

ISOPRENE EMISSION FROM PLANTS

Thomas D Sharkey and Sansun Yeh

Department of Botany, University of Wisconsin, Madison, Wisconsin 53706;

e-mail: tsharkey@facstaff.wisc.edu, syeh2@students.wisc.edu

Key Words deoxyxylulose 5-phosphate/methyl erythritol 4-phosphate, evolution, photosynthesis, thermoprotection, volatile organic carbon

■ **Abstract** Very large amounts of isoprene are emitted from vegetation, especially from mosses, ferns, and trees. This hydrocarbon flux to the atmosphere, roughly equal to the flux of methane, has a large effect on the oxidizing potential of the atmosphere. Isoprene emission results from de novo synthesis by the deoxyxylulose phosphate/methyl erythritol 4-phosphate pathway in plastids. Dimethylallyl pyrophosphate made by this pathway is converted to isoprene by isoprene synthase. Isoprene synthase activity in plants has a high pH optimum and requirement for Mg^{2+} that is consistent with its location inside chloroplasts. Isoprene emission costs the plant significant amounts of carbon, ATP, and reducing power. Researchers hypothesize that plants benefit from isoprene emission because it helps photosynthesis recover from short high-temperature episodes. The evolution of isoprene emission may have been important in allowing plants to survive the rapid temperature changes that can occur in air because of the very low heat capacity of isoprene relative to water.

CONTENTS

INTRODUCTION	408
Atmospheric Chemistry	409
BIOCHEMISTRY OF ISOPRENE SYNTHESIS	410
Isoprene Emission Requires De Novo Synthesis	410
Isoprene Synthase	411
The Methylerythritol Phosphate Pathway Is the Source of DMAPP	412
Isotopic Fractionation	413
Isoprene Degradation	414
PHYSIOLOGY OF ISOPRENE EMISSION	414
Relative Rate of Isoprene Metabolism	414
Relationship Between Isoprene Emission and Photosynthesis	415
Wound Signals	417
Environmental Effects	417
BENEFITS OF ISOPRENE EMISSION	417
The Thermotolerance Hypothesis	418
Antioxidant	419

Getting Rid of Excess Energy or Carbon	420
REGULATION OF ISOPRENE EMISSION	420
Isoprene Emission and Stomatal Closure	420
The Guenther Model for Predicting Isoprene Emission	421
Physiological Regulation	422
EVOLUTIONARY CONSIDERATIONS	423
Phylogenetics of Isoprene Emission	423
Costs Versus Benefits of Isoprene Emission	423
Paleoclimate	425
FUTURE RESEARCH	427

INTRODUCTION

Isoprene (C₅H₈, 2-methyl 1,3-butadiene) is a natural product of many organisms (128). Sanadze & Kursunov discovered isoprene emission from plants in the 1950s (117). In a series of papers, he and his colleagues showed that a number of plants, especially trees, emit isoprene in a highly light- and temperature-dependent manner (112, 113, 116, 117). Rasmussen & Went discovered isoprene emission from plants independently of the work of Sanadze (106). It was not immediately accepted that plants made isoprene, and both Sanadze & Rasmussen eventually proved by mass spectrometry that the compound they saw was isoprene (102, 112).

Isoprene is well-known chemically as the root of the isoprenoid class of compounds. Researchers first made it by burning rubber and later by heating turpentine. The structural formula was identified in 1882 (152, also see citation in 111). Kekulé recognized that many components of turpentine oil had a C:H ratio of 5:8 and coined the term terpenoids for this class of compounds (85). Otto Wallach pointed out that the terpenes consist of a repeating, branched, five-carbon unit similar to isoprene, so terpenes are also known as isoprenoids. The generalization that terpenes could be seen as composed of isoprene units became known as the isoprene rule (85), which investigators used to work out structures of isoprenoids.

However, isoprenoids are not made from isoprene. The biological precursors to the isoprenoids are isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP), sometimes called the active isoprenes. Isoprene is a hemiterpene and is made from DMAPP (138). Monoterpenes (10 carbons) include many of the scents we associate with plants such as pine, lemon, and the smell of a freshly peeled orange. Adding IPP to DMAPP makes monoterpenes. Additional IPP molecules can be added to make sterols (C₃₀, made from two C₁₅ compounds) in the cytosol of plants and carotenoids (C₄₀, made from two C₂₀ compounds) in plant plastids. There are many other isoprenoids in plants (74, 159), but they are not discussed in this review.

Although the isoprenoids are very well known, much less is known about biological isoprene production because isoprene is not an intermediate in isoprenoid production and its concentration in the environment is low. These facts have led some authors to conclude, "Isoprene itself does not occur free in nature" (55, p. 10).

In fact, free isoprene is widespread in nature, and biogenic isoprene from plants is very important, both for plants and the atmosphere.

Recent estimates suggest that isoprene emission from plants is among the most important biosphere-atmosphere interactions. The total hydrocarbon flux from the biosphere to the atmosphere was estimated by Rasmussen & Went in 1965 (106) to be 432 Tg C yr⁻¹. The estimate for global isoprene emission is now about 500 Tg C yr⁻¹, making it the dominant hydrocarbon that moves from plants to the air, roughly equal to the flux of methane to the atmosphere (41, 162).

Atmospheric Chemistry

One of the more important reasons to understand isoprene emission from plants is its role in atmospheric chemistry (31, 151). Though isoprene is not a greenhouse gas, it can alter atmospheric chemistry, affecting the residence times of gases that do contribute to the greenhouse effect. Isoprene oxidation in the atmosphere can give rise to ozone and smog if nitrogen oxides are present in the atmosphere (18, 44). Some of the breakdown products include organic acids, CO, methacrolein, and methyl vinyl ketone (2, 158). When the nitrogen oxide levels are high, isoprene breakdown can lead to the formation of peroxyacetyl nitrate (PAN) and methyl peroxyacetyl nitrate (MPAN). Isoprene breakdown is the primary source of MPAN in the atmosphere (168). Isoprene breakdown in the atmosphere can be estimated from MPAN concentrations, which is useful because the concentration of isoprene in the atmosphere is normally very low (<10 ppb) (53, 105).

Another phenomenon associated with volatile isoprenoids from plants is the blue haze described by Went (164). Oxidation of monoterpenes causes them to stick together until they form a solid particle. These particles can act as cloud condensation nuclei. This is important because in some areas rainfall is limited by a lack of cloud condensation nuclei, which has given rise to the practice of cloud seeding in these areas. Raindrop size is also affected by the concentration of cloud condensation nuclei. These particles that are formed from monoterpenes also scatter light. When the particles are very small, blue light is scattered more than other colors, giving rise to the natural blue haze (164). By itself, isoprene does not cause particle formation, but it may contribute to their growth (64).

Despite early warnings from Rasmussen (103), Zimmerman (174), and then presidential candidate Reagan¹ (100), the role of isoprene emitted from plants (and other biogenic hydrocarbons) in atmospheric chemistry was ignored in early attempts to reduce ozone pollution. Early air quality regulations emphasized reduction of anthropogenic hydrocarbons, even if that meant some increase in nitrogen oxide emissions. This strategy was not effective (122). In 1987, Trainer et al (157) pointed out the importance of biogenic isoprene in clean environments. In 1988,

¹“Approximately 80% of our air pollution stems from hydrocarbons released by vegetation, so let’s not go overboard in setting and enforcing tough emission standards from man-made sources.”

Chamedies et al (12) suggested that so much isoprene was coming from vegetation in the Atlanta, Georgia (USA) area that air pollution control strategies had to switch to reducing NO_x to be effective.

The 1988 paper by Chamedies et al made many people aware of isoprene emission from plants, but not much more information was available. Since then significant progress has been made. Estimates and reviews concerning the magnitude of plant isoprene emission are available (41, 99). This review is the first to cover this topic in this series, but reviews of plant isoprene emissions have been published elsewhere recently (31, 32, 48, 57, 67, 75, 94).

BIOCHEMISTRY OF ISOPRENE SYNTHESIS

Isoprene Emission Requires De Novo Synthesis

Isoprene emission is different from emissions of the related and better-known monoterpenes such as the pinenes and limonene. Emission of monoterpenes is, in most cases, from pools of hydrocarbon stored in resin ducts, glands, or trichomes. Monoterpene emission is therefore dependent in large measure on its volatility (66) and on damage of leaves and needles (72, 155, 156). However, isoprene emission requires de novo synthesis; this is demonstrated by the following two examples. First, researchers made estimates of the amount of isoprene stored in leaves by changing environmental conditions from those conditions that promote isoprene emission to those conditions that do not. Taking the extreme assumption that synthesis stopped instantaneously, the amount of isoprene emission that occurred after the switch is an upper limit of the amount of isoprene stored in the leaf (82). In addition, leaves can be flash frozen, and then researchers can extract and measure isoprene to determine the total pool (28, 76). Both measures indicate very little isoprene storage in leaves.

Second, investigators measured the rate at which ^{13}C appeared in isoprene when the $^{12}\text{CO}_2$ in air flowing past leaves was replaced with $^{13}\text{CO}_2$ (20) to assess the contribution of de novo synthesis. The stable isotope of carbon appeared in all five carbon atoms of the isoprene molecule with kinetics similar to the rate of 3-phosphoglycerate labeling. The results of Delwiche & Sharkey (20) differ from those of Sanadze and his colleagues (114, 115) who had found that only one or two carbon atoms were labeled. Sanadze et al used a closed labeling system in which the carbon dioxide concentration most likely fell to the compensation point, and so most of the isoprene was made from carbon derived from starch breakdown. The steady-state labeling system used by Delwiche & Sharkey kept the CO_2 concentration constant throughout the labeling period, so the isotopic composition reflects the normal relationship between photosynthesis and isoprene emission.

Dependence on de novo synthesis means that the temperature dependence of isoprene emission is not related to its volatility but rather to its metabolism. Isoprene emission increases with increasing leaf temperature. However, two distinct

phases can be separated. The fastest phase has a time constant of 8.2 s and is followed by a phase with a time constant of 116 s (142). Thus, for small changes in rate, isoprene emission changes as quickly as leaf temperature; though for larger rate changes, enzyme activation and other metabolic adjustments are needed. This observation is consistent with emission from de novo synthesis rather than control of emission rate by volatility.

The light requirement for isoprene synthesis is also consistent with a dependence on de novo synthesis. Recent work has shown that some species, especially some Mediterranean oaks, also emit monoterpenes in a light-dependent manner (80, 145). These trees emit monoterpenes from recently synthesized carbon like the case with isoprene (77, 79). A few western United States conifer species will emit the closely related molecule 2-methyl-3-buten-2-ol in a light-dependent manner (3), but researchers have not yet determined whether this is by de novo synthesis. In a few cases trees will emit some monoterpenes as a result of de novo synthesis, though other monoterpenes are emitted from storage pools (77, 79). *Trans* β -ocimene in particular can be emitted from Mediterranean Pine in a light-dependent manner while other monoterpenes are released from wounded needles (81).

