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RESEARCH IN CONTEXT

A unifying conceptual model for the environmental responses of isoprene emissions from plants

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Key results

- **Background and Aims** Isoprene is the most important volatile organic compound emitted by land plants in terms of abundance and environmental effects. Controls on isoprene emission rates include light, temperature, water supply and CO2 concentration. A need to quantify these controls has long been recognized. There are already models that give realistic results, but they are complex, highly empirical and require separate responses to different drivers. This study sets out to find a simpler, unifying principle.
- **Methods** A simple model is presented based on the idea of balancing demands for reducing power (derived from photosynthetic electron transport) in primary metabolism versus the secondary pathway that leads to the synthesis of isoprene. This model’s ability to account for key features in a variety of experimental data sets is assessed.
- **Key results** The model simultaneously predicts the fundamental responses observed in short-term experiments, namely: (1) the decoupling between carbon assimilation and isoprene emission; (2) a continued increase in isoprene emission with photosynthetically active radiation (PAR) at high PAR, after carbon assimilation has saturated; (3) a maximum of isoprene emission at low internal CO2 concentration (ci) and an asymptotic decline thereafter with increasing ci; (4) maintenance of high isoprene emissions when carbon assimilation is restricted by drought; and (5) a temperature optimum higher than that of photosynthesis, but lower than that of isoprene synthase activity.
- **Conclusions** A simple model was used to test the hypothesis that reducing power available to the synthesis pathway for isoprene varies according to the extent to which the needs of carbon assimilation are satisfied. Despite its simplicity the model explains much in terms of the observed response of isoprene to external drivers as well as the observed decoupling between carbon assimilation and isoprene emission. The concept has the potential to improve global-scale modelling of vegetation isoprene emission.

Key words: Isoprene, modelling, electron transport, photosynthesis, temperature, carbon dioxide, isoprene emission, volatile organic compounds.

INTRODUCTION

Isoprene (2-methyl-1,3-butadiene; C5H8) is a highly volatile and reactive unsaturated hydrocarbon that is produced continuously in daylight by many terrestrial plants, and in great abundance by broadleaved trees. On a mass basis, it is the most important biogenic volatile organic compound (BVOC) emitted by vegetation, with an annual global emission of approximately 0.5 × 1015 g C. This is similar in magnitude to the total annual emissions of CO2 and ozone (O3) (Fan and Zhang, 2004), and consequently influences the atmospheric lifetime and concentration of CH4 (Poisson et al., 2000; Collins et al., 2002, 2010; Pike and Young, 2009). The influence of isoprene on atmospheric oxidation capacity has been proposed as one of the controls of the glacial–interglacial variations of atmospheric CH4, as recorded in ice cores (Valdes et al., 2005; Singhary et al., 2011). Isoprene also enhances the production of tropospheric ozone (O3), a potent greenhouse gas and toxic pollutant, under high-NOx conditions (Sanderson et al., 2003; Hauglustaine et al., 2005), and can significantly affect the atmosphere’s radiative balance through the generation of secondary organic aerosols (Claeys et al., 2004; Heald et al., 2008; Carlton et al., 2009; Nozière et al., 2011).

Isoprene emissions by plants at the leaf scale respond to changes in photosynthetically active radiation (PAR), temperature, ambient CO2 concentration and drought (Sharkey and Yeh, 2001; Laathawornkitkul et al., 2009; Pacifico et al., 2009; Niinemets, 2010). Despite general agreement between
models under the present climate, simulations of future isoprene emissions, and their potential impact on atmospheric chemistry, change dramatically depending on the temperature and light responses of the model (Keenan et al., 2009) and whether the model includes a physiological response of isoprene emission to CO₂ (Heald et al., 2009; Young et al., 2009; Pacifico et al., 2012). Given the continuously increasing atmospheric CO₂ concentration and its impact on future temperature, we need to understand the processes behind observed responses, and use that understanding to build better models.

