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INVITED REVIEW

Isoprene Emission from Plants: Why and How

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• *Background* Some, but not all, plants emit isoprene. Emission of the related monoterpenes is more universal among plants, but the amount of isoprene emitted from plants dominates the biosphere–atmosphere hydrocarbon exchange.
• *Scope* The emission of isoprene from plants affects atmospheric chemistry. Isoprene reacts very rapidly with hydroxyl radicals in the atmosphere making hydroperoxides that can enhance ozone formation. Aerosol formation in the atmosphere may also be influenced by biogenic isoprene. Plants that emit isoprene are better able to tolerate sunlight-induced rapid heating of leaves (heat flecks). They also tolerate ozone and other reactive oxygen species better than non-emitting plants. Expression of the isoprene synthase gene can account for control of isoprene emission capacity as leaves expand. The emission capacity of fully expanded leaves varies through the season but the biochemical control of capacity of mature leaves appears to be at several different points in isoprene metabolism.
• *Conclusions* The capacity for isoprene emission evolved many times in plants, probably as a mechanism for coping with heat flecks. It also confers tolerance of reactive oxygen species. It is an example of isoprenoids enhancing membrane function, although the mechanism is likely to be different from that of sterols. Understanding the regulation of isoprene emission is advancing rapidly now that the pathway that provides the substrate is known.

Key words: Atmospheric chemistry, isoprene, methylerythritol 4-phosphate pathway, thermotolerance.

INTRODUCTION

It surprises most people to learn that plants emit much more hydrocarbon into the atmosphere than that coming from human activities, especially during extended warm weather (Purves *et al.*, 2004), when hydrocarbon inputs into the atmosphere can be especially deleterious (Monson and Holland, 2001; Purves *et al.*, 2004). This fact is behind the famous quote of Ronald Reagan that 'approximately 80 % of our air pollution stems from hydrocarbons released by vegetation' (Pope, 1980). The large amount of hydrocarbon coming from plants was used to suggest that air pollution control was not needed, quoting further: 'so let's not go overboard in setting and enforcing tough emission standards from man-made sources'. Thus, hydrocarbon emission from vegetation is of immediate societal significance. Most of the hydrocarbon flux from the biosphere to the atmosphere is just one compound, isoprene. Despite the more obvious emissions of pleasant smells such as pine scent and lemon scent (resulting from monoterpenes), isoprene emission is the predominant biogenic source of hydrocarbon in the atmosphere, roughly equal to global emission of methane from all sources (Guenther *et al.*, 2006; Kesselmeier and Staudt, 1999). This surprising finding of such a large flux of isoprene from plants to the atmosphere raises a number of questions, including what happens to the isoprene in the atmosphere and why plants emit isoprene. What is known and new information on why isoprene emission matters and why plants emit isoprene will be discussed and then new information on the biochemical regulation of emission rate will be presented. The history of the discovery of isoprene emission has been described elsewhere (Sanadze, 1991, 2004; Sharkey and Yeh, 2001).

WHY ISOPRENE EMISSIONS MATTER

Isoprene emission from plants has a significant effect on atmospheric chemistry. In the atmosphere, NO and NO_2 (collectively NO_x) cycle under the influence of sunlight. At night, all NO_x is in the form of NO_2. Sunlight photolyses the NO_2 and this leads to one ozone molecule per NO_2 (Jacob, 1999; Monson and Holland, 2001). In the absence of hydrocarbon, the total NO_x level in the atmosphere determines the amount of ozone that can be formed. However, oxidation of isoprene by atmospheric hydroxyl radicals can lead to hydroperoxides (RO_2) that can convert NO to NO_2 allowing more ozone production. Further reactions can form HO_2 which can also convert NO to NO_2 and generate the OH radical. If isoprene is shown as RH, then the following reactions describe the loss of one H from the isoprene molecule (adapted from Jacob, 1999):

$$RH + OH + O_2 \rightarrow RO_2 + H_2O$$
$$RO_2 + NO \rightarrow RO + NO_2$$
$$RO + O_2 \rightarrow R'CHO + HO_2$$
$$HO_2 + NO \rightarrow OH + NO_2$$
$$2(NO_2 + O_2 \rightarrow NO + O_3)$$
$$\text{Net} : RH + 4O_2 \rightarrow R'HCO + 2O_3 + H_2O$$

As long as there are C–H bonds, the resulting hydrocarbon can undergo further cycles to make even more ozone. The net effect is creation of many ozone molecules from one

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isoprene molecule. The reactions involve radicals and can go in many different directions and have highly non-linear dependencies on concentrations making it difficult to model how isoprene emission from plants will affect the atmosphere. However, the contribution of isoprene to ozone formation has been reported in a number of studies (Trainer *et al.*, 1987; Chameides *et al.*, 1988; Fehsenfeld *et al.*, 1992; Williams *et al.*, 1997). One isoprene molecule can lead to the formation of many ozone molecules when the NO_x levels are high. When there is very little NO_x, different reactions can dominate and isoprene emission from plants can reduce ozone in the atmosphere (Trainer *et al.*, 1987).

A second effect of isoprene in the atmosphere is the growth of aerosols. Aerosols are particles in the atmosphere which give rise to Frits Went's famous natural blue hazes (Went, 1960) but also to significant health problems. The yield of aerosol per molecule in the atmosphere is much lower for isoprene than for monoterpenes and larger molecules, but because there is so much more isoprene entering the atmosphere than other molecules, isoprene may be a significant factor in aerosol formation (Claeys *et al.*, 2004; Edney *et al.*, 2005; Kroll *et al.*, 2005, 2006; Ng *et al.*, 2006; Olcese *et al.*, 2007).

WHICH PLANTS EMIT ISOPRENE?

The taxonomic distribution of isoprene emission is broad. Mosses (Hanson *et al.*, 1999), ferns (Tingey *et al.*, 1987), gymnosperms and angiosperms (see http://www.es.lancs.ac.uk/cnhgroup/iso-emissions.pdf for a comprehensive list) all have members that make isoprene but also have members that do not. Isoprene synthase has been sequenced from several *Populus* species (Miller *et al.*, 2001; Sasaki *et al.*, 2005; Sharkey *et al.*, 2005) and from kudzu (*Pureria lobata*) (Sharkey *et al.*, 2005). The sequences and gene structures indicate they are part of the TPS-b family (Bohlmann *et al.*, 1998; Trapp and Croteau, 2001) of terpene synthases. Members of this gene family also code for monoterpene and sesquiterpenes synthases in angiosperms but are not found in gymnosperms. Because of this, the evolution of angiosperm isoprene synthases (IspSs) must have occurred after the split between angiosperms and gymnosperms, and so isoprene emission capacity in gymnosperms and angiosperms must have evolved independently. Less is known about the sequences of genes coding for IspS enzymes in mosses and ferns, so no conclusions can be reached at present. As fern and moss sequences become available, comparative genomics will help make the evolutionary origins of isoprene emission and terpene synthases more clear.

Even among angiosperms, isoprene emission capacity may have evolved multiple times. The sequences for IspSs among poplars are very similar (sequences are known for *P. alba*, *P.* × *canescens*, *P. tremuloides* and *P. trichocarpa*) (Miller *et al.*, 2001; Sasaki *et al.*, 2005; Sharkey *et al.*, 2005), but this group is very different from the IspS of kudzu (Sharkey *et al.*, 2005). Antibodies against poplar IspS do not cross react with kudzu IspS and vice versa, and neither antibody recognizes oak IspS (Schnitzler *et al.*, 2005; T. D. Sharkey, A. E. Wiberley and A. R. Donohue, unpub. res.). It is likely that isoprene synthesis capacity has evolved multiple times (Harley *et al.*, 1999), possibly from a reservoir of monoterpene synthase genes (Sharkey *et al.*, 2005). Small changes in gene sequence can easily alter both substrate and product specificity of IspS genes (El Tamer *et al.*, 2003; Tholl, 2006; Kampranis *et al.*, 2007). Gene sequences do not support the idea of a single origin of all IspS genes as had been proposed by Hanson *et al.* (1999). Instead, it appears that isoprene emission is more like the evolution of C_4 metabolism, which arose numerous times in response to an environmental constraint (Sage, 2001; Sage and Pearcy, 2000).

Within any particular group of plants, there are some traits that loosely correlate with isoprene emission but there is significant variability. Indeed, there are some peculiar disjunctions. North American oaks all emit isoprene, but many European oaks do not. Instead, among European oaks a variety of behaviours is found. Some clades emit isoprene, some emit monoterpenes in a light-dependent manner, and some emit very little terpene (Loreto *et al.*, 1998; Csiky and Seufert, 1999; Kesselmeier and Staudt, 1999).

WHY PLANTS EMIT ISOPRENE

To ask 'why' plants emit isoprene is really asking what advantage isoprene emission provides to the plant that makes it. The energy cost of isoprene emission is quite significant (starting from CO_2, 20 ATP and 14 NADPH per isoprene molecule; Sharkey and Yeh, 2001). The balance between cost and benefit likely will vary such that isoprene emission is favoured in some species but not others. This could be a significant influence on the distribution of the capacity for isoprene emission among plants. The nitrogen cost of isoprene emission is small; data for experiments reported in Wiberley *et al.* (2005) are about 5 mg m^{-2} IspS of a total of 2·2 g m^{-2} soluble protein or about 0·2 % of soluble protein is IspS.

Thermotolerance

Thermotolerance has been most often discussed as the advantage plants gain by synthesizing isoprene. The first evidence for thermotolerance was based on a photosystem II chlorophyll fluorescence assay (Sharkey and Singsaas, 1995). This experiment indicated that isoprene had some relationship to temperature effects. Leaf discs that do not show damage in this assay below 45 °C do not respond to isoprene (Logan and Monson, 1999). There are many heat-tolerance mechanisms in plants (Sharkey and Schrader, 2006) and many of them are found in all species. The obvious example is heat shock proteins and factors (Vierling, 1991; Nover *et al.*, 2001). These considerations indicate that isoprene may protect against a specific type of heat stress. Isoprene is emitted from leaves, is light dependent (Sanadze and Kalandaze, 1966; Sanadze, 1969; Rasmussen and Jones, 1973; Tingey *et al.*, 1979; Monson and Fall, 1989; Loreto and Sharkey, 1990) and uses carbon directly from the Calvin cycle of photosynthesis (Delwiche and Sharkey, 1993; Affek and Yakir, 2003;

Schnitzler *et al.*, 2004; Ferrieri *et al.*, 2005). While these aspects of isoprene emission pointed toward thermoprotection of leaves and specifically photosynthesis, the temperature environment of leaves was not well known.

It is difficult to measure the temperature of leaves because they are typically very thin and have very little heat capacity. The low heat capacity of leaves and high radiant energy fluxes of sunlight make large, rapid changes in leaf temperature a possibility. A system for measuring leaf temperature under natural conditions was devised (Fig. 1) that is similar to one reported by Drake *et al.* (1970). A very fine-wire thermocouple (0·079 mm diameter) was threaded through two veins of the abaxial surface of leaves. One joint of the thermocouple was pressed against the leaf surface while the other hung about 3 cm below the leaf. The small diameter of the thermocouple wire reduced heating from sunlight, and in any case, both joints were in the same radiation environment (below the leaf), so any radiation errors would be small. Also, the wire was in contact with the leaf for some distance on either side of the measuring joint, reducing conductivity errors.