Isoprene Synthase

Isoprene emitted from plants is made from DMAPP by isoprene synthase (138). This enzyme has a relatively high pH optimum and a requirement for Mg^{2+} (118, 139), consistent with its location inside chloroplasts (90, 166, 167). The molecular weight of isoprene synthase has been reported at 95 in *Quercus robur* (118), 73 in *Salix discolor* (165), and a doublet of 58 and 62 in aspen (139) and kudzu (CA Downs & TD Sharkey, unpublished data). A contributing factor to the difficulty in working with this enzyme is that plants that make substantial amounts of isoprene tend to be difficult to work with biochemically. Repeated attempts to measure isoprene emission from *Arabidopsis* have failed. (Note added in proof: The gene for isoprene synthase was sequenced after this review was written. *Arabidopsis* does not have an isoprene synthase gene.)

Wildermuth & Fall found some isoprene synthase activity bound to thylakoid membranes, although other isoprene synthase activity was found to be soluble (166, 167). The membrane-bound form appeared to have the same kinetics as the soluble form. Whether the soluble form is converted to the bound form or vice versa and what effect this might have on the activity of isoprene synthase is not known. Wildermuth (165) showed that thylakoid-bound isoprene synthase activity could be stimulated threefold by the addition of GTP and palmitoyl CoA. There is potential for regulation by this mechanism, but studies to investigate this have not yet been carried out.

Investigators have suggested that DMAPP is converted to isoprene noncatalytically in animals (22). Acid will catalyze the conversion of DMAPP to isoprene, but this reaction is slow and it seems unlikely to explain isoprene emission from

humans. Bacteria also make isoprene (60, 160). However, *Escherichia coli* makes more isoprene when fed tryptone media than when fed minimal media. Perhaps isoprene is a product of the breakdown of higher isoprenoids in *E. coli*. This could explain why there is no gene sequence in the fully sequenced *E. coli* genome that corresponds to isoprene synthase of *Populus* sp. (W Zimmer, D Gong, & TD Sharkey, unpublished). In *Bacillus subtilis*, isoprene emission occurs during distinct phases of culture growth (161).

The Methylerythritol Phosphate Pathway Is the Source of DMAPP

Two major metabolic pathways make DMAPP, the mevalonic acid (MVA) pathway and the recently discovered 2-deoxyxylulose 5-phosphate/2-methylerythritol 4-phosphate (MEP) pathway (108) (Figure 1). There are other ways that the five-carbon branched chain can be made, especially during leucine metabolism, but these are probably only minor sources of isoprenoids. The source of DMAPP within the chloroplast is the MEP pathway (109). The MEP pathway begins with pyruvate and glyceraldehyde 3-phosphate (109) and involves skeletal rearrangement to make the branched chain (1). Lichtenthaler and colleagues showed that this pathway is responsible for most, and probably all, isoprenoids made in plastids (70).

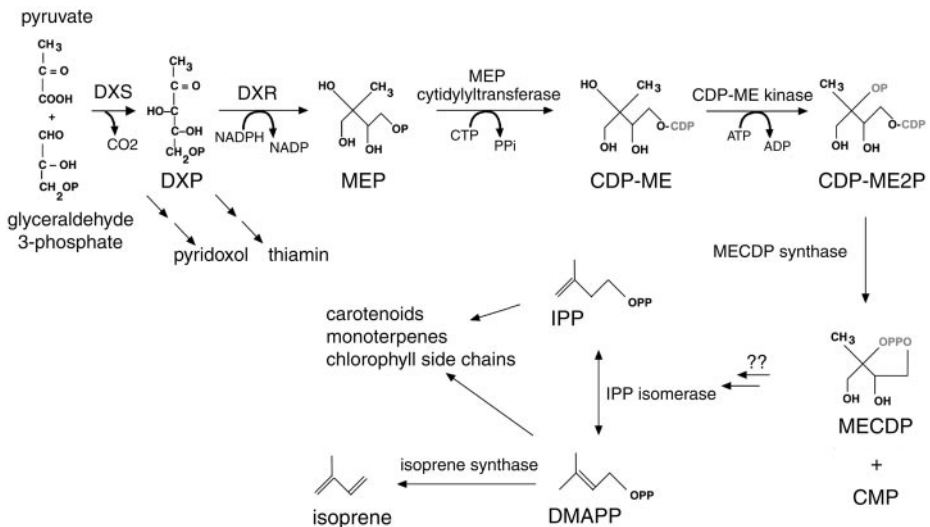


Figure 1 The MEP pathway is the source of DMAPP for isoprene synthesis. It is currently unknown whether this pathway makes IPP first, then DMAPP by isomerization, or if it makes DMAPP without first making IPP. CDP-ME, 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol; CDP-ME2P, 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol; DMAPP, dimethylallyl pyrophosphate; DXR, deoxyxylulose-5-phosphate reductoisomerase; DXS, deoxyxylulose-5-phosphate synthase; IPP, isopentenyl pyrophosphate; MECDP, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate.

TABLE 1 Energetics of the MEP pathway in photosynthetic organisms. It is assumed that each carbon in the starting intermediates costs 3 ATPs plus 2 NADPH if at the redox level of a triose phosphate with CTP equivalent to ATP. This estimate differs from that of Niinemets et al (96) because of the additional steps now known in the MEP pathway

	ATP	NADPH
Glyceraldehyde 3-phosphate	9	6
Pyruvate	8	5
Deoxyxylulose reductoisomerase		1
MEP cytidyltransferase	2	
4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase	1	
Two hypothetical reduction steps	2	
Total	20	14

They specifically proved that this pathway is responsible for isoprene synthesis (120, 171). This pathway was recently reviewed in this series (69), and since that review three additional steps in the pathway have been reported. These steps include the addition of CTP to MEP (62), a phosphorylation (63), and cyclization (148). The MEP pathway can make IPP (88), which researchers presume can be isomerized to DMAPP. However, in *E. coli* the MEP pathway can make both IPP and DMAPP without the need for IPP isomerase (107). Investigators have yet to determine whether IPP isomerase is required for isoprene synthesis in higher plants.

The new knowledge of the biosynthetic pathway for isoprene allows the energy cost of isoprene emission (132) to be revised (Table 1). Using the MVA pathway, isoprene emission costs 9 carbon atoms, 24 ATP, and 14 NADPH. However, the MEP pathway is more efficient than the MVA pathway in photosynthetic organisms, and isoprene emission based on the MEP pathway costs only 6 carbon atoms, 20 ATP, and 14 NADPH (Table 1). Although the cost is less when calculated for the MEP pathway, it is still substantial, and any benefits ascribed to isoprene emission will have to be weighed against this cost in both carbon and energy.

Isotopic Fractionation

Isoprene synthesis discriminates against the heavier natural isotope of carbon, ^{13}C (133). This was originally interpreted in terms of the mevalonic acid pathway, which starts with acetyl CoA. However, the current finding that isoprene is made by the MEP pathway results in a need to reinterpret these data. Isoprene is isotopically lighter than the carbon it is presumably made from, but it is not clear where the discrimination occurs. One candidate for the cause of this discrimination is the deoxyxylulose synthase because it decarboxylates pyruvate in a reaction similar to the one that is responsible for discrimination in the formation of acetyl CoA (23).

Isoprene Degradation

Plants do not break down isoprene. When radioactive isoprene was passed over leaves of oak (an isoprene emitter) or *Phaseolus vulgaris* (a nonemitter), no radioactivity stayed in the plant (PJ Vanderveer & TD Sharkey, unpublished data). In humans and other animals, isoprene is probably converted to an epoxide. The diepoxide may be toxic; but isoprene is less likely to form the diepoxide than is 1,3 butadiene, and so isoprene is less toxic (7, 16, 17, 89).

Bacteria can metabolize isoprene (13), and some types of bacteria can use it as their sole carbon source (27). This metabolism involves addition of glutathione to the epoxide formed from isoprene, as well as additional steps that might lead to acetyl CoA through β -oxidation. Globally, this metabolism is likely to be small relative to emission from plants, but this metabolism could be important in soil ecology (27).

PHYSIOLOGY OF ISOPRENE EMISSION

Isoprene emission is a major metabolic pathway in some plants. Because the flux through isoprene metabolism is high relative to other processes such as carotenoid synthesis, it is likely that the regulation of the MEP pathway is geared to the requirements of isoprene synthesis. Isoprene synthesis physiology also includes sensitivity to a number of environmental parameters, most notably temperature and light.

Relative Rate of Isoprene Metabolism

Isoprene synthesis of major emitting plant species such as oak and aspen is typically 2% of photosynthesis at 30°C. In some cases, however, isoprene emission can account for substantially more of photosynthesis (130, 136). In nonphysiological conditions, leaves can easily be in negative carbon balance (net loss of carbon by isoprene emission) by having substantial isoprene emission at or below the CO₂ compensation point (82, 91).

Relative to the rate of isoprenoid synthesis in leaves, isoprene synthesis is by far the dominant product of the MEP pathway. Although isoprene emission is typically 2% of photosynthesis, carotenoid synthesis is 0.02 % of photosynthesis (119). Moreover, isoprene synthesis begins sometime after the leaf has reached full photosynthetic competence (92) and is no longer accumulating carotenoids (except in cases of changes in growth light levels).

Sanadze had postulated that isoprene synthesis competes with fatty acid synthesis for acetyl CoA. He found that feeding cerulenin, a fatty acid synthesis inhibitor, increased the rate of isoprene emission (113) (confirmed by F Loreto & TD Sharkey, unpublished data). However, the increase in isoprene emission was much greater than the normal rate of fatty acid synthesis [fatty acid synthesis may be about 0.03% of photosynthesis (119) or about 1% of the rate of isoprene emission]. In any case, researchers now know that isoprene is made by the MEP

pathway and so does not directly compete with fatty acid synthesis for acetyl CoA. Why cerulenin increases isoprene emission remains unclear.

Relationship Between Isoprene Emission and Photosynthesis

Light Isoprene emission is dependent on photosynthesis, especially the photosynthetic carbon reduction cycle (PCRC or Calvin cycle). The earliest reports of isoprene emission described its dependence on light (112, 116, 117), and the wavelength dependence was shown to be similar to that of photosynthesis (104). In most cases, isoprene emission saturates with the same light level as photosynthesis, but in some cases isoprene emission increases with increasing light intensity after photosynthesis is saturated (46, 68, 130, 131). The increase in the rate of isoprene emission can be caused by light activation of isoprene synthase, activation of the MEP pathway providing substrate, or some combination of these effects (29). For example, Fall & Wildermuth (29) reported that changes in pH and Mg that normally occur in thylakoids in response to light can cause an 11-fold stimulation in isoprene synthase activity, and a further 3-fold stimulation of activity was obtained by adding GTP and palmitoyl CoA.