The adaptive significance of isoprene emission is thought to be connected with enhancing membrane stability at high temperatures, and protection against oxidative stress – including that induced by high temperatures (Sharkey and Yeh, 2001; Vickers et al., 2009; Velikova et al., 2011, 2012). On time scales of weeks to years, acclimation mechanisms acting at the level of gene transcription may operate, possibly in such a way as to match isoprene synthase activity to adaptive requirements (Grote and Niinemets, 2008; Monson et al., 2012; Harrison et al., 2013). Here, however, we focus on the immediate responses of isoprene emission to environmental variations, as observed in experiments conducted over a time scale of minutes to hours, and the basic metabolic mechanisms that may be responsible for them.

The biosynthesis of isoprene occurs via the chloroplastic methylyerythritol 4-phosphate (MEP) pathway (Lichtenthaler, 1999; Logan et al., 2000; Sharkey et al., 2008). ¹³C labelling experiments have shown that the majority of the C in isoprene comes directly from photosynthesis, with the remainder coming from cytosolic C pools depending upon the environmental conditions (Delwiche and Sharkey, 1993; Kreuzwieser et al., 2002; Karl et al., 2002; Affek and Yakir, 2003; Loreto et al., 2004). However, the metabolic controls of the MEP pathway are only beginning to be elucidated (Li and Sharkey, 2012). With incomplete understanding of the metabolic controls of the pathway, models have been developed on the basis of experimental studies of the relationships between isoprene emission and environmental variables. The approach with the longest pedigree combines empirically derived functions for each environmental effect: this is the principle of the MEGAN model (Guenther et al., 2006), developed from the pioneer work of Guenther et al. (1993). Other approaches have made more direct use of the limited available information at the biochemical process level, e.g. SIM-BIM (Zimmer et al., 2000, 2003) and the models of Niinemets et al. (1999) and Martin et al. (2000). Aside from the model from Martin et al. (2000), which has an ATP limitation for isoprene production at high internal CO₂ concentration (cI), all these models need an empirical parameterization to reproduce the observed CO₂ response. This is potentially quite a severe limitation because there may be unforeseen interactions between the effects of different environmental drivers. Empirical models such as MEGAN include a multiplicity of functions for each environmental response of isoprene emission. More mechanistic approaches such as SIM-BIM, on the other hand, require information on many parameters. This might also be an issue because there is a generally accepted trade-off between the multiplicity of required parameter values and model robustness. We set out to identify a unifying principle that might transcend these limitations.

Our starting point was the model of Niinemets et al. (1999), which is based on quantifying the NADPH requirement of isoprene synthesis. Niinemets et al. (1999) assumed that a certain fraction of the total electron flux generated by Photosystem II is allocated to this function. The model we present here, initially proposed in Harrison et al. (2013), builds on Niinemets’ work but differs in one fundamental respect: it links isoprene emission to the electron availability for isoprene emission, relative to the needs of CO₂ assimilation. Therefore, the model predicts higher isoprene emissions when absorbed radiant energy (leading to the ‘supply’ of NADPH) exceeds the ‘demand’ for CO₂ assimilation. An excess of energy arises because of a mismatch between light availability and carboxylation capacity, which typically occurs daily – especially at high PAR, associated high temperature and under water stress. We compare the model’s predictions of observed, published environmental responses of isoprene emission to changes in PAR and the leaf-internal concentration of CO₂ (cI) with those obtained with the Guenther et al. (1993) algorithm, hereafter called G93, which is the basis of the widely used MEGAN model (Guenther et al., 2006), and with the model of Niinemets et al. (1999), hereafter called the Niinemets model. We also compare the theoretical temperature responses of our model with G93 and the Niinemets model. We focus on these two models as they have been widely used at the global scale (Guenther et al., 2006; Lathière et al., 2010; Arnth et al., 2011; Pacifico et al., 2012). However, other isoprene models have been developed. Reviews can be found in Arnth et al. (2007a), Grote and Niinemets (2008), and Monson et al. (2012).