Using the system shown in Fig. 1, leaf temperature in natural conditions has been measured in oaks (Singsaas *et al.*, 1999), aspen (R. R. Wise, Univ. Wisc.-Oshkosh, Oshkosh Wisc, unpubl. res.), cotton (Wise *et al.*, 2004), and mosses (Hanson *et al.*, 1999). In all cases, sunlight caused very large and rapid changes in leaf temperature. In the example shown in Fig. 2, a range of $>10\,^{\circ}C$ is seen to occur throughout the day, except for three periods which correspond with clouds passing overhead. This temperature range was confirmed several times using a hand-held infrared thermometer. The finding of these very large heat flecks allowed a refinement of the thermotolerance hypothesis for isoprene emission. Specifically, isoprene synthesis (and consequent emission) protects against heat flecks. This hypothesis is consistent with the distribution of isoprene emission capacity among plant species.

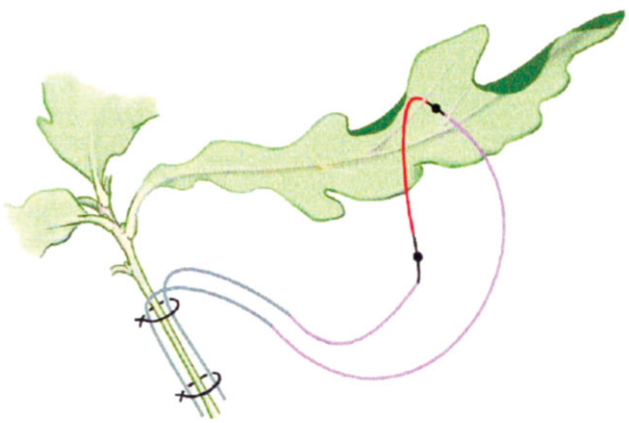

FIG. 1. Method for measuring leaf temperature. Copper and constantan wires of 0·079 mm diameter were made into a thermocouple that was threaded through adjacent veins so that the thermocouple measuring joint was pressed against the leaf. The use of very small diameter wire increased the response time and decreased radiation errors. This system is similar to one used by Drake *et al.* (1970) but different from the suggestion of Ehleringer (1991) who recommended that the thermocouples be inserted into the part of the leaf to be measured.

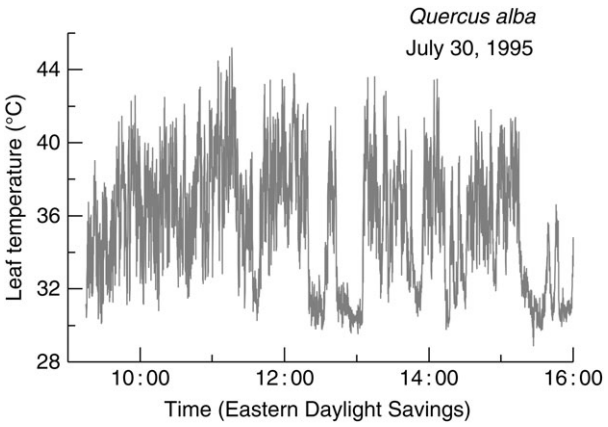

FIG. 2. Temperature of a white oak leaf. Measurement was made at the top of a 30-m *Quercus alba* tree in Duke Forest, North Carolina.

However, like the distribution of C_4 metabolism, both environmental and phylogenetic influences can be seen and strict correlations are not the rule.

The thermotolerance hypothesis is also consistent with the distribution of isoprene emission capacity through a canopy. Leaves at the top of a canopy are much more likely to suffer heat flecks and, when measured under identical conditions, leaves at the top of a canopy emit as much as four times more isoprene relative to leaves at the bottom of the canopy (Harley *et al.*, 1996; Sharkey *et al.*, 1996; Singsaas *et al.*, 1999). Differences in IspS activity can account for the canopy position effect (Lehning *et al.*, 2001).

Focusing the thermotolerance hypothesis on heat fleck damage protection made it possible to devise more targeted experimental tests. Additional improvements in experimental design were made possible by the discovery that isoprene is made by the methylerythritol 4-phosphate (MEP) pathway (Schwender *et al.*, 1997), which is sensitive to a specific inhibitor, fosmidomycin (Kuzuyama *et al.*, 1998; Zeidler *et al.*, 1998). As a result, it was possible to test whether plants that had the ability to make isoprene could withstand repeated, short high temperature episodes better than plants that did not. The answer is yes, the capacity for isoprene emission confers tolerance to short high temperature episodes (Sharkey *et al.*, 2001; Velikova and Loreto, 2005). For example, the data in Fig. 3 show that a leaf in which isoprene emission was inhibited by feeding fosmidomycin suffered more heat damage and recovered less than leaves not fed fosmidomycin (endogenous isoprene) or fed fosmidomycin but supplied with isoprene in the gas phase (exogenous isoprene). The control experiment of showing that adding back isoprene in the gas stream restores the thermotolerance of a fosmidomycin-poisoned leaf proves that nonspecific effects of fosmidomycin are not responsible for the results and makes the use of fosmidomycin a very strong experimental system.

In other experiments, exogenous isoprene treatment could restore all of the thermoprotection found in leaves emitting isoprene (table 1 of Sharkey *et al.*, 2001). It was shown that this is a general effect of compounds with double bonds (alkenes) and that alkanes enhance thermal damage. Some monoterpenes can also provide

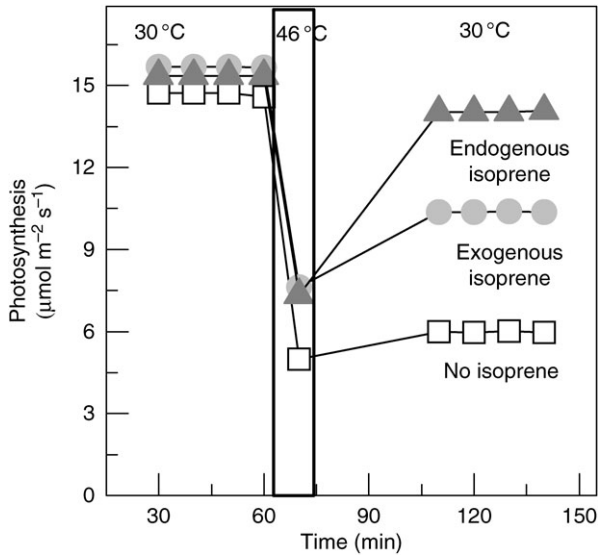

FIG. 3. Thermoprotection of photosynthetic capacity by isoprene. Photosynthesis of detached kudzu leaves was measured at the indicated temperatures. One leaf was fed water and so made isoprene from endogenous sources. Two other leaves were fed 4 μM fosmidomycin and isoprene emission was monitored until >90% of the isoprene emission capacity was lost. One of these leaves was then provided with 2 μL L^{-1} isoprene in the air stream (exogenous isoprene treatment).

thermoprotection (Delfine *et al.*, 2000; Peñuelas and Llusià, 2002; Copolovici *et al.*, 2005). Methyl butenol, which is related to isoprene and emitted by some pine trees (Harley *et al.*, 1998; Gray *et al.*, 2003), has not been tested for its ability to provide thermotolerance.

Feeding isoprene in the gas stream to leaves in which isoprene emission has been poisoned by feeding fosmidomycin, or leaves that do not normally emit isoprene, confers thermotolerance (Sharkey *et al.*, 2001). The protection provided by isoprene can be seen even 24 h after heat stress. For example, Sharkey *et al.* (2001) reported that photosynthesis of *Phaseolus vulgaris* was reduced to 80 ± 11 % (mean ± s.e., $n = 3$) 24 h after a 2 min 46 °C heat spike, but if 2 μL L^{-1} isoprene (a physiologically relevant level for isoprene inside leaves; Singsaas *et al.*, 1997) was provided in the gas stream during the heat spike, photosynthesis was 96 ± 1 % of the pre-stress value after 24 h. This 16 % difference in photosynthetic capacity can offset the cost of isoprene production in plants that experience such heating episodes.

Genetic engineering has allowed creation of poplar trees that lack the capacity for isoprene emission and these trees show increased damage to photosynthesis by heat spikes relative to control trees (Behnke *et al.*, 2007). Arabidopsis plants transformed with an IspS gene from kudzu (Sharkey *et al.*, 2005) can tolerate heat stress that kills untransformed plants (C. Barta and F. Loreto, Consiglio Nazionale delle Ricerche (CNR), Rome, unpubl. res.). Sasaki *et al.* (2007) report that arabidopsis expressing poplar IspS are much better able to tolerate heat stress. One the other hand, arabidopsis plants expressing an IspS gene from *Populus* × *canescens* did not show enhanced tolerance to heat spikes (Loivamäki *et al.*, 2007*a*), although the assay did not result in heat spike-induced damage to wild-type plants, and the isoprene emission rate was not very much higher than the background emission from wild-type plants so it is difficult to interpret this experiment. Thus, (*a*) providing isoprene to plants that do not normally make isoprene, (*b*) using genetic approaches to induce non-emitting species to make isoprene, (*c*) using genetic approaches to suppress isoprene synthesis, or (*d*) using an inhibitor to reduce isoprene emission, all confirm that isoprene provides leaves with the ability to tolerate brief high temperature episodes.

Tolerance of heat flecks can help explain the distribution of the capacity to emit isoprene among plants. Crop plants are selected for rapid growth and this requires open stomata. High stomatal conductance allows high rates of latent heat loss, buffering against heat flecks. Therefore, crop plants should not, and generally do not, emit isoprene. Sustained high temperature presents a type of stress that isoprene emission may not help plants to tolerate. Plants from hot deserts do not emit significant amounts of isoprene. On the other hand, leaves at the tops of trees are subject to intense sunlight and the light (and associated heat gain) can vary over very short periods. Trees are generally the biggest isoprene emitters, especially oak and aspen trees. In the tropics, plant leaves can grow very large, and this creates a large boundary layer insulating the leaf from air temperature, allowing the leaf temperature to exceed air temperature by 10 °C and more. Also, in humid air, heat loss by latent heat of evaporation is reduced. The humid tropics are known to have many isoprene-emitting species (Sharkey and Yeh, 2001). Thus, there is a correspondence between the distribution of isoprene emission capacity among plant species and its presumed function in increasing tolerance of heat flecks suffered by leaves.