Temperature The temperature dependence of isoprene emission is very different from that of photosynthesis. Photosynthesis of C₃ plants in 350 ppm CO₂ normally exhibits a very broad temperature optimum with maximal rates at 30°C or below. The V_{max} of rubisco increases with approximately the same temperature dependence as the K_m for CO₂, and the overall rate is proportional to V_{max}/K_m at 350 ppm CO₂ (30). Isoprene emission, on the other hand, is very sensitive to temperature. The activation energy is between 60 and 90 kJ mol⁻¹ (46, 135), which corresponds to a Q₁₀ of 2 to 4 depending on the temperature range. The proportion of fixed carbon emitted as isoprene increases rapidly with temperature. Because photosynthesis is constant or declining and isoprene emission is increasing with temperature above 30°C, at 30°C typically 2% of carbon fixed by photosynthesis is emitted as isoprene; but at 40°C 15% is emitted (136).

Carbon Dioxide The responses of photosynthesis and isoprene emission to carbon dioxide are reverse to their temperature responses: Photosynthesis is highly sensitive but isoprene is relatively insensitive. In CO₂-free air, isoprene emission can be reduced 50% or more from its peak rate (82, 91). With 50 ppm CO₂, isoprene emission reaches a broad maximum and sometimes declines slightly at high (500 ppm) CO₂. The decline at high CO₂ is more pronounced in low oxygen atmospheres and at low temperature (82), consistent with an ATP dependence of isoprene emission. At high CO₂ and moderate to low temperature, feedback from carbon metabolism can limit ATP synthesis (137).

Isoprene emission in CO₂-free air can be 50% of maximal rates; but if oxygen is also removed, isoprene emission stops (82). The interpretation of this phenomenon is that isoprene emission requires an active PCRC. In CO₂-free air containing

oxygen, photorespiration will keep the PCRC active as starch reserves are mobilized. However, without oxygen or CO₂, the PCRC is inhibited causing isoprene emission to cease (141), which also explains away the evidence for a link between photorespiration and isoprene emission (74) or carotenoid synthesis (54, 123). By feeding intermediates of photorespiration to CO₂-starved leaves, Jones & Rasmussen provided a carbon source that was essential for isoprene synthesis. The fact that these were intermediates of photorespiration did not indicate a link between photorespiration and isoprenoid metabolism, only that it requires an active photosynthetic carbon reduction cycle. Hewitt et al showed that photorespiration and isoprene emission are not linked (50).

Inhibitors The requirement for an active PCRC probably explains why almost all inhibitors of photosynthesis inhibit isoprene emission (82). Among inhibitors that inhibit both processes are some that affect one or the other process a little more rapidly (82). One inhibitor in this class is methyl viologen, which catalyzes pseudocyclic electron transport. Methyl viologen initially reduces the availability of NADPH but increases availability of ATP. Methyl viologen fed to oak leaves caused isoprene emission to increase as photosynthesis started to decline, but the effect was for only a short time. As the PCRC stopped so did isoprene emission (82). The methyl viologen results are consistent with a big role for ATP availability in regulating the rate of isoprene emission; a conclusion strengthened by finding a correlation between whole leaf ATP content and isoprene emission rate (84). These results have been interpreted in terms of the requirement of the MVA pathway for ATP at the final steps. However, the presumed ATP cost of the MEP pathway is less than that of the MVA pathway (Table 1). Nevertheless, ATP status probably affects the rate of isoprene synthesis, perhaps through the 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase or through regulatory effects on isoprene synthase. Another compound that causes an initial increase in isoprene emission before it is inhibited is D,L glyceraldehyde (113).

There are two exceptions to the rule that photosynthesis (or at least the PCRC) and isoprene are always inhibited together. The first is an unpublished observation by S Gosh & TD Sharkey. They found that cytosolic protein synthesis inhibitors (e.g. cycloheximide) reduced photosynthesis without reducing isoprene emission. In those experiments it appeared that the inhibitor was stopping photosynthesis without stopping the PCRC. There is one way this could happen. If both starch and sucrose synthesis were stopped, carbon assimilation would have to slow to the rate at which amino acids could serve as the end products of photosynthesis. In other words, leaves fed these inhibitors would become limited by triose phosphate utilization (126). Loss of oxygen sensitivity confirmed this hypothesis (124). S Gosh & TD Sharkey (unpublished results) found that photosynthesis reduced by feeding cycloheximide became insensitive to changes in oxygen level, confirming that the leaves were limited by triose phosphate use. The interpretation of these experiments is that triose phosphate use limitation does not restrict the PCRC and so does not restrict isoprene emission. The second case of an inhibitor inhibiting

only one of the two processes is fosmidomycin, the inhibitor of 2-deoxyxylulose 5-phosphate reductoisomerase (61, 170). Zeidler et al (170) showed that isoprene emission was eliminated by low levels of fosmidomycin, and Sharkey et al (129) showed that photosynthesis was unaffected by fosmidomycin.

Wound Signals

Isoprene emission is a sensitive indicator of wound signals that can travel through plants (83). Wounding was inflicted by puncturing, smashing, cutting, and, burning leaves, the last of these being the most effective. The effect was greatest when a large interface between damaged and undamaged leaf surface was left. By wounding one leaf while monitoring isoprene emission from a different leaf, researchers could show the transmission of a signal. The time between wounding one leaf and a change in rate of isoprene emission of a different leaf was linearly related to the distance between the two leaves, allowing a calculation of travel rate of the signal. The signal traveled about 2 mm s^{-1} , which is likely to result from electrical signals traveling through the plant. The calcium chelator EGTA substantially delayed the wound signal effect on isoprene emission, indicating that the electrical signal may have caused calcium fluxes that ultimately affected isoprene emission.

Environmental Effects

Isoprene emission can be affected by nitrogen nutrition (47, 71). Trees with low nitrogen availability had lower rates of isoprene emission than did trees with higher nitrogen nutrition. This effect interacted with light. Trees grown in sun or shade but with low nitrogen availability had similar, low rates of isoprene emission; but trees with high nitrogen availability emitted substantially more isoprene when grown in the sun than when grown in the shade.

When plants were water-stressed, isoprene emission changed little, even when photosynthesis fell to zero (153). However, upon rewatering, isoprene emission increased several fold above the prestress rate and stayed high for several weeks (130). Apparently the reduced photosynthesis caused by water stress did not prevent increased rates of isoprene emission. On the other hand, defoliation may reduce isoprene emission by restricting whole plant carbon availability. Funk et al (35) defoliated trees to examine stress responses to whole tree carbon balance. They found that reducing the foliage carried by a tree reduced isoprene emission.

BENEFITS OF ISOPRENE EMISSION

What benefit, if any, do plants derive from isoprene emission? In other words, why do plants make isoprene? Explanations for other volatile emissions from plants are one place to look for answers (58). For example, acetone is probably lost as an unavoidable by-product of fatty acid metabolism (86); and methanol is emitted, possibly as a waste product of pectin methyl esterase (87, 95). However, neither

of these explanations seems to apply to isoprene emission. Some of the reasons suggested for isoprene emission include a flowering hormone (150), an antioxidant (171), and a metabolite overflow to get rid of excess carbon (73a, 161). However, the hypothesis that has been studied most is the thermotolerance hypothesis.

The Thermotolerance Hypothesis

Sharkey & Singaas (134) proposed that isoprene protects photosynthesis from damage caused by high leaf temperature. Holding leaves in darkness or in a nitrogen atmosphere to control endogenous isoprene synthesis, they assessed damage to photosynthesis as the temperature at which chlorophyll fluorescence increased (121). Singaas et al (141) showed that adding isoprene to an air stream (or nitrogen gas) that passed over these leaves could increase the temperature at which damage occurred from as low as 35°C to as high as 45°C. Another series of experiments determined the temperature at which photosynthesis, measured as CO₂ assimilation, fell to zero as leaves were heated (141). The zero temperature was related to the endogenous isoprene produced by the leaf. Leaf-to-leaf variation in isoprene emission provided a range of isoprene treatments. The zero-photosynthesis temperature increased with increasing endogenous isoprene emission. These experiments indicated that isoprene was having some effect on the temperature tolerance of photosynthesis, but none of the experiments were conclusive.

Given the tentative nature of the evidence, Logan & Monson's published statement that, "Thermotolerance of leaf discs from four isoprene-emitting species is not enhanced by exposure to exogenous isoprene," (73) was of substantial concern. They found that chlorophyll fluorescence of leaf discs held in darkness or light plus nitrogen did not increase until 45°C, regardless of whether isoprene was in the air stream. In their experiments the control leaf pieces did not exhibit photosynthetic damage below 45°C. Measurements of the temperature where CO₂ uptake fell to zero were not reported.

Two improvements have allowed definitive experiments. First, the thermotolerance hypothesis has been refined. Now, researchers hypothesize that isoprene protects against short high-temperature episodes (140, 142). Therefore, instead of heating leaves and determining where irreversible damage occurs (cook and look), investigators used a new protocol of measuring the recovery from a short high-temperature episode. The second improvement was the use of fosmidomycin, the inhibitor that eliminates isoprene production without affecting photosynthesis. With these improvements, much stronger evidence for the thermotolerance hypothesis has been obtained (129). Heating kudzu leaves to 46°C for two minutes caused photosynthesis to be eliminated. Twenty minutes after returning the leaf to 30°C, photosynthesis recovered by 90%. Photosynthesis of leaves fed fosmidomycin recovered only 60%. To show that the reduced recovery resulted from the loss of isoprene, researchers gave fosmidomycin-fed leaves physiological levels of isoprene in the air stream. With exogenous isoprene, recovery was similar to

leaves in which isoprene emission had not been inhibited (129). This result shows that isoprene synthesis provides tolerance of short high-temperature episodes. Monoterpenes emitted in a light-dependent manner also provide this type of thermoprotection. Repeated cycles of high-temperature stress give reduced recovery in leaves without isoprene or monoterpene, although leaves with isoprene or monoterpene maintain high rates of photosynthesis, especially after repeated periods of high temperature (19, 80, 129).

What property of the isoprene molecule provides thermotolerance? Molecules similar to isoprene were tested to see what is important for thermotolerance. One rule was apparent in the results. All alkenes tested (1,3-butadiene, 1-butene, and *cis* 2-butene) provided thermotolerance though alkanes (2-methyl-butane, n-butane, and *iso*-butane) did not; they even increased the damage caused by heat (129).

Speculation concerning the mechanism by which isoprene protects against short high-temperature episodes depends upon characteristics of the high temperature damage. An attractive idea for which data exists is that thylakoid membranes become leaky at moderately high temperature (9, 97). Isoprene could reside in the thylakoid membrane for a (short) time and enhance hydrophobic interactions. It could even block the formation of water channels because of the large volume of the double bonds. Another possibility is that excursions to high temperature allow separation of the mono- and digalactosyldiacylglycerides of the thylakoid membrane, resulting in nonbilayer structures (37). Another alternative is that high temperature excursions could allow large membrane-bound protein complexes (e.g. photosystem II) to fragment. Isoprene could enhance hydrophobic interactions within either membranes or protein complexes. Because each excursion to high temperature could result in more nonbilayer structures or more disrupted protein complexes, repeated high temperature episodes would progressively reduce the photosynthetic capacity. As leaves can be subject to dozens of high-temperature episodes each day (140, 142), the increased recovery from each episode afforded by isoprene could become very important to the plant.