**HYPOTHESIS**

In isoprene-emitting plants with the C₃ pathway of photosynthesis, over 90% of isoprene production takes place in the chloroplast via the MEP pathway (Lichtenthaler et al., 1997; Sharkey et al., 2008). The final stage is the enzymatic synthesis of isoprene from its precursor, dimethylallyl diphosphate (DMADP). On a per-molecule basis, isoprene synthesis is energetically expensive, and has a high requirement for reducing power (14 NADPH for one molecule of isoprene). For comparison, only six NADPH are needed to synthesize glyceraldehyde 3-phosphate (G3P), and only five for pyruvate. NADPH consumption for G3P and pyruvate synthesis takes place within the Calvin cycle and therefore is linked to the electron cost for carbon assimilation. Three additional reducing steps are needed within the MEP pathway to reduce G3P and pyruvate to DMADP. These supplementary reducing steps consume one further NADPH, and two additional reducing equivalents in the form of either NADPH or ferredoxin (Fd) (Charon et al., 1999; Hecht et al., 2001; Seemann et al., 2006; Li and Sharkey, 2012). Our hypothesis focuses on these additional reduction steps, which are directly linked to the production of isoprene.

Isoprene production is typically measured in nanomoles per second while photosynthesis and respiration rates (to which G3P and pyruvate production are linked) are measured in micromoles per second. Hence, the major consumption of reducing power takes place within the Calvin cycle and associated photorespiration while the diversion of reducing power to the MEP pathway is very small. Yet there is abundant circumstantial evidence for a link between the availability of reducing power (after the requirements of carbon assimilation have been accounted for) and the magnitude of this diversion. The MEP pathway is tightly
linked to the photosynthetic apparatus, involves light-dependent reactions and takes place in the chloroplast. Higher isoprene emission capacity is encountered under conditions when photo-inhibition occurs, including high light intensities, low \( c_i \) and high temperatures. Isoprene emissions decrease if plants are fed with nitrate (note that nitrate reduction to ammonia occurs mainly in the cytoplasm and consumes NAPDH) instead of being fed with ammonia directly (Campbell, 1988; Rosenstiel et al., 2004). Li and Sharkey (2012) measured an extremely high level of the intermediate metabolite, methylerythritol cyclodiphosphate (MEcDP), in an N2 atmosphere, where the carbon assimilation and photorespiration sinks for NADPH are blocked. Thus, it might be that the MEP pathway acts as a ‘branch circuit’ with the amount of NAPDH allocated to it increasing in proportion to the amount of reducing power to spare from other functions.

Thus, we hypothesize that isoprene emission is regulated in the short term by variations of the DMADP pool size, linked to the excess or deficit of electrons (and so also reducing power) relative to the needs of carbon assimilation. Figure 1 provides a schematic of the processes involved. When the chloroplast is illuminated, light absorbed by the thylakoids generates the electron flux \( J_{\text{tot}} \) that finally reduces NADP\(^+\) to NADPH. Most of the NADPH is used in the Calvin cycle for carbon fixation, but the total NADPH thus generated (\( \approx 0.5 J_{\text{tot}} \)) exceeds the amount consumed in the Calvin Cycle (\( \approx 0.5 J_{\text{CO2+O2}} \)). When assimilation is light-limited (at high \( c_i \) and/or low PAR) there is still some NADPH available for other functions, which include nitrate reduction (Canvin and Atkins, 1974; Niinemets, 2004; Eichelmann et al., 2011) and DMADP synthesis. When assimilation is Rubisco-limited (at low \( c_i \) and/or high PAR) this excess of NADPH becomes larger, allowing more NADPH to be used in DMADP synthesis.