Reactive oxygen

A second role for isoprene is in tolerance of ozone and other reactive oxygen species (ROS). Isoprene can prevent visible damage caused by ozone exposure (Loreto and Velikova, 2001; Loreto *et al.*, 2001) and can prevent measurable loss in photosynthetic capacity by ROS (Affek and Yakir, 2002; Peñuelas and Llusià, 2002; Velikova *et al.*, 2004; Peñuelas *et al.*, 2005). However, there is also a report that isoprene emission can exacerbate ozone damage (Hewitt *et al.*, 1990). While IspS gene expression and protein amount are stimulated by high temperature, they are decreased in elevated ozone (Fares *et al.*, 2006; Calfapietra *et al.*, 2007). Therefore, while isoprene protects against both ROS and heat flecks, the physiology of isoprene emission appears related to the protection against heat flecks. It is unlikely that ozone levels were significant over evolutionary time (Jacob, 1999), so it may be that physiological responses to the ROS protection afforded by isoprene has not yet had time to evolve. Lerdau has pointed out that if isoprene emission can increase ozone production when NO_x is present, and simultaneously help plants tolerate ozone, ecosystem composition could change as isoprene-emitting species lead to high levels of ozone that they are better able to tolerate (Lerdau, 2007).

Mechanism of isoprene action

The mechanism by which isoprene protects against heat flecks and ROS is unknown. It is tempting to speculate that the same mechanism accounts for both effects of isoprene. Velikova and Loreto (2005) showed that heat flecks caused leaves to accumulate more H_2O_2 and malondialdehyde (a membrane oxidation product) when isoprene emission was inhibited by fosmidomycin. It is speculated that heat damage to photosynthesis is mediated by ROS, and isoprene protects against ROS by protecting both against experimentally induced ROS and heat-induced ROS. Death of yeast cells by heat shock involves ROS (Davidson *et al.*, 1996). However, H_2O_2 accumulation can be a signal for inducing gene expression that leads to stress tolerance (Kovtun *et al.*, 2000). It can be difficult to tease apart signals of heat stress from the damage caused by heat stress.

There are significant effects of heat stress on photosynthesis not easily explained by ROS. For example, heat flecks cause more damage when given to leaves in the dark than in the light (Weis, 1982; Schrader *et al.*, 2004). Heat can cause thylakoid membranes to become leaky and stimulate cyclic electron flow (Pastenes and Horton, 1996; Bukhov *et al.*, 1999; Schrader *et al.*, 2004). The cyclic electron flow maintains the proton motive force needed for ATP synthesis (Schrader *et al.*, 2007). The loss of membrane integrity could lead to enhanced levels of malondialdehyde. Thus, it is possible that the mechanism of action of isoprene is to protect membrane integrity, and this protects against heat fleck damage and the effects of ROS. In other words, isoprene could reduce ROS by reducing heat damage directly rather than acting only through quenching of ROS generated by heating. Lui and Huang (2002) reported that cytokinin given to heat-stressed *Agrostis palustris* (creeping bentgrass) reduced the heat stress and ROS but it was not suggested that the mechanism was quenching the ROS. Isoprene may work through one mechanism that helps leaves tolerate heat and ROS stress or the two mechanisms may be unrelated.

The only direct study of the mechanism by which isoprene might function is that of Siwko *et al.* (2007). They showed that a moderate amount of isoprene dissolved in a model membrane caused an increase in membrane order equivalent to a 10 °C decrease in temperature. Siwko *et al.* (2007) conclude that isoprene stabilizes lipid membranes and that their experiments provide a mechanistic basis for the suitability of isoprene for protection against heat spike damage. We agree.

Other hypothesized effects of isoprene

At high concentration, isoprene was shown in one study to speed flowering in arabidopsis (Terry *et al.*, 1995). Isoprene emission also consumes certain metabolites, and it has been proposed that this may be the function of isoprene emission, a 'safety valve' to get rid of unwanted metabolites (Rosenstiel *et al.*, 2004) or energy (Sanadze, 2004). However, in both cases these functions have no predictive power; they do not explain why some plants do and some do not emit isoprene. They do not explain why isoprene emission is greater at the tops of trees than lower in the canopy. The metabolite 'safety valve' hypothesis, that isoprene emission allows phosphate intermediates that get 'stuck' in the MEP pathway, is a futile cycle; futile cycles are normally avoided in metabolism. The first enzyme in the isoprene synthesis pathway is very sensitive to feedback from metabolites further in the pathway (Wolfertz *et al.*, 2004) which is the classic regulatory method of preventing futile cycles in a pathway of this type. The problem of insufficient phosphate turnover is common at low temperature but uncommon at high temperature (Sage and Sharkey, 1987). Therefore, there are mechanisms to prevent the accumulation of dimethylallyl diphosphate (DMADP) and the problem would be expected to be worse at low temperature, when isoprene emission is very low.

It has been hypothesized that isoprene emission can dissipate excess energy when leaves receive more light than they can use (Magel *et al.*, 2007). However, the well-known dissipation mechanisms that give rise to energy-dependent quenching of chlorophyll fluorescence and even photorespiration consume many more times the energy consumed by isoprene emission, making this function for isoprene emission quantitatively insignificant.

Another hypothesis concerning the role of isoprene emission is the 'opportunist hypothesis' of Owen and Peñuelas (2005). Clearly, isoprene emission capitalizes on the opportunity to use DMADP, presented by the fact that this metabolite is needed to synthesize many other compounds. The K_m of IspS for DMADP is in the millimolar range while the K_m of geranyl diphosphate synthase for DMADP is in the micromolar range (Tholl *et al.*, 2001). This difference effectively separates these two metabolic fates of DMADP. This rules out the second component of the opportunist hypothesis as put forward by Owen and Peñuelas (2005), that longer chain isoprenoids will determine the rate of isoprene emission. The opportunist hypothesis has been criticized by Pichersky *et al.* (2006).

REGULATION OF ISOPRENE EMISSION CAPACITY

Given the importance of isoprene in atmospheric chemistry, it is essential to understand how plants regulate their isoprene emission. Isoprene is synthesized by the action of IspS on DMADP (Silver and Fall, 1991) produced by the MEP pathway (Fig. 4) (Schwender *et al.*, 1997).

Isoprene emission is modelled as a base (or basal) rate corrected for differences between the conditions of the measurement and the conditions used to determine the basal rate (normally 30 °C and 1000 μmol m^{-2} s^{-1}). The basal rate was considered to be fairly constant once leaves were fully developed although species dependent (Guenther *et al.*, 1993, 1995). The instantaneous response to temperature was found to be similar among species and in different environments, but the basal rate has turned out to vary considerably. It is well known now that the capacity for isoprene emission is delayed developmentally, with leaves becoming photosynthetically competent as much as weeks before isoprene emission begins (Sharkey and Loreto, 1993; Monson *et al.*, 1994; Goldstein *et al.*,

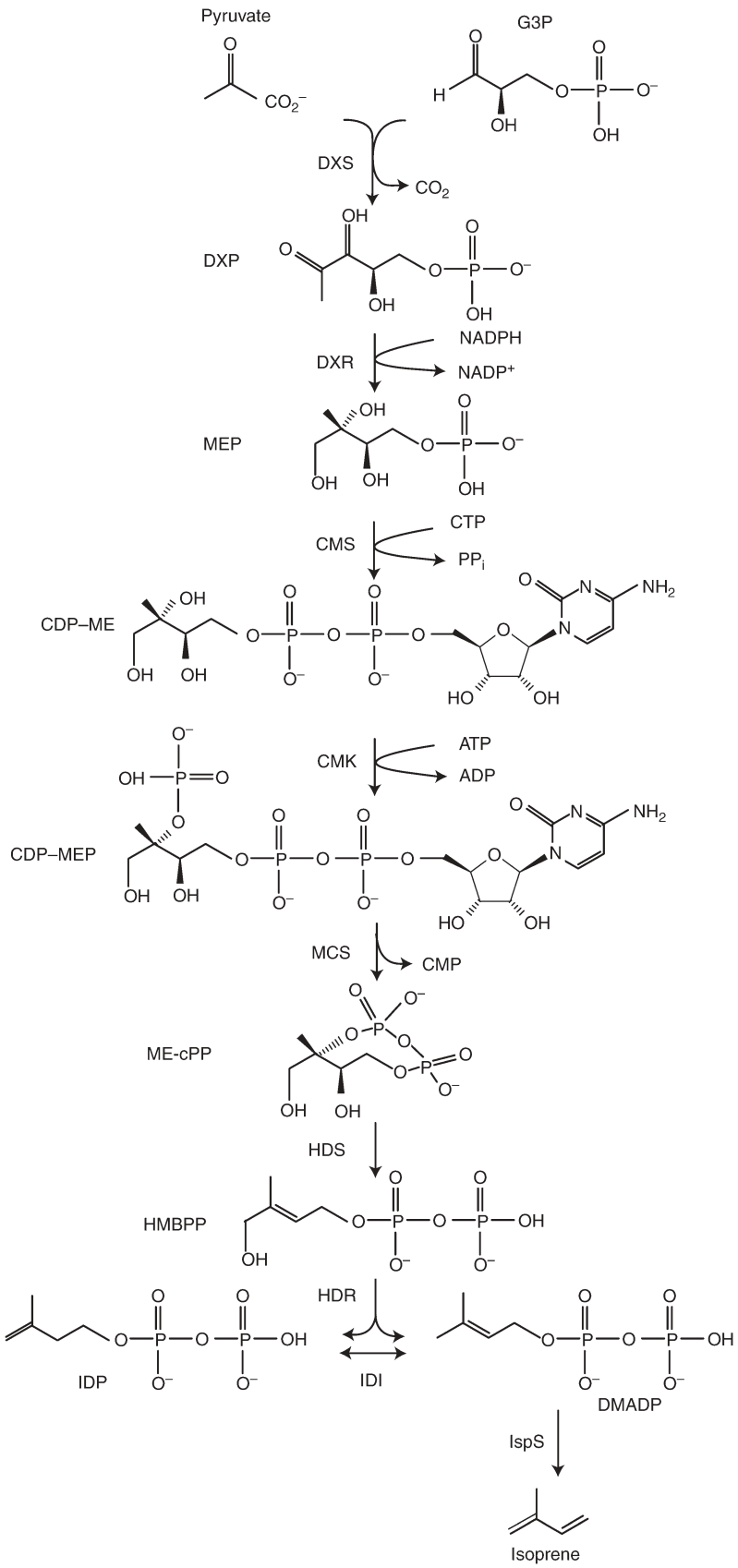

FIG. 4. The methylerythritol 4-phosphate pathway. G3P = glyceraldehyde 3-phosphate; DXS = deoxyxylulose 5-phosphate (DXP) synthase; DXR = DXP reductoisomerase; MEP = methylerythritol 4-phosphate; CMS = diphosphocytidylyl methylerythritol (CDP-ME) synthase; CMK = CDP-ME kinase; CDP-MEP = CDP-ME 2-phosphate; MCS = methylerythritol 2,4-cyclodiphosphate (ME-cPP) synthase; HDS = hydroxymethylbutenyl diphosphate (HMBPP) synthase; HDR = HMBPP reductase; IDP = isopentenyl diphosphate; DMADP = dimethylallyl diphosphate; IDI = IDP isomerase; IspS = isoprene synthase.