Antioxidant

A second hypothesis that investigators have considered is that isoprene serves as an antioxidant in leaves. This idea is normally put forward on the basis of the rapid reaction of isoprene with ozone and hydroxyl radicals. Although often discussed by researchers (147), this hypothesis has little evidence to support or reject it. Hewitt et al (49) found that isoprene-emitting plants were more sensitive to ozone damage because of hydroperoxides formed on the plants, which points out the weakness with the antioxidant hypothesis: Isoprene will propagate radicals not quench them. The result of isoprene interaction with hydroxyl radicals in the atmosphere is simply more radicals, and the same could be true inside of leaves.

On the other hand, F Loreto (unpublished data) has found that exogenous isoprene can protect leaves against a short, acute exposure to a high concentration of ozone (300 ppb). On the day following exposure, the leaves without isoprene

developed large necrotic areas where cells had collapsed; leaves given isoprene with ozone showed little necrosis. One question that arises is whether the effect of isoprene is radical quenching or membrane strengthening. More work is required on the antioxidant hypothesis.

Getting Rid of Excess Energy or Carbon

Some people have speculated that isoprene emission may serve the plant as a safety valve, releasing excess carbon and energy (73a). However, the amount of energy that can be lost by this mechanism is small compared to the energy dissipation mechanisms associated with nonphotochemical quenching of chlorophyll fluorescence, zeaxanthin, (21) and possibly other thermal dissipation mechanisms (10). Similarly, Rubisco is deactivated when the leaf cannot use carbon as fast as the photosynthetic reactions can produce it (110, 125), which is a much more effective method of regulating carbon uptake during those rare times when there is excess carbon fixation capacity. Wagner et al (161) have proposed a more restricted version of this hypothesis to explain isoprene emission from *B. subtilis*. In this case, they proposed that a mismatch between DMAPP production and its use in higher isoprenoid synthesis or prenylation reactions can be remedied by isoprene emission. Metabolic pathways are commonly regulated to prevent carbon from accumulating in unusable intermediates. However, nothing is known about the regulation of the MEP pathway by which DMAPP for isoprene emission from bacteria is made (160), so it is possible that bacteria might need an overflow valve.

REGULATION OF ISOPRENE EMISSION

The regulation of isoprene emission is important for understanding physiological controls and for modeling isoprene emission in air quality models. Most work up to now has been directed toward improved air-quality modeling. For isoprene emission, two environmental controls are normally considered: light and temperature (26, 153, 154). Stomatal control need not be considered.

Isoprene Emission and Stomatal Closure

Isoprene emission is not controlled by stomatal closure (91), even though isoprene exits the leaf through the stomata (82), because isoprene synthesis is not affected by its concentration inside the leaf and its emission reflects synthesis (127). In other words, the leaf is analogous to a current generating circuit where the current output is constant regardless of the resistance downstream of the current generator. Fall & Monson showed that when stomata are closed by feeding abscisic acid to their leaves through the petiole, then the concentration of isoprene inside the leaf builds up to counteract the increased diffusion resistance until emission matches synthesis (28).

The Guenther Model for Predicting Isoprene Emission

Current models are primarily based on algorithms published by Guenther et al (42), although mechanistic models have also been proposed (43, 96, 173). The Guenther algorithms specify three parameters for predicting isoprene emission. First is a basal emission rate or capacity for emission, second is a light correction term, and third is a temperature correction term. The basal rate is the rate in arbitrary conditions agreed upon by people working in this field. The conditions chosen were 30°C and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation. By choosing one set of conditions, researchers could compare measurements made by many different researchers at different times. The initial assumptions were that this rate reflected the capacity for isoprene emission and that it would not change over the normal growing season.

The correction for light was a rectangular hyperbola that was fit to data from a number of measured light responses. Generally, isoprene emission and photosynthesis exhibit similar light responses, but in some cases isoprene saturates at much higher light levels than does photosynthesis (68, 130).

The temperature correction incorporated an activation energy and a deactivation energy that described a high-temperature falloff. The activation energy is similar in many different situations. However, the falloff depends on how the measurements are made (140). When leaf temperature was raised to 35°C or more, isoprene emission at first increased then decreased. In some cases, the rate of emission at 40°C was initially 2–3 times greater than at 30°C; but over 30 min, the rate continually fell until it was less at 40°C than at 30°C (143). Because of this time dependence in the isoprene response to temperature, response curves measured over several hours show much less isoprene emission above 35°C than do curves measured over several minutes (140, 143). More isoprene is available when leaf temperature is rapidly fluctuating than when it is constant, even if the average temperature is the same. For predictive models, a simple activation energy correction algorithm may work well (143).

The basal rate was initially assumed constant for a given species over most of the growing season. Researchers soon recognized that leaves become photosynthetically competent before they begin to emit large amounts of isoprene (33, 39), especially when the temperature is low (47, 92). Similarly, the capacity for isoprene emission falls late in the season, giving a pronounced seasonality to both temperate and boreal forests (33, 34, 172). In predictive models this variation in the basal emission rate was taken into account by assuming a sine function for isoprene emission capacity over the growing season (40).

The basal rate is also dependent on light. Leaves that develop in the shade have lower basal emission rates than those that develop in the sun (131). As a result, leaves at the tops of trees emit more isoprene than leaves at the bottom because there is more sunlight at the top and leaves' capacity for emission is greater there (46, 136).

Even more basal rate variability has been reported recently (135, 146). By plotting basal rate measured on the same tree on six different occasions over three years of field work against several environmental variables, investigators have found that temperature measured over a few days could explain a great deal of variability in basal emission rate (135). Basal emission rates used in predictive models may need to be based on an algorithm that takes into account both seasonality and weather effects.

Physiological Regulation

The predictive modeling assumes either instantaneous changes in isoprene emission rates (the light and temperature correction factors) or very long-term (mostly seasonal) changes that are included in the basal emission rate. In the laboratory we see evidence for regulation over many different time frames. When leaf temperature is changed by changing the radiative heat load, isoprene emission initially increases as quickly as leaf temperature and then more slowly with a time constant of 116 s (142). The response to a change to 40°C is first an increase in emission rate that lasts for 5 to 15 min, followed by a decrease lasting for the next 15 min (143). Researchers have seen changes in isoprene emission capacity in response to temperature changes with a time constant of hours (DT Hanson & TD Sharkey, unpublished data), and the effect over a day or two of these changes has already been described (135).

The temperature response of isoprene emission was related to isoprene synthase activity (93). However, these measurements were made when the tools for measuring isoprene synthase were not as good as they are today. The results reported by Monson et al indicated that the drop-off temperature was likely caused by destruction of the isoprene synthase, but investigators now know that this is a regulatory phenomenon (143). The drop-off has also been ascribed to changes in electron transport activity of photosynthesis (96), but this model does not predict the variable temperature optima for isoprene emission rate that was reported by Singsaas et al (143).

The changes in basal emission rate have also been correlated with extractable isoprene synthase activity (59, 65, 165). In at least one case, the variation in extractable enzyme activity was not correlated with changes in protein amount as determined by Western blots (165). Much more work is still needed on measuring gene expression, message turnover, protein amount, and specific activity changes to see how the changes in isoprene emission are controlled.

In addition to changes in isoprene synthase activity, the activity of the MEP pathway must be regulated to accommodate the large changes in isoprene emission rates that occur. Preliminary results indicate that both deoxyxylulose synthase and deoxyxylulose reductoisomerase transcripts can be induced in kudzu leaves by switching to high temperature to induce isoprene emissions (findings based on RNA blots) (S Yeh & TD Sharkey, unpublished data). In *Arabidopsis*, which does not emit isoprene, high temperature did not induce changes in the abundance of

transcripts encoding these enzymes. On the other hand, isopentenyl pyrophosphate isomerase transcript abundance did not change when kudzu leaves were induced to make isoprene. If the MEP pathway can make DMAPP without first making IPP, this isomerase is not needed.

One level of regulation that is not likely to be relevant is the competition between carotenoid synthesis and isoprene synthesis for the products of the MEP pathway. Because isoprene emission consumes so much more DMAPP than does carotenoid synthesis, changes in carotenoid synthesis will not affect isoprene emission capacity.

EVOLUTIONARY CONSIDERATIONS

Phylogenetics of Isoprene Emission

Isoprene emission is a common but not universal plant trait. Nearly all plant species emit very low levels of isoprene, but only about onethird of angiosperm species tested emit isoprene at substantial rates (45). The term substantial is intentionally vague in this case and reflects the fact that emission rate measurements are hard to compare among the various reports. A number of lists of emitters have been published, and most or all have been compiled into a database available at <http://www.es.lancs.ac.uk/es/people/pg/pas/download.html> or from the author. A more exhaustive list is maintained by R Rasmussen (unpublished data).

Several groups have hypothesized about the meaning of the phylogenetic relationships that characterize isoprene emission. In one case, Harley et al (48) concluded that high rates of isoprene emission are a derived character that evolved many times because, they believe, basal lineages of many plant groups often do not emit isoprene. On the other hand, Hanson et al (45) concluded that isoprene emission evolved once when mosses evolved and that the trait can be either suppressed, resulting in the very low levels of emission found in most plants, or not suppressed, resulting in the high levels found in those plants considered isoprene emitters. One difference in assumptions between these two reports is that Hanson et al assumed that loss of function was more parsimonious than gain of function, although Harley et al considered gain and loss of function equally probable.

Isoprene is also emitted from fungi, bacteria (27, 60, 160, 161), and animals (11, 98), especially humans (14, 36, 52, 149). A relationship between these emissions has not been clearly established. Because isoprene is made in plastids and is closely associated with photosynthesis, a connection between human isoprene emission and plant isoprene emission is unlikely, unless human emission is a product of bacteria resident in humans.

Costs Versus Benefits of Isoprene Emission

Isoprene emission is a substantial cost to the plant in terms of both carbon and energy loss. Given the cost, plants that do not emit would outcompete those that do

emit, unless isoprene emission provides a benefit that exceeds the cost of emission. The thermotolerance hypothesis provides the explanation of what plants may gain from isoprene emission, and the effect can be large relative to the cost of emission. This hypothesis suggests plants that suffer short high-temperature episodes should emit isoprene. Trees often fit this description; the leaves at the tops of trees exposed to full sun can heat up substantially if the air is still (24). The heat of the leaves themselves would then cause convective air movement, cooling the leaf. Once the leaf was cooled, the air movement could stop and the cycle could be repeated. Thus, leaves at the tops of trees are potentially subject to numerous large swings in temperature. On the other hand, desert plants have very small leaves so that the boundary layer is small and the leaves cannot heat up much above air temperature. Generally, desert plants do not make isoprene. Crop plants that have been selected for high rates of photosynthesis will also have been selected for open stomata. When a leaf with open stomata begins to heat up, water will evaporate, keeping any leaf temperature change small relative to the case where stomata are more closed. Finally, when the humidity is high, heat loss by evaporation is reduced. Therefore, plants in tropical environments should, and do (56), emit relatively more isoprene than do most plants in temperate or cool climates.