This reasoning suggests the following simple model:

\[
Iso = \max \left\{ [aJ + b(J - J_v)]f(c_i), 0 \right\}
\]  

where \( Iso \) is the rate of isoprene emission; \( f(c_i) \) is a function of internal CO2 concentration; \( J \) is an estimate of the total electron flux, taken to be a non-rectangular hyperbolic function of absorbed PAR and the maximum electron flux \( J_{\text{max}} \), following Farquhar et al. (1980); \( J_v \) is the electron flux required to support Rubisco-limited carbon assimilation; and \( a \) and \( b \) are parameters. The electron flux required to support carbon assimilation is derived as follows (from Farquhar et al., 1980):

\[
A_j = \frac{(J/4)(c_i - \Gamma^*)}{(c_i + 2\Gamma^*)}
\]

where \( A_j \) is the gross (light-limited) assimilation rate and \( \Gamma^* \) is the CO2 compensation point in the absence of dark respiration. Hence

\[
J = 4A_j(c_i + 2\Gamma^*)/(c_i - \Gamma^*).
\]

When Rubisco limits photosynthesis, then

\[
A_v = \frac{V_{\text{cmax}}(c_i - \Gamma^*)}{(c_i + K_m)}
\]

where \( A_v \) is the gross (Rubisco-limited) assimilation rate, \( V_{\text{cmax}} \) is the Rubisco capacity and \( K_m = K_c(1 + [O_2]/K_o) \) where \( K_c \) and

**Fig. 1.** Schematic of the processes underlying the model of isoprene emissions. The availability of reducing power (NADPH) for CO\(_2\) assimilation is represented by a colour scheme, from dark blue (deficit of NADPH) to dark red (excess of NADPH). Symbols: NADPH and NADP\(^+\), nicotinamide adenine dinucleotide phosphate; DMADP, dimethylallyl diphosphate; Pyr, pyruvate; G3P, glyceraldehyde 3-phosphate; DOXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate.
\( K_r \) are the Michaelis coefficients of Rubisco for CO2 and O2 respectively (Farquhar et al., 1980). Substituting this into eqn (3) gives:

\[
J_v = 4V_{\text{cmax}}(c_i + 2G^*)/(c_i + K_m). \tag{5}
\]

It should be noted that \( J \) in eqn (1) is used as an estimate of \( J_{\text{tot}} \) and could be an underestimate (Singsaas et al., 2001; Niinemets, 2004). More details of the photosynthetic model, as used in this paper, can be found in the Supplementary Data.

The term \( aJ \) in eqn (1) represents a ‘baseline’ of isoprene emission under light-limited conditions under the equilibrium conditions for carbon assimilation (\( J = J_v \), energy supply = Rubisco demand), while \( b(J - J_v) \) represents variation in isoprene emission due to the disequilibrium between supply and demand.

The function \( f(c_i) \) in eqn (1) is chosen to take the value \( c_i/G^* \) when \( c_i \leq G^* \) and 1 otherwise. Because of this function, the model differs slightly from the one we proposed in Harrison et al. (2013). The function \( f(c_i) \) reflects the idea that a minimum rate of supply of carbon chains is required for isoprene synthesis, and the common observation that isoprene emission ceases abruptly when \( c_i < G^* \) (Wolfertz et al., 2003; Rasulov et al., 2009, 2011; Monson et al., 2012; Sun et al., 2012). This fall-off of isoprene at low \( c_i \) is not always observed: emission of isoprene in CO2-free air has been reported in a few studies (Monson and Fall, 1989; Affek and Yakir, 2003; Li and Sharkey, 2012), but comparable conditions are not found in natural environments.

Although based conceptually on the NADPH requirements of isoprene synthesis and the Farquhar photosynthesis model, our approach differs from that of Niinemets et al. (1999, 2004) in which isoprene production was assumed to be closely linked to the light-limited carbon assimilation rate (\( A_j \)). This difference has important consequences, as we will show.