1998; Kuhn *et al.*, 2004; Mayrhofer *et al.*, 2005; Wiberley *et al.*, 2005). The delay is significantly affected by growth temperature (Monson *et al.*, 1994; Wiberley *et al.*, 2005). Even after leaves are fully developed, air temperature of the previous few hours to weeks affects the base rate of isoprene emission (Goldstein *et al.*, 1998; Fuentes and Wang, 1999; Fuentes *et al.*, 1999; Sharkey *et al.*, 1999; Pétron *et al.*, 2001). Changes in the activity of IspS can be seen in response to temperature of the previous few days (Lehning *et al.*, 2001).

Isoprene emission is remarkably resistant to water stress (Tingey *et al.*, 1981). Water stress that causes nearly complete loss of photosynthetic capacity has only a minor effect on isoprene emission (Tingey *et al.*, 1981; Sharkey and Loreto, 1993; Fang *et al.*, 1996; Pegoraro *et al.*, 2004*b*; Funk *et al.*, 2005; Monson *et al.*, 2007). Following re-watering, isoprene emission capacity sometimes exceeds the capacity before the stress (Sharkey and Loreto, 1993; Brilli *et al.*, 2007). Isoprene synthase activity is quite robust in response to water stress (Brüggemann and Schnitzler, 2002*a*; Brilli *et al.*, 2007). The maintenance of isoprene emission and stimulation by water stress can be interpreted as adaptive in light of the thermotolerance hypothesis, since water stress is likely to lead to more frequent heat stress as latent heat loss is reduced with reduced water availability.

Isoprene emission is reduced when plants are grown under elevated ozone and the expression of IspS can be shown to be reduced (Fares *et al.*, 2006; Calfapietra *et al.*, 2007). It is not clear why isoprene emission would be reduced in plants grown in elevated ozone if the adaptive significance of isoprene emission were quenching ozone. Evolution may have resulted in isoprene emission as a mechanism of thermoprotection with the happy consequence that leaves that emit isoprene are also protected against the very recent stress of ozone.

The control of isoprene emission by temperature, water stress, and elevated CO_2 or ozone will rest with regulation of IspS and regulation of the supply of DMADP. It was originally assumed that the mevalonic acid pathway was the source of substrate (Sharkey *et al.*, 1991; Sanadze, 2004), but it was demonstrated that a newly discovered pathway for making isoprenoids was the real source (Zeidler *et al.*, 1997). The MEP pathway supplies plastids with DMADP (Schwender *et al.*, 2001). There is evidence for crosstalk between the mevalonic acid pathway in the cytosol and the MEP pathway in chloroplasts (Laule *et al.*, 2003; Dudareva *et al.*, 2005) but movement of substrates from the chloroplast to the cytosol has been demonstrated more often than movement in the other direction. Plants that lack the first enzyme of the MEP pathway are not viable (Estévez *et al.*, 2001) and fosmidomycin, the inhibitor of the second enzyme in the pathway completely (Sharkey *et al.*, 2001) or nearly completely (Loreto *et al.*, 2004) eliminates isoprene emission. The elimination of isoprene emission by fosmidomycin is not consistent with the hypothesis of Sanadze (2004) that a second carboxylation system exists. Therefore, understanding the regulation of the rate of isoprene emission requires understanding the regulation of IspS and the MEP pathway. Molecular tools have become available for dissecting the control of isoprene emission, and these studies plus other biochemical studies of isoprene synthesis regulation are discussed below.

Isoprene synthase regulation

Since the initial discovery of IspS, the link between IspS and isoprene emission has been studied extensively. Several studies have indicated that extractable IspS activity correlates with isoprene emission (Monson *et al.*, 1992; Kuzma and Fall, 1993; Schnitzler *et al.*, 1996, 1997, 2005; Lehning *et al.*, 1999; Brüggemann and Schnitzler, 2002*a*; Mayrhofer *et al.*, 2005). Recent molecular studies have shown that introduction of an *IspS* gene into arabidopsis is sufficient to cause the plant to emit isoprene (Sharkey *et al.*, 2005; Loivamäki *et al.*, 2007*a*; Sasaki *et al.*, 2007).

Thus far, studies of the regulation of IspS have shown that, during leaf development, the onset of isoprene emission is controlled by *IspS* transcription or mRNA turnover (Mayrhofer *et al.*, 2005; Wiberley *et al.*, 2005). More recently, the same has been shown in developing *Populus trichocarpa* leaves. The study of emissions from developing and mature leaves is simplified in *P. trichocarpa* because young stems flush continuously, so an entire series of leaves, from just-emerged to weeks past full expansion, can be studied on one stem. When grown at high temperature, leaves begin to emit isoprene at least 1 week after acquisition of photosynthetic competence, and *IspS* mRNA and protein begin to accumulate at the same developmental stage. The same transcriptional regulation is observed in leaves growing at low temperature, but such leaves do not begin to emit or accumulate *IspS* mRNA or protein until several days later than their high-temperature counterparts (Fig. 5) (experimental procedures were as described in Wiberley *et al.*, 2005). This is useful in the creation of mechanistic models of isoprene emission: the amount of isoprene that leaves will emit early in their lives, and how soon they begin to emit, are functions of the temperature at which they develop.

Isoprene synthase has a high K_m for DMADP and in some cases exhibits sigmoidal kinetics (Sharkey *et al.*, 2005; Schnitzler *et al.*, 2005). As a result, it is easy for isoprene emission to be co-regulated by both the enzyme amount and the substrate amount. There is no direct evidence for post-translational regulation of the activity of IspS but no evidence ruling it out either. In some species, IspS can be found in the soluble fraction and in membrane fractions and the amount in each fraction can vary (Wildermuth *et al.*, 1996; Wiberley *et al.*, 2005). The mechanisms for this and its possible role in regulating isoprene emission are not yet known.

MEP pathway regulation

The MEP pathway provides substrate for the synthesis of numerous terpenoids in addition to isoprene, and has been implicated in regulation of their synthesis. Deoxyxylulose 5-phosphate synthase (DXS), DXP reductoisomerase (DXR) and hydroxymethylbutenyl diphosphate reductase (HDR) have had regulatory roles suggested in production of

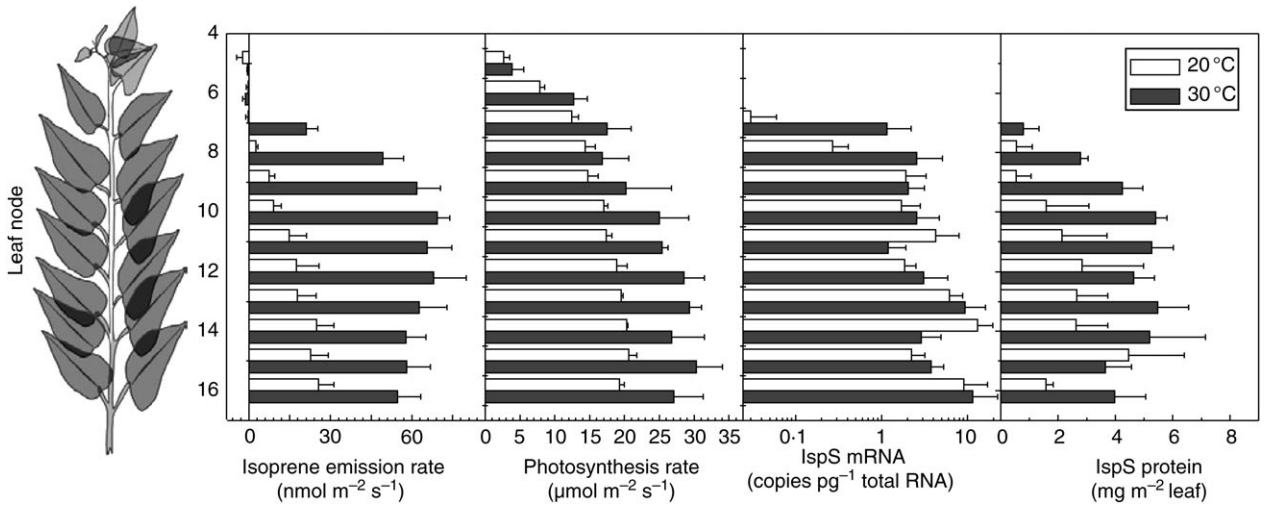

FIG. 5. Isoprene emission and photosynthesis rates, and *IspS* mRNA and protein levels for developing *P. trichocarpa* leaves. Emission rates were measured at 30 °C and 1000 μmol m^{-2} s^{-1} light. Experimental methods were similar to those reported in Wiberley *et al.* (2005) for kudzu.

terpenoids such as carotenoids (Albrecht and Sandmann, 1994; Sun *et al.*, 1998; Lois *et al.*, 2000; Estévez *et al.*, 2001; Carretero-Paulet *et al.*, 2002, 2006; Guevara-Garcia *et al.*, 2005; Muñoz-Bertomeu *et al.*, 2006). Some evidence has linked deoxyxylulose 5-phosphate synthase and isopentenyl diphosphate isomerase to regulation of isoprene emission as well (Brüggemann and Schnitzler, 2002*b*; Wolfertz *et al.*, 2003, 2004). Given these results and observations of isoprene emission capacities of mature leaves subjected to temperature changes, the role of the MEP pathway in regulation of isoprene emission bears further investigation.

Molecular regulation. Populus trichocarpa is well suited to studies of the molecular regulation of isoprene emission: in addition to producing a continual supply of new leaf tissue and being easy to propagate, its genome has been completely sequenced (Tuskan *et al.*, 2006) making it ideal for molecular studies.

Another advantage of having a sequenced genome available is the ability to do preliminary *in silico* studies that may indicate regulatory factors testable *in vitro*. For example, the promoter regions of the MEP pathway genes and IspS have been analysed to identify potential testable regulatory elements. The sequences of these regions (2000 nucleotides upstream of the start codon) were found on the *P. trichocarpa* genome website (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html) and analysed with PLACE (Higo *et al.*, 1999) and PlantCARE (Lescot *et al.*, 2002), which search DNA sequences for transcription factor binding sites.

Isoprene emission is regulated by heat and exhibits circadian regulation (Wilkinson *et al.*, 2006; Loivamäki *et al.*, 2007*b*), so a search of the promoters of MEP pathway genes for transcription factor binding sites related to these responses was performed. In *Chlamydomonas* it has been found that the promoters of heat-shock genes often contain heat-responsive elements within about 300 nucleotides of their start codons (von Gromoff *et al.*, 2006); such genes in arabidopsis and soybean also frequently have a pair of similar elements repeated at least twice, about 20 nucleotides upstream of their transcription start site, and the response of these elements may be increased if they are within 10–30 nucleotides downstream of a CCAAT box (Gurley and Key, 1991; Haralampidis *et al.*, 2002). According to PLACE and PlantCARE analyses, the promoters of most of the MEP pathway genes contain at least one of these elements, but only DXS, methylerythritol 2,4-cyclodiphosphate synthase (MCS) and IspS have two heat shock-response sites between a CCAAT box and putative transcription start site. Only diphosphocytidylyl methylerythritol synthase (CMS), isopentenyl diphosphate isomerase (IDI) and IspS have *Chlamydomonas*-like heat-responsive elements within a few hundred nucleotides of their start codons. Based on these analyses and consistent with studies described above, IspS is an especially strong candidate for heat-induced expression.