Hanson et al (45) measured moss temperature and found that moss growing at some distance from the water surface experienced wide temperature changes, though moss growing close to water (within 2.5 cm) had much less temperature variation. They speculated that isoprene emission might have been an important step in the evolution of land plants. As plant progenitors started to stand up in the air, the low heat capacity of air allowed plant temperatures to vary much more than when the organisms were in water, which has a high heat capacity. Isoprene emission is common in mosses (45) but uncommon and possibly nonexistent in algae. Although investigators have reported some level of isoprene from algae and they have found isoprene in seawater (8), given the likely presence of bacteria in the algae, it is not clear whether marine algae or bacteria associated with the algae are responsible for the significant amounts of isoprene in the atmosphere above the oceans (169).

Liverworts may be the most primitive land plants, based on an analysis of three mitochondrial introns (101). The liverworts tested to date do not emit isoprene (45). Many mosses do emit isoprene, and so Hanson et al postulated that there was a major gain of function somewhere between the liverworts and mosses. There are some large-scale patterns concerning which lineages of plants emit and which do not. For example, cycads and ginkgo do not emit, but emission is common in conifers, ferns, and angiosperms. In addition to this large-scale pattern, there is a small-scale pattern in isoprene emission capacity. The best example is in the genus *Quercus*. All species endemic to North America emit isoprene, but a number of Eurasian species do not emit it (15, 48, 78). Other genera, such as *Picea*, also show a disjunct between North American species and Eurasian species as researchers have found for a number of flowering plant genera (163). Researchers believe this disjunct occurred 20 to 30 million years ago when a number of climatic

changes caused the loss of land bridges that connected the Eurasian and American populations. Thus, we see both large-scale patterns that could have had their origins 200–300 million years ago and small-scale patterns that may have arisen 20–30 million (or fewer) years ago.

Paleoclimate

Climatic changes can be inferred from isotope and other geological records. In some cases, plant evolution can be interpreted in light of paleoclimatic changes. One of the best examples is the association of the evolution of the C_4 pathway of photosynthesis with lowered CO_2 and increased temperature that occurred between 10 and 30 million years ago (25). To make a similar analysis for isoprene, we must first consider the costs and benefits of isoprene emission. The cost is clearly the loss of carbon and energy needed for isoprene synthesis. The benefit can be assumed to be protection against short high-temperature episodes. Thus, when CO_2 is plentiful and the temperature is warm, the costs are less and the benefits more than when CO_2 levels and temperature are low.

The changes in CO_2 , global temperature, and oxygen (which affects how costly the energy loss is because oxygen leads to photorespiration) were reconstructed from several models (4, 5, 6) (Figure 2). Two periods can be identified where the cost/benefit ratio for isoprene emission may have been high. When land plants first evolved, the CO_2 level and temperature were high while the oxygen level was less than the present day level of 20%. Under these conditions, isoprene emission may have been highly favored and, as argued by Hanson et al (45), possibly necessary

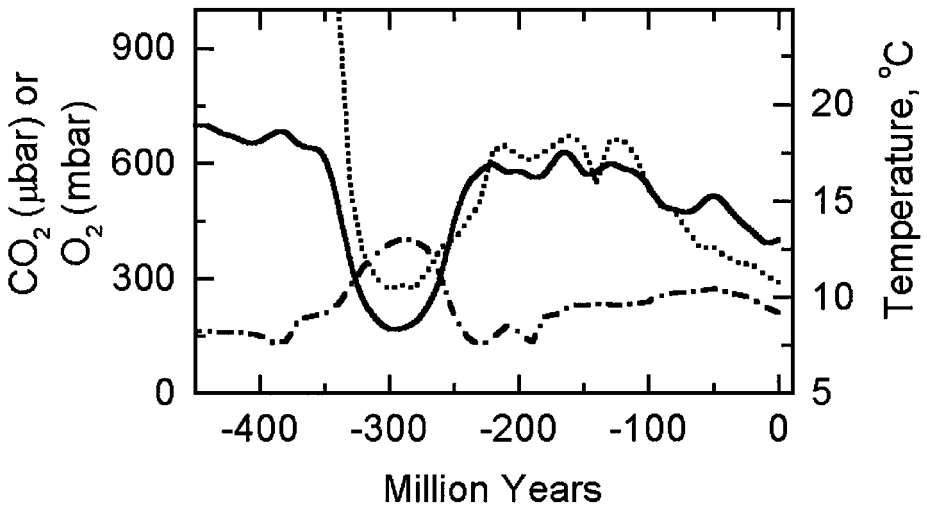


Figure 2 Possible levels of CO_2 , O_2 , and temperature over the time that land plants have existed. Derived from data of Berner (4, 5, 6). CO_2 = ●●●●●●, O_2 = -●-●, temperature = ———.

for the emergence of mosses. Because air has a very low heat capacity, as mosses started to grow up and away from the ground, they would be subject to large changes in temperature. Isoprene emission may have been required to cope with these large temperature changes.

During the Permian period, CO₂ levels were low, temperature was low, and oxygen levels were high. Therefore, the carbon and energy cost of isoprene was more important and the benefit (which occurs in high temperature) was less important. Perhaps during the Permian period, plant lineages that arose (e.g. cycads) (38) did not have the capability to emit isoprene because the cost benefit ratio was not favorable (Figure 3). By the time angiosperms evolved, the CO₂ level and temperature had gone back up and oxygen had gone back down. Many angiosperms have the capacity to make isoprene, and some researchers have argued that the first angiosperms had this ability (45); but this is not a universal view (48).

The CO₂ level fell and oxygen rose during the mid to late Miocene epoch of the Tertiary period (~5–20 million years ago), the time that C₄ plants are believed to have evolved. Perhaps a similar evolutionary pressure led to loss of isoprene emission capacity in some lineages of angiosperms (and other plant groups). Variation in loss of function during this period could lead to the evolution of certain groups within a genus that emit isoprene though other groups lack this trait, as seen in *Quercus*. In summary, the colonization of land by plants may have depended on the evolution of isoprene emission to cope with the low-heat capacity of air, but

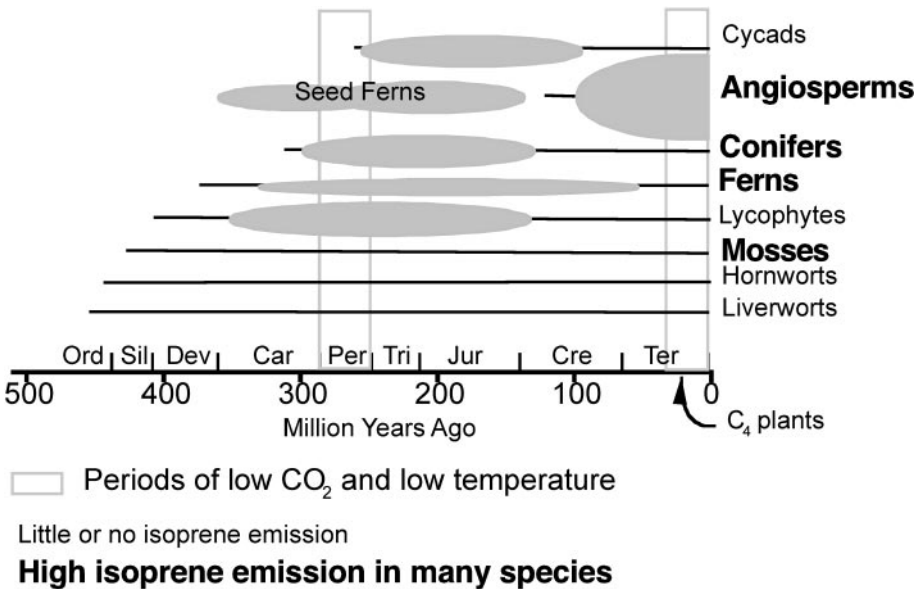


Figure 3 Relative abundances of major land plant groups (modified from Graham, 38).

then isoprene emission may have been lost in many land plants, especially during the Permian and Miocene (Figure 3).

FUTURE RESEARCH

From a physiological standpoint, the most important next step is to clone the isoprene synthase gene and study its regulation. This step will answer questions about metabolic regulation and provide tools for the further study of the regulation of isoprene emission capacity. The enzymes of the MEP pathway should also be studied in isoprene-emitting species. Because isoprene synthesis is perhaps 100 times faster than carotenoid synthesis, the regulation of the MEP pathway in isoprene-emitting species may be different from that in nonemitting species. How much regulation needs to be changed in order to change a nonemitting plant into an emitting plant and vice versa?

Studies are needed on the possible mechanisms by which isoprene can protect membranes against thermal stress. Whether thermotolerance is ultimately accepted as the driving force behind the evolution of isoprene synthesis in higher plants will depend on a clear understanding of its mechanism. These studies could lead to new discoveries concerning the effect of temperature on membranes.

Finally, additional work is needed to determine the complete MEP pathway. This could provide insight into why there are two independent pathways to make IPP. It would be interesting to know whether the lowered energy cost of the MEP pathway in photosynthetic organisms contributed to the evolution of this pathway.

ACKNOWLEDGMENTS

The National Science Foundation Integrative Plant Biology Program and the US Department of Agriculture National Research Initiative have supported this work. I thank Ray Fall, Deming Gong, Francesco Loreto, Eric Singasaas, Peter Vanderveer, and Mary Wildermuth for helpful reviews of the manuscript.