**Tests of the Hypothesis**

We consider the observed environmental responses of isoprene emission (\( \text{Iso} \)) and also the ratio of isoprene emission to carbon gross assimilation (\( \text{Iso}/A_{\text{gross}} \)), which is a sensitive indicator of the allocation of reducing power to the MEP pathway versus the Calvin cycle. We will also consider changes in the ratio of isoprene emission to carbon net assimilation (\( \text{Iso}/A_{\text{net}} \)).

**Responses to PAR**

Equation (1) predicts an increase of isoprene emission with PAR, but also an increase of the ratio \( \text{Iso}/A_{\text{gross}} \) (Fig. 2A). The

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**Fig. 2.** Modelled responses of the normalized ratio of isoprene to CO2 assimilation to changes in PAR for (A) our model, (B) G93 and (C) the Niinemets model. \( T = 30 \, ^\circ \text{C}, c_i = 273 \, \text{mol} \, \text{m}^{-2} \, \text{s}^{-1}, V_{\text{cmax, 25}^\circ \text{C}} = 70 \, \text{mol} \, \text{m}^{-2} \, \text{s}^{-1}, J_{\text{max, 25}^\circ \text{C}} = 130 \, \text{mol} \, \text{m}^{-2} \, \text{s}^{-1} \) based on values from Arneth et al. (2007a). The solid line represents the ratio of isoprene emission to gross assimilation, the dashed line to net assimilation. Normalized ratio of isoprene to CO2 net assimilation was simulated for two extreme values of dark respiration to illustrate the potential effect of the magnitude of \( R_d \) on how \( \text{Iso}/A_{\text{net}} \) varies with PAR; grey short-dashed line, low \( R_d; R_d_{25}^\circ \text{C} = 0.5 \, \text{mol} \, \text{m}^{-2} \, \text{s}^{-1}; \) black long-dashed line, high \( R_d; R_d_{25}^\circ \text{C} = 2 \, \text{mol} \, \text{m}^{-2} \, \text{s}^{-1} \). Isoprene model parameters \( a \) and \( b \) (eqn 1) are based on data of Possell and Hewitt (2011; fig. 6).
predicted behaviour of $\text{Iso}A_{\text{net}}$ ($A_{\text{net}} = A - R_d$, where $R_d$ is mitochondrial respiration) is substantially different at low PAR, as shown in Fig. 2A. At saturating PAR, the difference becomes less important. This is due to the introduction of the $R_d$ term, which affects the assimilation independently from the allocation of reducing power between carbon fixation and secondary metabolism. Most laboratory experiments have reported only $A_{\text{net}}$; this should be kept in mind while interpreting the results.

The response of normalized $\text{Iso}A_{\text{gross}}$ (and $\text{Iso}A_{\text{net}}$) with PAR is predicted to take place in three stages (Fig. 2A).

Stage 1: light-limited carbon assimilation. This stage occurs when PAR absorbed is insufficient to generate an electron flux to satisfy Rubisco capacity. It is characterized by an initial steep increase of $\text{Iso}A_{\text{gross}}$ with PAR, becoming gradually less steep at higher PAR. For $\text{Iso}A_{\text{net}}$ the form of the response at low PAR depends on the magnitude of $R_d$.

Stage 2: transition between light- and Rubisco-limited carbon assimilation. This stage is characterized by a discontinuity (abrupt increase) in the slope of $\text{Iso}A_{\text{gross}}$ (and $\text{Iso}A_{\text{net}}$) versus PAR.

Stage 3: Rubisco-limited carbon assimilation. When the electron requirement for carbon assimilation is fully satisfied, the additional reducing power generated by increasing PAR allows $\text{Iso}A_{\text{gross}}$ to continue increasing while $A_{\text{gross}}$ remains constant. In this stage, $\text{Iso}A_{\text{net}}$ follows a similar pattern of the $\text{Iso}A_{\text{gross}}$ and increases with PAR. With still further increases in PAR, $\text{Iso}A_{\text{gross}}$ and $\text{Iso}A_{\text{net}}$ eventually saturate, as $J$ tends to its maximal value ($J_{\text{max}}$).