The promoters of some circadian-regulated genes in tomato have a 'CAA(N)$_4$ATC' motif within about 300 nucleotides of their start codons (Piechulla *et al.*, 2001); so do poplar DXS, CMS, MCS, HDS and IspS. IspS also has the CCA1/LHY-binding motif (A)$_5$TCT, which controls dawn-phased expression (reviewed by Hotta *et al.*, 2007). A conserved 'TATTCT' ten nucleotides upstream of the transcription start site in barley light-responsive genes has been shown to be important in circadian regulation (Thum *et al.*, 2001). This sequence is also found in the proper position in the promoters of poplar DXS, CMS, MCS, HDS, HDR and IspS. In addition, the poplar DXS promoter contains a series of repeated GATA boxes with spacing and position similar to those required for circadian regulation of some arabidopsis genes (Anderson *et al.*, 1994), and the IspS promoter may, also. These findings are consistent with observations to date on the circadian regulation of isoprene emission: isoprene emission and *IspS* transcript levels show strong circadian rhythms (Wilkinson *et al.*, 2006; Loivamäki *et al.*, 2007*b*), while *DXR* transcript levels do

not (Mayrhofer *et al.*, 2005). The role of the LHY motif for the *IspS* promoter was confirmed by an electrophoretic mobility shift assay (Loivamäki *et al.*, 2007*b*). In both of the cases examined here, DXS and IspS show strong potential for important regulation. These *in silico* analyses help pinpoint genes that may be key in regulation of isoprene emission under varying conditions, identifying suitable targets for further *in vivo* and *in vitro* work.

Regulation through energetics. The MEP pathway requires a significant amount of reducing power and ATP, and this could link regulation of the pathway with photosynthesis. There usually is no emission of isoprene from leaves in darkness. Correlations have been found between leaf ATP content and isoprene emission rate (Loreto and Sharkey, 1993). Models of isoprene emission rate often rely on predictions of photosynthetic electron transport rates to predict isoprene emission rate (Niinemets *et al.*, 1999; Martin *et al.*, 2000; Zimmer *et al.*, 2000).

There are three redox reactions in the MEP pathway. DXR is known to use NADPH, readily available during photosynthetic electron transport. In plants, the HDS enzyme can use electrons transferred directly from the electron transport chain through ferredoxin (Seemann *et al.*, 2006) further connecting MEP pathway activity to photosynthesis. The HDR reductant has not been identified (Seemann *et al.*, 2002). One ATP and one CTP are required in the MEP pathway. The CTP loses two phosphates, and so, presuming the CTP is regenerated by ATP, the total ATP cost is three. If isoprene emission is typically 2 % of photosynthesis on a carbon basis (Sharkey and Yeh, 2001), and there are five carbons per isoprene, the ATP used in the MEP pathway for isoprene synthesis is 0·4 % of that being used for carbon fixation; for reducing power, 0·6 % of that used for carbon fixation is used for MEP pathway reactions. If all of the energy needed to reduce CO_2 to sugars is included in the cost of isoprene the totals rise to 2·7 % of ATP and 3·4 % of NADPH is required for isoprene emission at a rate of 2 % of photosynthesis on a carbon basis, not including photorespiration. While these amounts of energy use could lead to loss of the capacity for isoprene emission through evolution if isoprene emission had no value to the plant, these are trivial amounts of energy compared with, for example, photorespiration, where 20–40 % of the total ATP and NADPH usage can be used (Sharkey, 1988). This is why the suggestion that one function of isoprene emission is to dissipate unused energy (Magel *et al.*, 2007) does not hold up under quantitative scrutiny. Because of the very small proportion of energy used by the MEP pathway under normal conditions, the control of the MEP pathway by energetics of the chloroplast is likely to be regulatory rather than by mass action effects. Thus, competition between carbon fixation and the MEP pathway for energy is less likely to be a useful predictor of isoprene emission than is energy status of the chloroplast, which can be unrelated to electron transport rates. Of course, when isoprene emission increases to a large proportion of the carbon fixed the energy cost increases.

Regulation by carbon supply. The MEP pathway draws on the Calvin cycle for carbon. Feeding $^{13}CO_2$ to leaves results in a rapid appearance of ^{13}C in isoprene (Sanadze *et al.*, 1972; Delwiche and Sharkey, 1993; Loreto *et al.*, 1996; Karl *et al.*, 2002; Affek and Yakir, 2003; Loreto *et al.*, 2004). Similar results have been obtained using $^{11}CO_2$ (Funk *et al.*, 2004). One of the puzzling findings has been that isoprene does not become completely labelled. In oak and poplar trees it was shown that sugar arriving in the transpiration stream can contribute carbon to isoprene (Kreuzwieser *et al.*, 2002). During water stress, as the availability of carbon in the Calvin cycle becomes limited, more carbon comes from other sources (Brilli *et al.*, 2007). However, Delwiche and Sharkey (1993) pointed out that the first carbon product of photosynthesis, phosphoglyceric acid, shows similar incomplete labelling. Thus, the incomplete labelling may be a general phenomenon related to the availability of carbon within the chloroplast and may not have special significance to isoprene synthesis. The incomplete labelling need not indicate a decoupling between plastid carbon metabolism and isoprene synthesis. It is tempting to assume that the incomplete labelling of isoprene reflects the cytosolic source of pyruvate [through phosphoenolpyruvate (PEP) import into chloroplasts]. However, this has not been directly demonstrated, and given incomplete labelling of PGA, the explanation that incomplete labelling of isoprene is caused by the pyruvate source in the cytosol should be viewed with caution.

Isoprene emission capacity is reduced at high CO_2 (Rosenstiel *et al.*, 2003; Centritto *et al.*, 2004; Pegoraro *et al.*, 2004*a*, *b*, 2005*a*; Scholefield *et al.*, 2004). It is not easy to see an adaptive explanation for this response based on the thermotolerance hypothesis, since high CO_2 should lead to stomatal closure and increased leaf temperature. Rosenstiel *et al.* (2003) proposed and presented evidence for a mechanistic explanation. They showed that PEP carboxylase competed for substrate with isoprene emission. Inhibiting PEP carboxylase stimulated isoprene emission (Rosenstiel *et al.*, 2003, 2004). Loreto *et al.* (2007) found a positive relationship between dark respiration and isoprene emission. This is inconsistent with mitochondrial activity competing with isoprene emission for PEP during the day, and this could indicate that mitochondrial respiration during the day is low or uses substrates other than pyruvate derived from PEP. In their experiments there was a negative correlation between isoprene emission and PEP carboxylase activity, consistent with the hypothesis of competition between PEP carboxylation and isoprene synthesis (Loreto *et al.*, 2007).

On the other hand, Wolfertz *et al.* (2004) showed that isoprene emission was strongly controlled by the activity of DXS. Aspen grown in elevated CO_2 have reduced amounts of IspS, which partially accounts for a long-term reduction in isoprene emission capacity (Calfapietra *et al.*, 2007). This and other data make it clear that there can be several factors controlling the rate of isoprene emission simultaneously. This makes modelling isoprene emission potentially more complex than modelling photosynthesis, where either Rubisco activity or ribulose bisphosphate regeneration dominate the control of the rate of photosynthesis at any given instant (Farquhar *et al.*, 1980). The

effect of elevated CO_2 on isoprene emission may provide some insights into the control of isoprene emission and has implications for predicting global change effects on the atmosphere (Monson *et al.*, 2007; Possell *et al.*, 2005).

SYNTHESIS

Isoprene emission from plants is an unseen but highly significant component of atmosphere–biosphere interaction. Because it is possible for plants to survive without significant isoprene production and emission, we presume that those plants that do emit derive some benefit that outweighs the cost. Thermotolerance has significant explanatory power and experimental support. Inhibitor studies and genetic approaches have confirmed that thermotolerance is provided by isoprene. Isoprene-emitting plants are also protected against ozone but, given that significant ozone stress is a recent phenomenon, it may be that this is simply a happy coincidence. The regulation of the rate of isoprene emission should reflect the benefits derived from isoprene. It is not surprising, then, that temperature regulates isoprene emission at many different levels. Evidence for regulation at the level of gene transcription has been found but, more generally, the regulation has been difficult to understand. This is in part because there is a significant amount of DMADP in leaves in a compartment that is not accessible to IspS. While isoprene emission is significant at approx. 2 % of photosynthesis, it is hard to do detailed analyses of carbon flux regulation on a background activity 50 times greater than the process under study. Molecular tools are being developed and the use of stable isotopes has led to significant insights into isoprene emission rate regulation. Substantial progress is likely in the coming years. We should be able to answer with increasing depth 'how and why plants emit isoprene'.

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LITERATURE CITED

Affek HP, Yakir D. 2002. Protection by isoprene against singlet oxygen in leaves. *Plant Physiology* **129**: 269–277.

Affek HP, Yakir D. 2003. Natural abundance carbon isotope composition of isoprene reflects incomplete coupling between isoprene synthesis and photosynthetic carbon flow. *Plant Physiology* **131**: 1727–1736.

Albrecht M, Sandmann G. 1994. Light-stimulated carotenoid biosynthesis during transformation of maize etioplasts is regulated by increased activity of isopentenyl pyrophosphate isomerase. *Plant Physiology* **105**: 529–534.

Anderson SL, Teakle GR, Martino-Catt SJ, Kay SA. 1994. Circadian clock- and phytochrome-regulated transcription is conferred by a 78 bp *cis*-acting domain of the *Arabidopsis CAB2* promoter. *The Plant Journal* **6**: 457–470.

Behnke K, Ehlting B, Teuber M, Bauerfeind M, Louis S, Hänsch R, *et al.* 2007. Transgenic, non-isoprene emitting poplars don't like it hot. *The Plant Journal* **56**: 485–499.

Bohlmann J, Meyer-Gauen G, Croteau R. 1998. Plant terpenoid synthases: molecular biology and phylogenetic analysis. *Proceedings of the National Academy of Sciences of the USA* **95**: 4126–4133.

Brilli F, Barta C, Fortunati A, Lerdau M, Loreto F, Centritto M. 2007. Response of isoprene emission and carbon metabolism to drought in white poplar (*Populus alba*) saplings. *New Phytologist* **175**: 244–254.

Brüggemann N, Schnitzler JP. 2002*a*. Comparison of isoprene emission, intercellular isoprene concentration and photosynthetic performance in water-limited oak (*Quercus pubescens* Willd. and *Quercus robur* L.) saplings. *Plant Biology* **4**: 456–463.