Visit the Annual Reviews home page at www.AnualReviews.org

LITERATURE CITED

1. Arigoni D, Sagner S, Latzel C, Eisenreich W, Bacher A, Zenk MH. 1997. Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement. *Proc. Natl. Acad. Sci. USA* 94:10600–5
2. Atkinson R, Carter WPL. 1995. Kinetics and mechanisms of the gas-phase reactions of ozone with organic compounds under atmospheric conditions. *Chem. Rev.* 84:437–70
3. Baker B, Guenther A, Greenburg J, Goldstein A, Fall R. 1999. Canopy fluxes of 2-methyl-3-buten-2-ol over a ponderosa pine forest by relaxed eddy accumulation: Field data and model comparison. *J. Geophys. Res. Atmos.* 104:26107–14
4. Berner RA. 1991. A model for atmospheric

- CO₂ over phanerozoic time. *Am. J. Sci.* 291:339–76
5. Berner RA. 1994. 3Geocarb II: a revised model of atmospheric CO₂ over phanerozoic time. *Am. J. Sci.* 294:56–91
 6. Berner RA, Canfield DE. 1989. A new model for atmospheric oxygen over phanerozoic time. *Am. J. Sci.* 289:333–61
 7. Bleasdale C, Small RD, Watson WP, Wilson J, Golding BT. 1996. Studies on the molecular toxicology of buta-1,3-diene and isoprene epoxides. *Toxicology* 113:290–93
 8. Bonsang B, Polle C, Lambert G. 1992. Evidence for marine production of isoprene. *Geophys. Res. Lett.* 19:1129–32
 9. Bukhov NG, Wiese C, Neimanis S, Heber U. 1999. Heat sensitivity of chloroplasts and leaves: leakage of protons from thylakoids and reversible activation of cyclic electron transport. *Photosynth. Res.* 59:81–93
 10. Buschmann C. 1999. Thermal dissipation during photosynthetic induction and subsequent dark recovery as measured by photoacoustic signals. *Photosynthetica* 36:149–61
 11. Cailleux A, Cogny M, Allain P. 1992. Blood isoprene concentrations in humans and in some animal species. *Biochem. Med. Metab. Biol.* 47:157–60
 12. Chameides WL, Lindsay RW, Richardson J, Kiang CS. 1988. The role of biogenic hydrocarbons in urban photochemical smog: Atlanta as a case study. *Science* 241:1473–75
 13. Cleveland CC, Yavitt JB. 1998. Microbial consumption of atmospheric isoprene in a temperate forest soil. *Appl. Environ. Microbiol.* 64:172–77
 14. Conkle JP, Camp BJ, Welch BE. 1975. Trace composition of human respiratory gas. *Arch. Environ. Health* 30:290–95
 15. Csiky O, Seufert G. 1999. Terpenoid emissions of Mediterranean oaks and their relation to taxonomy. *Ecol. Appl.* 9:1138–46
 16. Dahl AR. 1996. Metabolism of isoprene in vivo. *Toxicology* 113:273–77
 17. Dahl AR, Birnbaum LS, Bond JA, Gervasi PG, Henderson RF. 1987. The fate of isoprene inhaled by rats: comparison to butadiene. *Toxicol. Appl. Pharmacol.* 89:237–48
 18. Daum PH, Kleinman LI, Nunnermacker LJ, Lee YN, Springston SR, et al. 2000. Analysis of O₃ formation during a stagnation episode in central Tennessee in summer 1995. *J. Geophys. Res. Atmos.* 105:9107–19
 19. Delfine S, Csiky O, Seufert G, Loreto F. 2000. Fumigation with exogenous monoterpenes of a non-isoprenoid-emitting oak (*Quercus suber*): monoterpene acquisition, translocation, and effect on the photosynthetic properties at high temperatures. *New Phytol.* 146:27–36
 20. Delwiche CF, Sharkey TD. 1993. Rapid appearance of ¹³C in biogenic isoprene when ¹³CO₂ is fed to intact leaves. *Plant Cell Environ.* 16:587–91
 21. Demmig-Adams B, Adams WW III. 1992. Photoprotection and other responses of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:599–626
 22. Deneris ES, Stein RA, Mead JF. 1985. Acid-catalyzed formation of isoprene from a mevalonate-derived product using a rat liver cytosolic fraction. *J. Biol. Chem.* 260:1382–85
 23. DeNiro MJ, Epstein S. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197:261–63
 24. Ehleringer JR. 1991. Temperature and energy budgets. In *Plant Physiological Ecology*, ed. RW Pearcy, J Ehleringer, HA Mooney, PW Rundel, pp. 117–35. London: Chapman & Hall
 25. Ehleringer JR, Cerling TE, Helliker BR. 1997. C₄ photosynthesis, atmospheric CO₂ and climate. *Oecologia* 112:285–99
 26. Evans RC, Tingey DT, Gumpertz ML,

- Burns WF. 1982. Estimates of isoprene and monoterpene emission rates in plants. *Bot. Gaz.* 143:304–10
27. Fall R, Copley SD. 2000. Bacterial sources and sinks of isoprene, a reactive atmospheric hydrocarbon. *Environ. Microbiol.* 2:123–30
28. Fall R, Monson RK. 1992. Isoprene emission rate and intercellular isoprene concentration as influenced by stomatal distribution and conductance. *Plant Physiol.* 100:987–92
29. Fall R, Wildermuth MC. 1998. Isoprene synthase: from biochemical mechanism to emission algorithm. *J. Geophys. Res.* 103:25599–609
30. Farquhar GD, Von Caemmerer S. 1982. Modelling of photosynthetic response to environmental conditions. In *Encyclopedia of Plant Physiology NS. Vol. 12B. Physiological Plant Ecology II Water Relations and Carbon Assimilation*, ed. OL Lange, PS Nobel, CB Osmond, H Ziegler, pp. 549–87. Berlin: Springer-Verlag
31. Fehsenfeld FC, Calvert J, Fall R, Goldan P, Guenther AB, et al. 1992. Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochem. Cycles* 6:389–430
32. Fuentes JD, Lerdau M, Atkinson R, Baldocchi D, Botteneheim JW, et al. 2000. Biogenic hydrocarbons in the atmospheric boundary layer: a review. *Bull. Am. Meteorol. Soc.* 81:1537–75
33. Fuentes JD, Wang D. 1999. On the seasonality of isoprene emission from a mixed temperate forest. *Ecol. Appl.* 9:1118–31
34. Fuentes JD, Wang D, Gu L. 1999. Seasonality variations in isoprene emissions from a boreal aspen forest. *J. Appl. Meteorol.* 38:855–69
35. Funk JL, Jones CG, Lerdau MT. 1999. Defoliation effects on isoprene emission from *Populus*. *Oecologia* 118:333–39
36. Gelmont D, Stein RA, Mead JF. 1981. Isoprene—the main hydrocarbon in human breath. *Biochem. Biophys. Res. Commun.* 99:1456–60
37. Gounaris K, Brain APR, Quinn PJ, Williams WP. 1984. Structural reorganization of chloroplast thylakoid membranes in response to heat stress. *Biochim. Biophys. Acta* 766:198–208
38. Graham LE. 1993. *Origin of Land Plants*. New York: Wiley
39. Grinspoon J, Bowman WD, Fall R. 1991. Delayed onset of isoprene emission in developing velvet bean (*Mucuna* sp.) leaves. *Plant Physiol.* 97:170–74
40. Guenther A. 1997. Seasonal and spatial variations in natural volatile organic compound emissions. *Ecol. Appl.* 7:34–45
41. Guenther A, Hewitt CN, Erickson D, Fall R, Geron C, et al. 1995. A global model of natural volatile organic compound emissions. *J. Geophys. Res.* 100:8873–92
42. Guenther AB, Monson RK, Fall R. 1991. Isoprene and monoterpene emission rate variability: observations with Eucalyptus and emission rate algorithm development. *J. Geophys. Res.* 96:10799–808
43. Guenther AB, Zimmerman PR, Harley PC. 1993. Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analysis. *J. Geophys. Res.* 98:12609–17
44. Haagen-Smit AJ. 1952. Chemistry and physiology of Los Angeles smog. *Ind. Eng. Chem.* 44:1342–46
45. Hanson DT, Swanson S, Graham LE, Sharkey TD. 1999. Evolutionary significance of isoprene emission from mosses. *Am. J. Bot.* 86:634–39
46. Harley PC, Guenther AB, Zimmerman PR. 1996. Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. *Tree Physiol.* 16:25–32
47. Harley PC, Litvak ME, Sharkey TD, Monson RK. 1994. Isoprene emission from velvet bean leaves. Interactions among nitrogen availability, growth photon flux

- density, and leaf development. *Plant Physiol.* 105:279–85
48. Harley PC, Monson RK, Lerdau MT. 1999. Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia* 118:109–23
49. Hewitt CN, Kok GL, Fall R. 1990. Hydroperoxides in plants exposed to ozone mediate air pollution damage to alkene emitters. *Nature* 344:56–58
50. Hewitt CN, Monson RK, Fall R. 1990. Isoprene emissions from the grass *Arundo donax* L. are not linked to photorespiration. *Plant Sci.* 66:139–44
51. Hill RE, Himmeldirk K, Kennedy IA, Pauloski RM, Sayer BG, et al. 1996. The biogenetic anatomy of vitamin B₆: a ¹³C NMR investigation of the biosynthesis of pyridoxol in *Escherichia coli*. *J. Biol. Chem.* 271:30426–35
52. Hyspler R, Crhová S, Gasparic J, Zadák Z, Cízková M, Balasová V. 2000. Determination of isoprene in human expired breath using solid-phase microextraction and gas chromatography-mass spectrometry. *J. Chromatogr. B* 739:183–90
53. Jacob DJ, Wofsy SC. 1988. Photochemistry of biogenic emissions over the Amazon forest. *J. Geophys. Res.* 93:1477–86
54. Jones CA, Rasmussen RA. 1975. Production of isoprene by leaf tissue. *Plant Physiol.* 55:982–87
55. Kaufman PB, Cseke LJ, Warber S, Duke JA, Briellmann HL. 1999. *Natural Products from Plants*. Boca Raton, FL: CRC Press. 343 pp.
56. Keller M, Lerdau M. 1999. Isoprene emission from tropical forest canopy leaves. *Global Biogeochem. Cycles* 13:19–29
57. Kesselmeier J, Staudt M. 1999. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *J. Atmos. Chem.* 33:23–88
58. Kreuzwieser J, Schnitzler J-P, Steinbrecher R. 2000. Biosynthesis of organic compounds emitted by plants. *Plant Biol.* 1:149–59
59. Kuzma J, Fall R. 1993. Leaf isoprene emission rate is dependent on leaf development and the level of isoprene synthase. *Plant Physiol.* 101:435–40
60. Kuzma J, Nemecek-Marshall M, Pollock WH, Fall R. 1995. Bacteria produce the volatile hydrocarbon isoprene. *Curr. Microbiol.* 30:97–103
61. Kuzuyama T, Shimizu T, Takahashi S, Seto H. 1998. Fosmidomycin, a specific inhibitor of 1-deoxy-D-xylulose 5-phosphate reductoisomerase in the nonmevalonate pathway for terpenoid biosynthesis. *Tetrahedron Lett.* 39:7913–16
62. Kuzuyama T, Takagi M, Kaneda K, Dairi T, Seto H. 2000. Formation of 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol from 2-C-methyl-D-erythritol 4-phosphate by 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase, a new enzyme in the nonmevalonate pathway. *Tetrahedron Lett.* 41:703–6
63. Kuzuyama T, Takagi M, Kaneda K, Watanabe H, Dairi T, Seto H. 2000. Studies on the nonmevalonate pathway: conversion of 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol to its 2-phospho derivative by 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase. *Tetrahedron Lett.* 41:2925–28
64. Leaitch WR, Bottenheim JW, Biesenthal TA, Li SM, Liu PSK, et al. 1999. A case study of gas-to-particle conversion in an eastern Canadian forest. *J. Geophys. Res. Atmos.* 104:8095–111
65. Lehning A, Zimmer I, Steinbrecher R, Brüggemann N, Schnitzler JP. 1999. Isoprene synthase activity and its relation to isoprene emission in *Quercus robur* L-leaves. *Plant Cell Environ.* 22:495–504
66. Lerdau M. 1991. Plant function and biogenic terpene emissions. In *Trace Gas Emissions from Plants*, ed. TD Sharkey, EA Holland, HA Mooney, pp. 121–34. San Diego, CA: Academic
67. Lerdau M, Guenther A, Monson R. 1997. Plant production and emission of volatile