Note that the PAR flux where the transition between light- and Rubisco-limited assimilation occurs (Stage 2) as well as the rate of increase of $\text{Iso}A_{\text{gross}}$ with increasing PAR are dependent on both the photosynthetic and the isoprene model parameters.

We also examined the normalized responses of $\text{Iso}A_{\text{gross}}$ and $\text{Iso}A_{\text{net}}$ to changes in PAR in G93 and Niinemets models (Fig. 2B, C). Under light-limited conditions (Stage 1), the picture differs dramatically depending on the model. In the Niinemets model, isoprene emissions are tightly linked to $A_i$ (see Supplementary Data) and therefore the response of $\text{Iso}$ to PAR necessarily has the same shape as that of $A_i$, irrespective of the chosen values of $V_{\text{max}}$ or $J_{\text{max}}$. As a result, the ratio $\text{Iso}A_{\text{gross}}$ in this model is always constant under light-limited conditions, where carbon assimilation is equal to $A_i$. The ratio $\text{Iso}A_{\text{net}}$, when simulated with the Niinemets model, always decreases with PAR under light-limited conditions. In G93, by contrast, changes in $\text{Iso}A_{\text{gross}}$ with PAR are strongly dependent on $V_{\text{max}}$, $J_{\text{max}}$ and temperature under light-limited conditions. Consequently, the increase followed by a decrease of $\text{Iso}A_{\text{gross}}$ under light-limited conditions, shown in Fig. 2B, is one of the possible responses of $\text{Iso}A_{\text{gross}}$ for G93, obtained for the temperature and photosynthetic parameters chosen for this simulation. Changing those parameters changes the shape of the response, and $\text{Iso}A_{\text{gross}}$ can decrease or increase at low PAR. Introducing a dark respiration term affects the shape of the response of $\text{Iso}A_{\text{net}}$ with PAR, as represented by the dashed lines in Fig. 2B. Hence, G93 can potentially show an $\text{Iso}A$ response to PAR similar to that of our model.

All three models predict increasing $\text{Iso}A_{\text{gross}}$ with PAR under Rubisco-limited conditions. Indeed, in the Niinemets model, as isoprene emissions are linked to $A_i$, they must continue to increase even when carbon assimilation is Rubisco-limited. In that sense, the Niinemets model implicitly allows consumption of extra NADPH above the needs for carbon assimilation (for the PAR response only). For G93, isoprene emission approaches an asymptotic value at high PAR, while the Farquhar model fully saturates under Rubisco-limited conditions at high PAR.

Most studies reporting the fraction of assimilated carbon that is re-emitted as isoprene have found that it increases with PAR, in line with our predictions (Sharkey and Loreto, 1993; Harley et al., 1996; Lerdau and Keller, 1997; Niinemets et al., 2010). However, one study (Lerdau and Throop, 1999) found no significant increase in $\text{Iso}A_{\text{net}}$ with PAR for most of the tropical taxa they investigated.

Figure 3A compares the relationships of $\text{Iso}A_{\text{net}}$ to PAR in our model and in digitized data from Sharkey and Loreto (1993) on kudzu leaves (Pueraria lobata). Assuming $J_r$ is constant (no variation in $c_i$; Fig. 3B), the observed isoprene emissions show a strong positive linear relationship with $J$ ($r^2 = 0.97$). The model parameters $a$ and $b$ (eqn 1) have been estimated from this linear regression. The Farquhar model parameters were estimated with a best data/model fit by minimizing the residual sum of squares (RSS). The comparison between our model and the data for $\text{Iso}A_{\text{net}}$ shows excellent agreement ($r^2 = 0.92$). In comparison, G93 and the Niinemets model both show poor agreement ($r^2 = 0.19$ and $r^2 = 0.06$, respectively). Yet the three models show a good agreement of the modelled isoprene alone ($\text{Iso}$) with data (our model: $r^2 = 0.97$; G93: $r^2 = 0.92$; Niinemets: $r^2 = 0.97$; results not shown).