Brüggemann N, Schnitzler J-P. 2002*b*. Relationship of isopentenyl diphosphate (IDP) isomerase activity to isoprene emission of oak leaves. *Tree Physiology* **22**: 1011–1018.

Bukhov NG, Wiese C, Neimanis S, Heber U. 1999. Heat sensitivity of chloroplasts and leaves: [eakage of protons from thylakoids and reversible activation of cyclic electron transport]. *Photosynthesis Research* **59**: 81–93.

Calfapietra C, Wiberley AE, Falbel TG, Linskey AR, Mugnozza GS, Karnosky DF, *et al.* 2007. Isoprene synthase expression and protein levels are reduced under elevated O_3 but not under elevated CO_2 (FACE) in field-grown aspen trees. *Plant, Cell and Environment* **30**: 654–661.

Carretero-Paulet L, Ahumada I, Cunillera N, Rodríguez-Concepción M, Ferrer A, Boronat A, *et al.* 2002. Expression and molecular analysis of the *Arabidopsis* DXR gene encoding 1-deoxy-D-xylulose 5-phosphate reductoisomerase, the first committed enzyme of the 2-C-methyl-D-erythritol 4-phosphate pathway. *Plant Physiology* **129**: 1581–1591.

Carretero-Paulet L, Cairó A, Botella-Pavía P, Besumbes O, Campos N, Boronat A, *et al.* 2006. Enhanced flux through the methylerythritol 4-phosphate pathway in *Arabidopsis* plants overexpressing deoxyxylulose 5-phosphate reductoisomerase. *Plant Molecular Biology* **62**: 683–695.

Centritto M, Nascetti P, Petrilli L, Raschi A, Loreto F. 2004. Profiles of isoprene emission and photosynthetic parameters in hybrid poplars exposed to free-air CO_2 enrichment. *Plant, Cell and Environment* **27**: 403–412.

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**: 1473–1475.

Claeys M, Graham B, Vas G, Wang W, Vermeylen R, Pashynska V, *et al.* 2004. Formation of secondary organic aerosols through photooxidation of isoprene. *Science* **303**: 1173–1176.

Copolovici LO, Filella I, Llusia J, Niinemets U, Penuelas J. 2005. The capacity for thermal protection of photosynthetic electron transport varies for different monoterpenes in *Quercus ilex*. *Plant Physiology* **139**: 485–496.

Csiky O, Seufert G. 1999. Terpenoid emissions of Mediterranean oaks and their relation to taxonomy. *Ecological Applications* **9**: 1138–1146.

Davidson JF, Whyte B, Bissinger PH, Schiestl RH. 1996. Oxidative stress is involved in heat-induced cell death in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the USA* **93**: 5116–5121.

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 Phytologist* **146**: 27–36.

Delwiche CF, Sharkey TD. 1993. Rapid appearance of ^{13}C in biogenic isoprene when $^{13}CO_2$ is fed to intact leaves. *Plant, Cell and Environment* **16**: 587–591.

Drake BG, Raschke K, Salisbury FB. 1970. Temperature and transpiration resistances of *Xanthium* leaves as affected by air temperature, humidity, and wind speed. *Plant Physiology* **46**: 324–330.

Dudareva N, Andersson S, Orlova I, Gatto N, Reichelt M, Rhodes D, *et al.* 2005. From the cover: the nonmevalonate pathway supports both monoterpene and sesquiterpene formation in snapdragon flowers. *Proceedings of the National Academy of Sciences of the USA* **102**: 933–938.

Edney EO, Kleindienst TE, Jaoui M, Lewandowski M, Offenberg JH, Wang W, *et al.* 2005. Formation of 2-methyl tetrols and 2-methylglyceric acid in secondary organic aerosol from laboratory irradiated isoprene/NO_X/SO_2/air mixtures and their detection in

ambient $PM_{2.5}$ samples collected in the eastern United States. *Atmospheric Environment* **39**: 5281–5289.

Ehleringer JR. 1991. Temperature and energy budgets. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel PW, eds. *Plant physiological ecology.* London: Chapman and Hall, 117–135.

El Tamer MK, Lucker J, Bosch D, Verhoeven HA, Verstappen FWA, Schwab W, *et al*. 2003. Domain swapping of *Citrus limon* monoterpene synthases: impact on enzymatic activity and product specificity. *Archives of Biochemistry and Biophysics* **411**: 196–203.

Estévez JM, Cantero A, Reindl A, Reichler S, Leon P. 2001. 1-Deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. *Journal of Biological Chemistry* **276**: 22901–22909.

Fang C, Monson RK, Cowling EB. 1996. Isoprene emission, photosynthesis, and growth in sweetgum (*Liquidambar styraciflua*) seedlings exposed to short- and long-term drying cycles. *Tree Physiology* **16**: 441–446.

Fares S, Barta C, Brilli F, Centritto M, Ederli L, Ferranti F, *et al*. 2006. Impact of high ozone on isoprene emission, photosynthesis and histology of developing *Populus alba* leaves directly or indirectly exposed to the pollutant. *Physiologia Plantarum* **128**: 456–465.

Farquhar GD, Von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149**: 78–90.

Fehsenfeld FC, Calvert J, Fall R, Goldan P, Guenther A, Hewitt C, *et al*. 1992. Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochemical Cycles* **6**: 389–430.

Ferrieri RA, Gray DW, Babst BA, Schueller MJ, Schlyer DJ, Thorpe MR, *et al*. 2005. Use of carbon-11 in *Populus* shows that exogenous jasmonic acid increases biosynthesis of isoprene from recently fixed carbon. *Plant, Cell and Environment* **28**: 591–602.

Fuentes JD, Wang D. 1999. On the seasonality of isoprene emission from a mixed temperate forest. *Ecological Applications* **9**: 1118–1131.

Fuentes JD, Wang D, Gu L. 1999. Seasonal variations in isoprene emissions from a boreal aspen forest. *Journal of Applied Meterology* **38**: 855–869.

Funk JL, Mak JE, Lerdau MT. 2004. Stress-induced changes in carbon sources for isoprene production in *Populus deltoides*. *Plant, Cell and Environment* **27**: 747–755.

Funk JL, Jones CG, Gray DW, Throop HL, Hyatt LA, Lerdau MT. 2005. Variation in isoprene emission from *Quercus rubra*: sources, causes, and consequences for estimating. *Journal of Geophysical Research – Atmospheres* **110**: doi:10·1029/2004JD005229.

Goldstein AH, Goulden ML, Munger JW, Wofsy SC, Geron CD. 1998. Seasonal course of isoprene emissions from a midlatitude deciduous forest. *Journal of Geophysical Research – Atmospheres* **103**: 31045–31056.

Gray DW, Lerdau MT, Goldstein AH. 2003. Influences of temperature history, water stress, and needle age on methylbutenol emissions. *Ecology* **84**: 765–776.

von Gromoff ED, Schroda M, Oster U, Beck CF. 2006. Identification of a plastid response element that acts as an enhancer within the *Chlamydomonas* HSP70A promoter. *Nucleic Acids Research* **34**: 4767–4779.

Guenther A, Hewitt CN, Erickson D, Fall R, Geron C, Graedel T, *et al*. 1995. A global model of natural volatile organic compound emissions. *Journal of Geophysical Research* **100**: 8873–8892.

Guenther A, Karl T, Harley P, Wiedinmyer C, Palmer PI, Geron C. 2006. Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). *Atmospheric Chemistry Physics* **6**: 3181–3210.

Guenther AB, Zimmerman PR, Harley PC. 1993. Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analysis. *Journal of Geophysical Research* **98**: 12609–12617.

Guevara-Garcia A, San Roman C, Arroyo A, Cortes ME, de la Luz Gutierrez-Nava M, Leon P. 2005. Characterization of the Arabidopsis clb6 mutant illustrates the importance of posttranscriptional regulation of the methyl-D-erythritol 4-phosphate pathway. *The Plant Cell* **17**: 628–643.

Gurley WB, Key JL. 1991. Transcriptional regulation of the heat-shock response: a plant perspective. *Biochemistry* **30**: 1–12.

Hanson DT, Swanson S, Graham LE, Sharkey TD. 1999. Evolutionary significance of isoprene emission from mosses. *American Journal of Botany* **86**: 634–639.

Haralampidis K, Milioni D, Rigas S, Hatzopoulos P. 2002. Combinatorial interaction of *cis* elements specifies the expression of the arabidopsis AtHsp90-1 gene. *Plant Physiology* **129**: 1138–1149.

Harley P, Fridd-stroud V, Greenberg J, Guenther A, Vasconcellos P. 1998. Emission of 2-methyl-3-buten-2-ol by pines: a potentially large natural source of reactive carbon to the atmosphere. *Journal of Geophysical Research* **103**: 25479–25486.

Harley PC, Guenther AB, Zimmerman PR. 1996. Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidamber styraciflua*) leaves. *Tree Physiology* **16**: 25–32.

Harley PC, Monson RK, Lerdau MT. 1999. Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia* **118**: 109–123.

Hewitt CN, Kok GL, Fall R. 1990. Hydroperoxides in plants exposed to ozone mediate air pollution damage to alkene emitters. *Nature* **344**: 56–58.

Higo K, Ugawa Y, Iwamoto M, Korenaga T. 1999. Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Research* **27**: 297–300.

Hotta CT, Gardner MJ, Hubbard KE, Baek SJ, Dalchau N, Suhita D, *et al*. 2007. Modulation of environmental responses of plants by circadian clocks. *Plant, Cell and Environment* **30**: 333–349.

Jacob DJ. 1999. *Introduction to Atmospheric Chemistry*. Cambridge, MA: Harvard University Press.

Kampranis SC, Ioannidis D, Purvis A, Mahrez W, Ninga E, Katerelos NA, *et al*. 2007. Rational conversion of substrate and product specificity in a *Salvia* monoterpene synthase: structural insights into the evolution of terpene synthase function. *The Plant Cell* **19**: 1994–2005.

Karl T, Fall R, Rosenstiel TN, Prazeller P, Larsen B, Seufert G, *et al*. 2002. On-line analysis of the $^{13}CO_2$ labeling of leaf isoprene suggests multiple subcellular origins of isoprene precursors. *Planta* **215**: 894–905.

Kesselmeier J, Staudt M. 1999. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *Journal of Atmospheric Chemistry* **33**: 23–88.

Kovtun Y, Chiu WL, Tena G, Sheen J. 2000. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proceedings of the National Academy of Sciences of the USA* **97**: 2940–2945.

Kreuzwieser J, Graus M, Wisthaler A, Hansel A, Rennenberg H, Schnitzler J-P. 2002. Xylem-transported glucose as an additional carbon source for leaf isoprene formation in *Quercus robur*. *New Phytologist* **156**: 171–178.

Kroll JH, Ng NL, Murphy SL, Flagen RC, Seinfeld JH. 2005. Secondary organic aerosol formation from isoprene photooxidation under high-NOx conditions. *Geophysical Research Letters* **32**: L18808.

Kroll JH, Ng NL, Murphy SM, Flagan RC, Seinfeld JH. 2006. Secondary organic aerosol formation from isoprene photooxidation. *Environmental Science and Technology* **40**: 1869–1877.