- organic compounds. *BioScience* 47:373–83
68. Lerdau M, Keller M. 1997. Controls on isoprene emission from trees in a subtropical dry forest. *Plant Cell Environ.* 20:569–78
69. Lichtenthaler HK. 1999. The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50:47–65
70. Lichtenthaler HK, Schwender J, Disch A, Rohmer M. 1997. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. *FEBS Lett.* 400:271–74
71. Litvak ME, Loreto F, Harley PC, Sharkey TD, Monson RK. 1996. The response of isoprene emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white oak trees. *Plant Cell Environ.* 19:549–59
72. Litvak ME, Madronich S, Monson RK. 1998. The potential impact of herbivore-induced monoterpene emissions from coniferous forests on local tropospheric chemistry dynamics. *Ecol. Appl.* 9:1147–59
73. Logan BA, Monson RK. 1999. Thermotolerance of leaf discs from four isoprene-emitting species is not enhanced by exposure to exogenous isoprene. *Plant Physiol.* 120:821–25
- 73a. Logan BA, Monson RK, Potosnak MJ. 2000. Biochemistry and physiology of foliar isoprene production. *Trends Plant Sci.* 5:477–81
74. Loomis WD, Croteau R. 1980. Biochemistry of Terpenoids. In *The Biochemistry of Plants. Vol. 4. Lipids: Structure and Function*, ed. PK Stumpf, pp. 363–418. New York: Academic
75. Loreto F. 1997. Emission of isoprenoids by plants: their role in atmospheric chemistry, response to the environment, and biochemical pathways. *J. Environ. Pathol. Toxicol. Oncol.* 16:119–24
76. Loreto F, Ciccioli P, Brancaleoni E, Cecinato A. 1998. Measurement of isoprenoid content in leaves of Mediterranean *Quercus* spp. by a novel and sensitive method and estimation of the isoprenoid partition between liquid and gas phase inside the leaves. *Plant Sci.* 136:25–30
77. Loreto F, Ciccioli P, Brancaleoni E, Cecinato A, Frattoni M, Sharkey TD. 1996. Different sources of reduced carbon contribute to form three classes of terpenoid emitted by *Quercus ilex* L leaves. *Proc. Natl. Acad. Sci. USA* 93:9966–69
78. Loreto F, Ciccioli P, Brancaleoni E, Valentini R, De Lillis M., et al. 1998. A hypothesis on the evolution of isoprenoid emission by oaks based on the correlation between emission type and *Quercus* taxonomy. *Oecologia* 115:302–5
79. Loreto F, Ciccioli P, Cecinato A, Brancaleoni E, Frattoni M, et al. 1996. Evidence of the photosynthetic origin of monoterpenes emitted by *Quercus ilex* L. leaves by ¹³C labeling. *Plant Physiol.* 110:1317–22
80. Loreto F, Förster A, Dürr M, Csiky O, Seufert G. 1998. On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. *Plant Cell Environ.* 21:101–7
81. Loreto F, Nascetti P, Graverini A, Mannozi M. 2000. Emission and content of monoterpenes in intact and wounded needles of the Mediterranean pine, *Pinus pinea*. *Funct. Ecol.* 14:589–95
82. Loreto F, Sharkey TD. 1990. A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L. *Planta* 182:523–31
83. Loreto F, Sharkey TD. 1993. Isoprene emission by plants is affected by transmissible wound signals. *Plant Cell Environ.* 16:563–70
84. Loreto F, Sharkey TD. 1993. On the relationship between isoprene emission and photosynthetic metabolites under different environmental conditions. *Planta* 189:420–24

85. Loudon GM. 1988. *Organic Chemistry*. Menlo Park, CA: Benjamin Cummings. 1259 pp.
86. MacDonald RC, Fall R. 1993. Acetone emission from conifer buds. *Phytochemistry* 34:991-94
87. MacDonald RC, Fall R. 1993. Detection of substantial emissions of methanol from plants to the atmosphere. *Atmos. Environ.* A27:1709-13
88. McCaskill D, Croteau R. 1999. Isopentenyl diphosphate is the terminal product of the deoxyxylulose-5-phosphate pathway for terpenoid biosynthesis in plants. *Tetrahedron Lett.* 40:653-56
89. Melnick RL, Sills RC, Roycroft JH, Chou BJ, Ragan HA, Miller RA. 1996. Inhalation toxicity and carcinogenicity of isoprene in rats and mice: comparisons with 1,3-butadiene. *Toxicology* 113:247-52
90. Mgaloblishvili MP, Khetsuriana ND, Kalandaze AN, Sanadze GA. 1979. Localization of isoprene biosynthesis in poplar leaf chloroplasts. *Sov. Plant Physiol.* 26:837-42
91. Monson RK, Fall R. 1989. Isoprene emission from aspen leaves. The influence of environment and relation to photosynthesis and photorespiration. *Plant Physiol.* 90:267-74
92. Monson RK, Harley PC, Litvak ME, Wildermuth M, Guenther AB, et al. 1994. Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. *Oecologia* 99:260-70
93. Monson RK, Jaeger CH, Adams WW III, Driggers EM, Silver GM, Fall R. 1992. Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. *Plant Physiol.* 98:1175-80
94. Monson RK, Lerdau MT, Sharkey TD, Schimel DS, Fall R. 1995. Biological aspects of constructing volatile organic compound emission inventories. *Atmos. Environ.* 29:2989-3002
95. Nemecek-Marshall M, MacDonald RC, Franzen JJ, Wojciechowski CL, Fall R. 1995. Methanol emission from leaves. Enzymatic detection of gas-phase methanol and relation of methanol fluxes to stomatal conductance and leaf development. *Plant Physiol.* 108:1359-68
96. Niinemets Ü, Tenhunen JD, Harley PC, Steinbrecher R. 1999. A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. *Plant Cell Environ.* 22:1319-35
97. Pastenes C, Horton P. 1996. Effect of high temperature on photosynthesis in beans .1. Oxygen evolution and chlorophyll fluorescence. *Plant Physiol.* 112:1245-51
98. Peter H, Wiegand HJ, Bolt HM, Greim H, Walter G, et al. 1987. Pharmacokinetics of isoprene in mice and rats. *Toxicol. Lett.* 36:9-14
99. Pierce T, Waldruff P. 1991. A personal computer version of the biogenic emissions inventory system. *J. Air Waste Manag. Assoc.* 41:937-41
100. Pope C. 1980. The candidates and the issues. *Sierra* 65:15-17
101. Qiu Y-L, Cho Y, Cox JC, Palmer JD. 1998. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. *Nature* 394:671-74
102. Rasmussen RA. 1970. Isoprene: identified as a forest-type emission to the atmosphere. *Environ. Sci. Technol.* 4:667-71
103. Rasmussen RA. 1972. What do the hydrocarbons from trees contribute to air pollution. *J. Air Pollut. Control Assoc.* 22:537-43
104. Rasmussen RA, Jones CA. 1973. Emission isoprene from leaf discs of *Hamamelis*. *Phytochemistry* 12:15-19
105. Rasmussen RA, Khalil MAK. 1988. Isoprene over the amazon basin. *J. Geophys. Res.* 93:1417-21
106. Rasmussen RA, Went FW. 1965. Volatile

- organic material of plant origin in the atmosphere. *Proc. Natl. Acad. Sci. USA* 53:215–20
107. Rodríguez-Concepción M, Campos N, Lois LM, Maldonado C, Hoeffler JF, et al. 2000. Genetic evidence of branching in the isoprenoid pathway for the production of isopentenyl diphosphate and dimethylallyl diphosphate in *Escherichia coli*. *FEBS Lett.* 473:328–32
108. Rohmer M. 1993. The biosynthesis of triterpenoids of the hopane series in the Eubacteria: a mine of new enzyme reactions. *Pure Appl. Chem.* 65:1293–98
109. Rohmer M, Knani M, Simonin P, Sahn H. 1993. Isoprenoid biosynthesis in bacteria: A novel pathway for the early steps leading to isopentenyl diphosphate. *Biochem. J.* 295:517–24
110. Sage RF. 1990. A model describing the regulation of ribulose-1,5-bisphosphate carboxylase, electron transport, and triose phosphate use in response to light intensity and CO₂ in C₃ plants. *Plant Physiol.* 94:1728–34
111. Saltman WM. 1981. Isoprene. In *Kirk-Othmer Encyclopedia of Chemical Technology*, ed. RE Kirk, DF Othmer, M Grayson, DV Eckroth, pp. 818–37. New York: Wiley
112. Sanadze GA. 1969. Light-dependent excretion of molecular isoprene. *Prog. Photosyn. Res.* 2:701–6
113. Sanadze GA. 1991. Isoprene effect-light dependent emission of isoprene by green parts of plants. See Ref. 129a, pp. 135–52
114. Sanadze GA, Dzhaiani GI, Baazov DI, Khakhubiya GT, Ebralidze SS, Gvantse-ladze LG. 1976. Probability-statistical model of distribution of the carbon of ¹³CO₂ in the isoprene molecule during photosynthesis. *Sov. Plant Physiol.* 23:580–86
115. Sanadze GA, Dzhaiani GI, Tevzadze IM. 1972. Incorporation into the isoprene molecule of carbon from ¹³CO₂ assimilated during photosynthesis. *Sov. Plant Physiol.* 19:17–20
116. Sanadze GA, Kalandaze AN. 1966. Light and temperature curves of the evolution of C₅H₈. *Fiziol. Rast.* 13:458–61
117. Sanadze GA, Kursanov AL. 1966. On certain conditions of the evolution of the diene C₅H₈ from poplar leaves. *Sov. Plant Physiol.* 13:184–89
118. Schnitzler J-P, Arenz R, Steinbrecher R, Lehning A. 1996. Characterization of an isoprene synthase from leaves of *Quercus petraea* (Mattuschka) Liebl. *Bot. Acta* 109:216–21
119. Schulze-Siebert D, Heintze A, Schultz G. 1987. Substrate flow from photosynthetic carbon metabolism to chloroplast isoprenoid synthesis in spinach evidence for a plastidic phosphoglycerate mutase. *Z. Naturforsch.* 42 Teil C:570–80
120. Schwender J, Zeidler J, Gröner R, Müller C, Focke M, et al. 1997. Incorporation of 1-deoxy-D-xylulose into isoprene and phytol by higher plants and algae. *FEBS Lett.* 414:129–34
121. Seemann JR, Berry JA, Downton WJS. 1984. Photosynthetic response and adaptation to high temperature in desert plants: a comparison of gas exchange and fluorescence methods for studies of thermal tolerance. *Plant Physiol.* 75:364–68
122. Seinfeld JH, Atkinson R, Berglund RL, Chameides WL, Cotton WR, et al. 1991. *Rethinking the Ozone Problem in Urban and Regional Air Pollution*. Washington, DC: Natl. Acad. Press.
123. Shah SPJ, Rogers LJ. 1969. Compartmentation of terpenoid biosynthesis in green plants. *Biochem. J.* 114:395–405
124. Sharkey TD. 1985. O₂-insensitive photosynthesis in C₃ plants. Its occurrence and a possible explanation. *Plant Physiol.* 78:71–75
125. Sharkey TD. 1989. Evaluating the role of rubisco regulation in C₃ photosynthesis. *Philos. Trans. R. Soc. London Ser. B* 323:435–48