We also compiled data on the response of $\text{Iso}A_{\text{net}}$ to PAR from the limited number of published studies to assess the generality of the pattern (Fig. 4). The publications reported $A_{\text{net}}$ rather than $A_{\text{gross}}$, and did not typically provide measurements of $R_d$. As the predicted response of $\text{Iso}A_{\text{net}}$ for low PAR is dependent on $R_d$, it is not surprising to observe an initial decline in $\text{Iso}A_{\text{net}}$ with PAR for some of the 18 experiments. More importantly, the great majority of the studies show increasing $\text{Iso}A_{\text{net}}$ up to the highest PAR fluxes, especially when photosynthesis saturates (open circles in Fig. 4). In some studies a drop in assimilation rate at high PAR contributed to this increase in $\text{Iso}A_{\text{net}}$ at high PAR; this was probably due to stomatal closure at high PAR, resulting in reduced $c_i$.

As shown in Fig. 2, the Niinemets model cannot reproduce the positive response of $\text{Iso}A_{\text{net}}$ to PAR that is generally observed under low PAR. Our model, along with G93, fully captures the shape of the observed response of $\text{Iso}A_{\text{net}}$ to PAR over the full range of PAR. But our model also provides a process-based explanation for this response.

We also examined the relationship between $\text{Iso}A_{\text{gross}}$ and PAR at the canopy scale, at which isoprene emission is more likely to be controlled by the DMADP pool size than by isoprene synthase activity (Vickers et al., 2010). We used simultaneous CO$_2$ and isoprene flux measurements made at Harvard Forest, Massachusetts, USA (42.54’N, 72.17’W) (Urbanski et al., 2007; McKinney et al., 2011). Data used were obtained during the 2007 growing seasons using eddy covariance, with proton transfer reaction mass spectrometry used to measure the isoprene mixing ratio (McKinney et al., 2011). Daytime data were selected for temperatures above 23°C where variations in isoprene emission were no longer significantly driven by
temperature (Fig. A1). Ecosystem respiration, estimated from night-time CO2 flux measurements, was used to convert the daytime measured net ecosystem CO2 exchanges into canopy-scale gross assimilation rates. Canopy-scale carbon assimilation shows a typical rectangular hyperbolic response to PAR, but the response of isoprene emission to PAR is closer to linearity, and emissions do not saturate at high PAR (Fig. 5A, B). Thus, above a PAR threshold of approx. 300 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \), \( \text{Iso}/A_{\text{gross}} \) increases with PAR even at high PAR, where assimilation is light-saturated, consistently with our hypothesis. Scaling from leaf to canopy involves additional processes, such as within-canopy chemistry and canopy structure effects (Grote, 2007; Keenan et al., 2011; Bryan et al., 2012). Therefore, a canopy model is needed to fully account for these results, especially for low PAR where deposition processes can influence the observed above-canopy isoprene emissions and possibly explain the observed drop in \( \text{Iso}/A_{\text{gross}} \). Nevertheless these results, along with those of laboratory experiments, corroborate the notion that isoprene emission is related to the availability of electrons generated in photosynthesis, relative to the demand for them to be used in carbon assimilation.

**Responses to \( c_i \)**

Responses of isoprene emission to ambient CO2 concentration have been widely reported. Plants grown at high atmospheric CO2 concentrations generally emit less isoprene than those grown at lower CO2 concentrations. On short time scales, isoprene emission has also been shown to respond strongly and rapidly to \( c_i \), with lower emission rates at higher \( c_i \) (Rosenstiel et al., 2003; Wilkinson et al., 2009; Possell and Hewitt, 2011; Sun et al., 2012). The fact that rapid changes in \( c_i \) evoke instantaneous responses in isoprene emission suggests that the driving mechanism must be tightly linked to processes in the chloroplast.