Kuhn U, Rottenberger S, Biesenthal T, Wolf A, Schebeske G, Ciccioli P, *et al*. 2004. Strong correlation between isoprene emission and gross photosynthetic capacity during leaf phenology of the tropical tree species *Hymenaea courbaril* with fundamental changes in volatile organic compounds emission composition during early leaf development. *Plant, Cell and Environment* **27**: 1469–1485.

Kuzma J, Fall R. 1993. Leaf isoprene emission rate is dependent on leaf development and the level of isoprene synthase. *Plant Physiology* **101**: 435–440.

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 Letters* **39**: 7913–7916.

Laule O, Furholz A, Chang HS, Zhu T, Wang X, Heifetz PB, *et al*. 2003. Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA* **100**: 6866–6871.

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 and Environment* **22**: 495–504.

Lehning A, Zimmer W, Zimmer I, Schnitzler J-P. 2001. Modeling of annual variations of oak (*Quercus robur* L.) isoprene synthase activity to predict isoprene emission rates. *Journal of Geophysical Research* **106D**: 3157–3166.

Lerdau M. 2007. ECOLOGY: a positive feedback with negative consequences. *Science* **316**: 212–213.

Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, *et al*. 2002. PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Research* **30**: 325–327.

Liu X, Huang B. 2002. Cytokinin effects on creeping bentgrass response to heat stress. II. Leaf senescence and antioxidant metabolism. *Crop Science* **42**: 466–472.

Logan BA, Monson RK. 1999. Thermotolerance of leaf discs from four isoprene-emitting species is not enhanced by exposure to exogenous isoprene. *Plant Physiology* **120**: 821–825.

Lois LM, Rodriguez-Concepción M, Gallego F, Campos N, Boronat A. 2000. Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. *The Plant Journal* **22**: 503–513.

Loivamäki M, Gilmer F, Fischbach RJ, Sorgel C, Bachl A, Walter A, *et al*. 2007*a*. Arabidopsis, a model to study biological functions of isoprene emission? *Plant Physiology* **144**: 1066–1078.

Loivamäki M, Louis S, Cinege G, Zimmer I, Fischbach RJ, Schnitzler JP. 2007*b*. Circadian rhythms of isoprene biosynthesis in grey poplar leaves. *Plant Physiology* **143**: 540–551.

Loreto F, Sharkey TD. 1990. A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L. *Planta* **182**: 523–531.

Loreto F, Sharkey TD. 1993. On the relationship between isoprene emission and photosynthetic metabolites under different environmental conditions. *Planta* **189**: 420–424.

Loreto F, Velikova V. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology* **127**: 1781–1787.

Loreto F, Ciccioli P, Cecinato A, Brancaleoni E, Frattoni M, Fabozzi C, *et al*. 1996. Evidence of the photosynthetic origin of monoterpenes emitted by *Quercus ilex* L. leaves by ^{13}C labeling. *Plant Physiology* **110**: 1317–1322.

Loreto F, Ciccioli P, Brancaleoni E, Valentini R, De Lillis M, Csiky O, *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**: 302–305.

Loreto F, Mannozzi M, Maris C, Nascetti P, Ferranti F, Pasqualini S. 2001. Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiology* **126**: 993–1000.

Loreto F, Pinelli P, Brancaleoni E, Ciccioli P. 2004. ^{13}C labelling reveals chloroplastic and extra-chloroplastic pools of dimethylallyl pirophosphate and their contribution to isoprene formation. *Plant Physiology* **135**: 1903–1907.

Loreto F, Centritto M, Barta C, Calfapietra C, Fares S, Monson RK. 2007. The relationship between isoprene emission rate and dark respiration rate in white poplar (*Populus alba* L.) leaves. *Plant, Cell and Environment* **30**: 662–669.

Magel E, Mayrhofer S, Muller A, Zimmer I, Hampp R, Schnitzler JP. 2007. Photosynthesis and substrate supply for isoprene biosynthesis in poplar leaves. *Atmospheric Environment* **40**: 5138–5151.

Martin MJ, Stirling CM, Humphries SW, Long SP. 2000. A process-based model to predict the effects of climatic change on leaf isoprene emission rates. *Ecological Modelling* **131**: 161–174.

Mayrhofer S, Teuber M, Zimmer I, Louis S, Fischbach RJ, Schnitzler JP. 2005. Diurnal and seasonal variation of isoprene biosynthesis-related genes in grey poplar leaves. *Plant Physiology* **139**: 474–484.

Miller B, Oschinski C, Zimmer W. 2001. First isolation of an isoprene synthase gene from poplar and successful expression of the gene in *Escherichia coli*. *Planta* **213**: 483–487.

Monson RK, Fall R. 1989. Isoprene emission from aspen leaves: the influence of environment and relation to photosynthesis and photorespiration. *Plant Physiology* **90**: 267–274.

Monson RK, Holland EA. 2001. Biospheric trace gas fluxes and their control over tropospheric chemistry. *Annual Review of Ecology and Systematics* **32**: 547–576.

Monson RK, Jaeger CH, Adams III WW, Driggers EM, Silver GM, Fall R. 1992. Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. *Plant Physiology* **98**: 1175–1180.

Monson RK, Harley PC, Litvak ME, Wildermuth M, Guenther AB, Zimmerman PR, *et al*. 1994. Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. *Oecologia* **99**: 260–270.

Monson RK, Trahan N, Rosenstiel TN, Veres P, Moore D, Wilkinson M, *et al*. 2007. Isoprene emission from terrestrial ecosystems in response to global change: minding the gap between models and observations. *Philosophical Transactions of the Royal Society A* **365**: 1677–1695.

Muñoz-Bertomeu J, Arrillaga I, Ros R, Segura J. 2006. Up-regulation of 1-deoxy-D-xylulose-5-phosphate synthase enhances production of essential oils in transgenic spike lavender. *Plant Physiology* **142**: 890–900.

Ng NL, Kroll JH, Keywood MD, Bahreini R, Varutbangkul V, Flagan RC, *et al*. 2006. Contribution of first- versus second-generation products to secondary organic aerosols formed in the oxidation of biogenic hydrocarbons. *Environmental Science and Technology* **40**: 2283–2297.

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 and Environment* **22**: 1319–1335.

Nover L, Bharti K, Döring P, Mishra SK, Ganguli A, Scharf K-D. 2001. *Arabidopsis* and the heat stress transcription factor world: how many heat stress transcription factors do we need? *Cell Stress & Chaperones* **6**: 177–189.

Olcese LE, Penner JE, Sillman S. 2007. Development of a secondary organic aerosol formation mechanism: comparison with smog chamber experiments and atmospheric measurements. *Atmospheric Chemistry and Physics Discussions* **7**: 8361–8393.

Owen SM, Peñuelas J. 2005. Opportunistic emissions of volatile isoprenoids. *Trends in Plant Science* **10**: 420–426.

Pastenes C, Horton P. 1996. Effect of high temperature on photosynthesis in beans. 2. CO_2 assimilation and metabolite contents. *Plant Physiology* **112**: 1253–1260.

Pegoraro E, Rey A, Bobich EG, Barron-Gafford G, Grieve KA, Malhi Y, *et al*. 2004*a*. Effect of elevated CO_2 concentration and vapour pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought. *Functional Plant Biology* **31**: 1137–1147.

Pegoraro E, Rey A, Greenberg J, Harley P, Grace J, Malhi Y, *et al*. 2004*b*. Effect of drought on isoprene emission rates from leaves of *Quercus virginiana* Mill. *Atmospheric Environment* **38**: 6149–6156.

Pegoraro E, Abrell L, Van Haren J, Barron-Gafford G, Grieve KA, Malhi Y, *et al*. 2005*a*. The effect of elevated atmospheric CO_2 and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm. *Global Change Biology* **11**: 1234–1246.

Pegoraro E, Rey A, Barron-Gafford G, Monson R, Malhi Y, Murthy R. 2005*b*. The interacting effects of elevated atmospheric CO_2 concentration, drought and leaf-to-air vapour pressure deficit on ecosystem isoprene fluxes. *Oecologia* **146**: 120–129.

Peñuelas J, Llusià J. 2002. Linking photorespiration, monoterpenes and thermotolerance in *Quercus*. *New Phytologist* **155**: 227–237.

Peñuelas J, Llusià J, Asensio D, Munné-Bosch S. 2005. Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. *Plant, Cell and Environment* **28**: 278–286.

Pétron G, Harley P, Greenberg J, Guenther A. 2001. Seasonal temperature variations influence isoprene emission. *Atmospheric Environment* **28**: 1707–1710.

Pichersky E, Sharkey TD, Gershenzon J. 2006. Plant volatiles: a lack of function or a lack of knowledge? *Trends in Plant Science* **11**: 421–421.

Piechulla B, Merforth N, Eibl C, Koop H-U, Mullet JE. 2001. Identification of tomato *Lhc* promoter regions necessary for circadian expression. *Plant Molecular Biology* **38**: 655–662.

Pope C. 1980. The candidates and the issues. *Sierra* **65**: 15–17.

Possell M, Nicholas Hewitt C, Beerling DJ. 2005. The effects of glacial atmospheric CO_2 concentrations and climate on isoprene emissions by vascular plants. *Global Change Biology* **11**: 60–69.

Purves DW, Caspersen JP, Moorcroft PR, Hurtt GC, Pacala SW. 2004. Human-induced changes in US biogenic volatile organic compound emissions: evidence from long-term forest inventory data. *Global Change Biology* **10**: 1737–1755.

Rasmussen RA, Jones CA. 1973. Emission isoprene from leaf discs of *Hamamelis*. *Phytochemistry* **12**: 15–19.

Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R, Monson RK. 2003. Increased CO_2 uncouples growth from isoprene emission in an agri-forest ecosystem. *Nature* **421**: 256–259.

Rosenstiel TN, Ebbets AL, Khatri WC, Fall R, Monson RK. 2004. Induction of poplar leaf nitrate reductase: a test of extrachloroplastic control of isoprene emission rate. *Plant Biology* **6**: 12–21.

Sage RF. 2001. Environmental and evolutionary preconditions for the origin and diversification of the C_4 photosynthetic syndrome. *Plant Biology* **3**: 202–213.

Sage RF, Pearcy RW. 2000. The physiological ecology of C_4 photosynthesis. In: Leegood RC, Sharkey TD, Von Caemmerer S, eds. *Photosynthesis: physiology and metabolism.* Dordrecht: Kluwer Academic Publishers, 497–532.

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 Physiology* **84**: 658–664.

Sanadze GA. 2004. Biogenic isoprene (a review). *Russian Journal of Plant Physiology* **51**: 729–741.

Sanadze GA. 1969. Light-dependent excretion of molecular isoprene. *Progress in Photosynthesis Research* **2**: 701–706.

Sanadze GA. 1991. Isoprene effect: light dependent emission of isoprene by green parts of plants. In: Sharkey TD, Holland EA, Mooney HA, eds. *Trace gas emissions by plants.* San Diego, CA: Academic Press, 135–152.