126. Sharkey TD. 1990. Feedback limitation of photosynthesis and the physiological role of ribulose biphosphate carboxylase carbamylation. *Bot. Mag. Tokyo* 2:87–105
127. Sharkey TD. 1991. Stomatal control of trace gas emissions. See Ref. 129a, pp. 335–39
128. Sharkey TD. 1996. Isoprene synthesis by plants and animals. *Endeavor* 20:74–78
129. Sharkey TD, Chen X, Yeh S. 2001. Isoprene increases thermotolerance of fosmidomycin-fed leaves. *Plant Physiol.* In press
- 129a. Sharkey TD, Holland EA, Mooney HA, eds. 1991 *Trace Gas Emissions by Plants*. San Diego, CA: Academic
130. Sharkey TD, Loreto F. 1993. Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. *Oecologia* 95:328–33
131. Sharkey TD, Loreto F, Delwiche CF. 1991. High carbon dioxide and sun/shade effects on isoprene emission from oak and aspen tree leaves. *Plant Cell Environ.* 14:333–38
132. Sharkey TD, Loreto F, Delwiche CF. 1991. The biochemistry of isoprene emission from leaves during photosynthesis. See Ref. 129a, pp. 153–84
133. Sharkey TD, Loreto F, Delwiche CF, Treichel IW. 1991. Fractionation of carbon isotopes during biogenesis of atmospheric isoprene. *Plant Physiol.* 97:463–66
134. Sharkey TD, Singaas EL. 1995. Why plants emit isoprene. *Nature* 374:769
135. Sharkey TD, Singaas EL, Lerdau MT, Geron C. 1999. Weather effects on isoprene emission capacity and applications in emissions algorithms. *Ecol. Appl.* 9:1132–37
136. Sharkey TD, Singaas EL, Vanderveer PJ, Geron CD. 1996. Field measurements of isoprene emission from trees in response to temperature and light. *Tree Physiol.* 16:649–54
137. Sharkey TD, Stitt M, Heineke D, Gerhardt R, Raschke K, Heldt HW. 1986. Limitation of photosynthesis by carbon metabolism. II O₂ insensitive CO₂ uptake results from limitation of triose phosphate utilization. *Plant Physiol.* 81:1123–29
138. Silver GM, Fall R. 1991. Enzymatic synthesis of isoprene from dimethylallyl diphosphate in aspen leaf extracts. *Plant Physiol.* 97:1588–91
139. Silver GM, Fall R. 1995. Characterization of aspen isoprene synthase, an enzyme responsible for leaf isoprene emission to the atmosphere. *J. Biol. Chem.* 270:13010–16
140. Singaas EL, Laporte MM, Shi J-Z, Monson RK, Bowling DR, et al. 1999. Leaf temperature fluctuation affects isoprene emission from red oak (*Quercus rubra*) leaves. *Tree Physiol.* 19:917–24
141. Singaas EL, Lerdau M, Winter K, Sharkey TD. 1997. Isoprene increases thermotolerance of isoprene-emitting species. *Plant Physiol.* 115:1413–20
142. Singaas EL, Sharkey TD. 1998. The regulation of isoprene emission responses to rapid leaf temperature fluctuations. *Plant Cell Environ.* 21:1181–88
143. Singaas EL, Sharkey TD. 2000. The effects of high temperature on isoprene synthesis in oak leaves. *Plant Cell Environ.* 23:751–57
144. Sprenger GA, Schörken U, Wiegert T, Grolle S, de Graaf AA, et al. 1997. Identification of a thiamin-dependent synthase in *Escherichia coli* required for the formation of the 1-deoxy-D-xylulose 5-phosphate precursor to isoprenoids, thiamin, and pyridoxol. *Proc. Natl. Acad. Sci. USA* 94:12857–62
145. Staudt M, Bertin N. 1998. Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (*Quercus ilex* L.) leaves. *Plant Cell Environ.* 21:385–95
146. Steinbrecher R, Hauff K, Rabong R,

- Steinbrecher J. 1997. Isoprenoid emission of oak species typical for the Mediterranean area: source strength and controlling variables. *Atmos. Environ.* 31:79–88
147. Stokes NJ, Terry GM, Hewitt CN. 1998. The impact of ozone, isoprene and propene on antioxidant levels in two leaf classes of velvet bean (*Mucuna pruriens* L.). *J. Exp. Bot.* 49:115–23
148. Takagi M, Kuzuyama T, Kaneda K, Watanabe H, Dairi T, Seto H. 2000. Studies on the nonmevalonate pathway: formation of 2-C-methyl-D-erythritol 2,4-cyclodiphosphate from 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol. *Tetrahedron Lett.* 41:3395–98
149. Taucher J, Hansel A, Jordan A, Fall R, Futrell JH, Lindinger W. 1997. Detection of isoprene in expired air from human subjects using proton-transfer-reaction mass spectrometry. *Rapid. Commun. Mass. Spectrom.* 11:1230–34
150. Terry GM, Stokes NJ, Hewitt CN, Mansfield TA. 1995. Exposure to isoprene promotes flowering in plants. *J. Exp. Bot.* 46:1629–31
151. Thompson AM. 1992. The oxidizing capacity of the Earth's atmosphere: probable past and future changes. *Science* 256:1157–65
152. Tilden WA. 1882. *Chem. News* 46:120
153. Tingey DT, Evans R, Gumpertz M. 1981. Effects of environmental conditions on isoprene emission from live oak. *Planta* 152:565–70
154. Tingey DT, Manning M, Grothaus LC, Burns WF. 1979. The influence of light and temperature on isoprene emission rates from live oak. *Physiol. Plant.* 47:112–18
155. Tingey DT, Manning M, Grothaus LC, Burns WF. 1980. Influence of light and temperature on monoterpene emission rates from slash pine. *Plant Physiol.* 65:797–801
156. Tingey DT, Turner DP, Weber JA. 1991. Factors controlling the emissions of monoterpenes and other volatile organic compounds. See Ref. 129a, pp. 93–119
157. Trainer M, Williams EJ, Parrish DD, Buhr MP, Allwine EJ, et al. 1987. Models and observations of the impact of natural hydrocarbons on rural ozone. *Nature* 329:705–7
158. Tuazon EC, Atkinson R. 1990. A product study of the gas-phase reaction of isoprene with OH radical in the presence of NO_x. *Int. J. Chem. Kin.* 22:1221–36
159. Vickery ML, Vickery B. 1981. *Secondary Plant Metabolism*. Baltimore: University Park. 335 pp.
160. Wagner WP, Helmig D, Fall R. 2000. Isoprene biosynthesis in *Bacillus subtilis* via the methylerythritol phosphate pathway. *J. Nat. Prod.* 63:37–40
161. Wagner WP, Nemecek-Marshall M, Fall R. 1999. Three distinct phases of isoprene formation during growth and sporulation of *Bacillus subtilis*. *J. Bacteriol.* 181:4700–3
162. Wang KY, Shallcross DE. 2000. Modelling terrestrial biogenic isoprene fluxes and their potential impact on global chemical species using a coupled LSM-CTM model. *Atmos. Environ.* 34:2909–25
163. Wen J. 1999. Evolution of Eastern Asian and Eastern North American disjunct distributions in flowering plants. *Annu. Rev. Ecol. Syst.* 30:421–55
164. Went FW. 1960. Blue hazes in the atmosphere. *Nature* 187:641–43
165. Wildermuth MC. 1997. *Subcellular location and biophysical regulation of foliar isoprene production (chloroplasts)*. PhD thesis. Univ. Colo. Boulder. 307 pp.
166. Wildermuth MC, Fall R. 1996. Light-dependent isoprene emission - characterization of a thylakoid-bound isoprene synthase in *Salix discolor* chloroplasts. *Plant Physiol.* 112:171–82
167. Wildermuth MC, Fall R. 1998. Biochemical characterization of stromal and thylakoid-bound isoforms of isoprene

- synthase in willow leaves. *Plant Physiol.* 116:1111–23
168. Williams J, Roberts JM, Fehsenfeld FC, Bertman SB, Buhr MP, et al. 1997. Regional ozone from biogenic hydrocarbons deduced from measurements of PAN, PPN, and MPAN. *Geophys. Res. Lett.* 24:1099–102
169. Yokuchi Y, Li HJ, Machida T. 1999. Isoprene in the marine boundary layer (Southeast Asian Sea, Eastern Indian Ocean, and Southern Ocean): Comparison with dimethyl sulfide and bromoform. *J. Geophys. Res. Atmos.* 104:8067–76
170. Zeidler J, Schwender J, Müller C, Wiesner J, Weidemeyer C, et al. 1998. Inhibition of the non-mevalonate 1-deoxy-D-xylulose-5-phosphate pathway of plant isoprenoid biosynthesis by fosmidomycin. *Z. Naturforsch. Teil C* 53:980–86
171. Zeidler JG, Lichtenthaler HK, May HU, Lichtenthaler FW. 1997. Is isoprene emitted by plants synthesized via the novel isopentenyl pyrophosphate pathway? *Z. Naturforsch. Teil C* 52:15–23
172. Zhang XS, Mu YJ, Song WZ, Zhuang YH. 2000. Seasonal variations of isoprene emissions from deciduous trees. *Atmos. Environ.* 34:3027–32
173. Zimmer W, Brüggemann N, Emeis S, Giersch C, Lehning A, et al. 2000. Process-based modelling of isoprene emission by oak leaves. *Plant Cell Environ.* 23:585–95
174. Zimmerman PR. 1979. Determination of emission rates of hydrocarbons from indigenous species of vegetation in the Tampa/St. Petersburg Florida area. *EPA* 904/977:1–104

NOTE ADDED IN PROOF

The DNA sequence for the gene encoding isoprene synthase has now been determined independently by W Zimmer and D Gong and TD Sharkey. The gene is similar to limonene synthases of many species. There is no similar gene in any of the bacterial genomes or the human genome, consistent with the idea that plant isoprene emission is unrelated to that of bacteria and humans.