emission from live oak. Geochem J **19:** 269–274
14. **Rasmussen RA** (1970) Isoprene: identified as a forest-type emission to the atmosphere. Environ Sci Technol **4:** 667–671
15. **Rasmussen RA** (1972) What do hydrocarbons from trees contribute to air pollution? J Air Pollut Control Assoc **22:** 537–543
16. **Rasmussen RA, Jones CA** (1973) Emission of isoprene from leaf discs of *Hamamelis*. Phytochemistry **12:** 15–19
17. **Sage RF, Sharkey TD** (1987) The effect of temperature on the occurrence of O_2 and CO_2 insensitive photosynthesis in field grown plants. Plant Physiol **84:** 658–664
18. **Sanadze GA** (1961) The absorption of molecular hydrogen by green leaves in light. Fiziol Rast **8:** 555–559
19. **Sanadze GA** (1969) Light-dependent excretion of molecular isoprene. Prog Photosynth Res **2:** 701–706
20. **Sanadze GA, Baazov DI** (1985) Enhancement of photosynthesis and isoprene emission in poplar leaves. Soobsch Akad Nauk Gruz SSR **118:** 597–600
21. **Sanadze GA, Tarkhnishvili GM** (1986) Effect of molecular oxygen on the biosynthesis of isoprene in leaves under conditions of photosynthesis-saturating light intensity. Dokl Acad Nauk SSR **286:** 501–503
22. **Schönherr J** (1982) Resistance of plant surfaces to water loss: transport properties of cutin, suberin, and associated lipids. *In* OL Lange, PS Nobel, CB Osmond, H Ziegler, eds, Physiological Plant Ecology II. Water Relations and Carbon Assimilation. Springer-Verlag, Berlin, pp 154–179
23. **Schulze-Siebert D, Schultz G** (1987) Full autonomy in isoprenoid synthesis in spinach chloroplasts. Plant Physiol Biochem 25: 145–153
24. **Shah SPJ, Rogers LJ** (1969) Compartmentation of terpene biosynthesis in green plants. Biochem J **114:** 395–405
25. **Sharkey TD** (1985) O_2-insensitive photosynthesis in C_3 plants. Its occurrence and a possible explanation. Plant Physiol **78:** 71–75
26. **Sharkey TD, Stitt M, Heineke D, Gerhardt R, Raschke K, Heldt HW** (1986) Limitation of photosynthesis by carbon metabolism. II. O_2-Insensitive CO_2 uptake results from limitation of triose phosphate utilization. Plant Physiol **81:** 1123–1129
27. **Sheu-Hwa C, Lewis CH, Walker DA** (1975) Stimulation of photosynthetic starch formation by sequestration of cytoplasmic orthophosphate. New Phytol **74:** 383–392
28. **Tingey DT, Evans RC, Bates EH, Gumpertz ML** (1987) Isoprene emissions and photosynthesis in three ferns—the influence of light and temperature. Plant Physiol **69:** 609–616
29. **Tingey DT, Evans R, Gumpertz M** (1981) Effects of environmental conditions on isoprene emission from live oak. Planta **152:** 565–570
30. **Tingey DT, Manning M, Grothaus LC, Burns WF** (1979) The influence of light and temperature on isoprene emission rates from live oak. Plant Physiol **47:** 112–118
31. **vonCaemmerer S, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153:** 376–387
32. **Weis E, Berry JA** (1987) Quantum efficiency of photosystem II in relation to energy dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta **894:** 198–208
33. **Zimmerman PR** (1979) Testing of hydrocarbon emissions from vegetation, leaf litter and aquatic surfaces, and development of a methodology for compiling biogenic emission inventories. Final Report, EPA-450/4-79-004
34. **Zimmerman PR, Chatfield RB, Fishman J, Crutzen PJ, Hanst PL** (1978) Estimation on the production of CO_2 and H_2 from the oxidation of hydrocarbon emission from vegetation. Geophys Res Lett **5:** 679–682