Sanadze GA, Kalandaze AN. 1966. Light and temperature curves of the evolution of C_5H_8. *Soviet Plant Physiology* **13**: 458–461.

Sanadze GA, Dzhaiani GI, Tevzadze IM. 1972. Incorporation into the isoprene molecule of carbon from $^{13}CO_2$ assimilated during photosynthesis. *Soviet Plant Physiology* **19**: 17–20.

Sasaki K, Ohara K, Yazaki K. 2005. Gene expression and characterization of isoprene synthase from *Populus alba*. *FEBS Letters* **579**: 2514–2518.

Schnitzler J-P, Arenz R, Steinbrecher R, Lehning A. 1996. Characterization of an isoprene synthase from leaves of *Quercus petraea* (Mattuschka) Liebl. *Botanical Acta* **109**: 216–221.

Schnitzler J-P, Lehning A, Steinbrecher R. 1997. Seasonal pattern of isoprene synthase activity in *Quercus robur* leaves and its significance for modeling isoprene emission rates. *Botanical Acta* **110**: 240–243.

Schnitzler JP, Graus M, Kreuzwieser J, Heizmann U, Rennenberg H, Wisthaler A, *et al*. 2004. Contribution of different carbon sources to isoprene biosynthesis in poplar leaves. *Plant Physiology* **135**: 152–160.

Schnitzler JP, Zimmer I, Bachl A, Arend M, Fromm J, Fischbach R. 2005. Biochemical properties of isoprene synthase in poplar (*Populus canescens*). *Planta* **222**: 777–786.

Scholefield PA, Doick KJ, Herbert BMJ, Hewitt CNS, Schnitzler JP, Pinelli P, *et al*. 2004. Impact of rising CO_2 on emissions of volatile organic compounds: isoprene emission from *Phragmites australis* growing at elevated CO_2 in a natural carbon dioxide spring. *Plant, Cell and Environment* **27**: 393–401.

Schrader SM, Wise RR, Wacholtz WF, Ort DR, Sharkey TD. 2004. Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. *Plant, Cell and Environment* **27**: 725–735.

Schrader SM, Kleinbeck KR, Sharkey TD. 2007. Rapid heating of intact leaves reveals initial effects of stromal oxidation on photosynthesis. *Plant, Cell and Environment* **30**: 671–678.

Schwender J, Zeidler J, Groner R, Muller C, Focke M, Braun S, *et al*. 1997. Incorporation of 1-deoxy-D-xylulose into isoprene and phytol by higher plants and algae. *FEBS Letters* **414**: 129–134.

Schwender J, Gemünden C, Lichtenthaler HK. 2001. Chlorophyta exclusively use the 1-deoxyxylulose 5-phosphate/2-*C*-methyl-erythritol 4-phosphate pathway for the biosynthesis of isoprenoids. *Planta* **212**: 416–423.

Seemann M, Tse Sum Bui B, Wolff M, Tritsch D, Campos N, Boronat A, *et al*. 2002. Isoprenoid biosynthesis through the methyl-erythritol phosphate pathway: the (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase (GcpE) is a [4Fe-4S] protein. *Angewandte Chemie International Edition* **41**: 4337–4339.

Seemann M, Tse Sum Bui B, Wolff M, Miginiac-Maslow M, Rohmer M. 2006. Isoprenoid biosynthesis in plant chloroplasts via the MEP pathway: direct thylakoid/ferredoxin-dependent photoreduction of GcpE/IspG. *FEBS Letters* **580**: 1547–1552.

Sharkey TD. 1988. Estimating the rate of photorespiration in leaves. *Physiologia Plantarum* **73**: 147–152.

Sharkey TD, Loreto F. 1993. Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. *Oecologia* **95**: 328–333.

Sharkey TD, Schrader SM. 2006. High temperature stress. In: Rao KVM, Raghavendra AS, Reddy KJ, eds. *Physiology and molecular biology of stress tolerance in plants.* Dordrecht: Springer, 101–130.

Sharkey TD, Singsaas EL. 1995. Why plants emit isoprene. *Nature* **374**: 769–769.

Sharkey TD, Yeh S. 2001. Isoprene emission from plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**: 407–436.

Sharkey TD, Loreto F, Delwiche CF. 1991. The biochemistry of isoprene emission from leaves during photosynthesis. In: Sharkey TD, Holland EA, Mooney HA, eds. *Trace gas emissions from plants.* San Diego, CA: Academic Press, 153–184.

Sharkey TD, Singsaas EL, Vanderveer PJ, Geron CD. 1996. Field measurements of isoprene emission from trees in response to temperature and light. *Tree Physiology* **16**: 649–654.

Sharkey TD, Singsaas EL, Lerdau MT, Geron C. 1999. Weather effects on isoprene emission capacity and applications in emissions algorithms. *Ecological Applications* **9**: 1132–1137.

Sharkey TD, Chen XY, Yeh S. 2001. Isoprene increases thermotolerance of fosmidomycin-fed leaves. *Plant Physiology* **125**: 2001–2006.

Sharkey TD, Yeh S, Wiberley AE, Falbel TG, Gong D, Fernandez DE. 2005. Evolution of the isoprene biosynthetic pathway in kudzu. *Plant Physiology* **137**: 700–712.

Silver GM, Fall R. 1991. Enzymatic synthesis of isoprene from dimethylallyl diphosphate in aspen leaf extracts. *Plant Physiology* **97**: 1588–1591.

Singsaas EL, Lerdau M, Winter K, Sharkey TD. 1997. Isoprene increases thermotolerance of isoprene-emitting species. *Plant Physiology* **115**: 1413–1420.

Singsaas EL, Laporte MM, Shi J-Z, Monson RK, Bowling DR, Johnson K, *et al*. 1999. Leaf temperature fluctuation affects isoprene emission from red oak (*Quercus rubra*) leaves. *Tree Physiology* **19**: 917–924.

Siwko ME, Marrink SJ, de Vries AH, Kozubek A, Schoot Uiterkamp AJM, Mark AE. 2007. Does isoprene protect plant membranes from thermal shock? A molecular dynamics study. *Biochimica et Biophysica Acta – Biomembranes* **1768**: 198–206.

Sun Z, Cunningham FX, Gantt E. 1998. Differential expression of two isopentenyl pyrophosphate isomerases and enhanced carotenoid accumulation in a unicellular chlorophyte. *Proceedings of the National Academy of Sciences of the USA* **95**: 11482–11488.

Terry GM, Stokes NJ, Hewitt CN, Mansfield TA. 1995. Exposure to isoprene promotes flowering in plants. *Journal of Experimental Botany* **46**: 1629–1631.

Tholl D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current Opinion in Plant Biology* **9**: 297–304.

Tholl D, Croteau R, Gershenzon J. 2001. Partial purification and characterization of the short-chain prenyltransferases, geranyl diphosphate synthase and farnesyl diphosphate synthase, from *Abies grandis* (grand fir). *Archives of Biochemistry and Biophysics* **386**: 233–242.

Thum KE, Kim M, Morishige DT, Eibl C, Koop HU, Mullet JE. 2001. Analysis of barley chloroplast psbD light-responsive promoter elements in transplastomic tobacco. *Plant Molecular Biology* **47**: 353–366.

Tingey DT, Manning M, Grothaus LC, Burns WF. 1979. The influence of light and temperature on isoprene emission rates from live oak. *Physiologia Plantarum* **47**: 112–118.

Tingey DT, Evans R, Gumpertz M. 1981. Effects of environmental conditions on isoprene emission from live oak. *Planta* **152**: 565–570.

Tingey DT, Evans RC, Bates EH, Gumpertz ML. 1987. Isoprene emissions and photosynthesis in three ferns: the influence of light and temperature. *Physiologia Plantarum* **69**: 609–616.

Trainer M, Williams EJ, Parrish DD, Buhr MP, Allwine EJ, Westberg HH, *et al*. 1987. Models and observations of the impact of natural hydrocarbons on rural ozone. *Nature* **329**: 705–707.

Trapp SC, Croteau RB. 2001. Genomic organization of plant terpene synthases and molecular evolutionary implications. *Genetics* **158**: 811–832.

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, *et al*. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**: 1596–1604.

Velikova V, Loreto F. 2005. On the relationship between isoprene emission and thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat stress. *Plant, Cell and Environment* **28**: 318–327.

Velikova V, Edreva A, Loreto F. 2004. Endogenous isoprene protects *Phragmites australis* leaves against singlet oxygen. *Physiologia Plantarum* **122**: 219–225.

Vierling E. 1991. The roles of heat shock proteins in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**: 579–620.

Weis E. 1982. Influence of light on the heat sensitivity of the photosynthetic apparatus in isolated spinach chloroplasts. *Plant Physiology* **70**: 1530–1534.

Went FW. 1960. Blue hazes in the atmosphere. *Nature* **187**: 641–643.

Wiberley AE, Linskey AR, Falbel TG, Sharkey TD. 2005. Development of the capacity for isoprene emission in kudzu. *Plant, Cell and Environment* **28**: 898–905.

Wilkinson MJ, Owen SM, Possell M, Hartwell J, Gould P, Hall A, *et al*. 2006. Circadian control of isoprene emissions from oil palm (*Elaeis guineensis*). *The Plant Journal* **47**: 960–968.

Williams J, Roberts JM, Fehsenfeld FC, Bertman SB, Buhr MP, Goldan PD, *et al*. 1997. Regional ozone from biogenic hydrocarbons deduced from measurements of PAN, PPN, and MPAN. *Geophysical Research Letters* **24**: 1099–1102.

Wise RR, Olson AJ, Schrader SM, Sharkey TD. 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. *Plant, Cell and Environment* **27**: 717–724.

Wolfertz M, Sharkey TD, Boland W, Kühnemann F, Yeh S, Weise SE. 2003. Biochemical regulation of isoprene emission. *Plant, Cell and Environment* **26**: 1357–1364.

Wolfertz M, Sharkey TD, Boland W, Kühnemann F. 2004. Rapid regulation of the methylerythritol 4-phosphate pathway during isoprene synthesis. *Plant Physiology* **135**: 1939–1945.

Zeidler JG, Lichtenthaler HK, May HU, Lichtenthaler FW. 1997. Is isoprene emitted by plants synthesized via the novel isopentenyl pyrophosphate pathway? *Zeitschrift fur Naturforschung C* **52**: 15–23.

Zeidler J, Schwender J, Müller C, Wiesner J, Weidemeyer C, Beck E, *et al*. 1998. Inhibition of the non-mevalonate 1-deoxy-D-xylulose-5-phosphate pathway of plant isoprenoid biosynthesis by fosmidomycin. *Zeitschrift fur Naturforschung C* **53**: 980–986.

Zimmer W, Brüggemann N, Emeis S, Giersch C, Lehning A, Steinbrecher R, *et al*. 2000. Process-based modelling of isoprene emission by oak leaves. *Plant, Cell and Environment* **23**: 585–595.