The mechanisms behind the decoupling between isoprene emission and carbon assimilation in the response to \( c_i \) are not well established. Niinemets et al. (1999) hypothesized that the dependency of isoprene emission on \( c_i \) might be due to the partitioning of reducing power and ATP into the MEP pathway. However, the model of Niinemets et al. (1999) does not allow for any greater partitioning of reducing power to the MEP pathway at low \( c_i \). Isotopic labelling studies have provided evidence for the existence of extra-chloroplastic sources of carbon to support isoprene production. Hence, competition for phosphoenolpyruvate (PEP) between cytosolic and chloroplastic processes has been proposed as an explanation for the drop in isoprene emission at high \( c_i \) due to the CO2-dependence of PEP carboxylase activity (Karl et al., 2002; Rosenstiel et al., 2003; Possell and Hewitt, 2011; Trowbridge et al., 2012). But these experiments compared plants grown at different CO2 concentrations. Gene expression involving changes in enzyme quantities cannot explain the observed fast (about 10-min) responses to changes in \( c_i \). We focus here only on the short-term responses to \( c_i \), in which isoprene emission appears to be tightly coupled to
changes in the pool size of DMADP (Rasulov et al., 2009). Specifically, we examine whether the fast responses of isoprene emission to $c_i$ could be explained in a simple way by our model, based on the same mechanisms we have proposed to explain the response to PAR.

At low $c_i$, carbon fixation is Rubisco-limited, resulting in an excess of NADPH (Figs1 and 6A). The excess of NADPH can be smaller or larger depending on PAR. This provides a simple explanation for why isoprene responses to changes in $c_i$ are light-dependent (Loreto and Sharkey, 1993; Fig.7D). Moreover, our model can indeed reproduce the isoprene emission response to changes in $c_i$. This is shown in Fig.6A using data on Acacia nigrescens from Possell and Hewitt (2011). Here, isoprene emission shows a strong negative linear relationship with the Rubisco-limited electron flux, $J_v$ ($r^2=0.70$), as shown in Fig. 6B. The parameters $a$ and $b$ (eqn 1) of our model were estimated from this linear regression. When plotted against $c_i$, our model shows a good agreement with the data ($r^2=0.70$).

Figure 6A also shows the response of the G93 and the Niinemets model with and without a CO2 inhibition effect (Arneth et al., 2007a; Pacifico et al., 2011). It is clear from Fig. 6A that these models do not reproduce the observed response of isoprene emission to $c_i$. Without an additional empirical function for CO2 inhibition, isoprene emissions simulated with the Niinemets model are quite out of range. Instead, the model shows a strong negative correlation with the data ($r^2=0.7$). The negative relationship can be explained by the fact that although the PAR and therefore the light-limited electron flux ($J$) are constant, light-limited assimilation ($A_j$) is strongly $c_i$-dependent. Adding an empirical function to represent the CO2 inhibition effect, as in Arneth et al. (2007a), changes the shape of the response (allowing a decrease at high $c_i$) but still the simulated emissions agree poorly with the data.

Again using the data from Possell and Hewitt (2011), we plotted $\text{Isol/A}_{\text{net}}$ versus $c_i$ (Fig. 7A) and $J – J_v$ (Fig. 7B). These plots confirm that the fraction of assimilated carbon allocated to isoprene production increases under conditions of NADPH excess. This provides a simple explanation for the response of isoprene emission to $c_i$. The extremely steep rise in $\text{Isol/A}_{\text{net}}$ when $J – J_v$ becomes positive is due to the combination of steeply increasing isoprene emission with decreasing assimilation rate as $c_i$ declines.
response shown in the data (Fig. 7D), as well as the effects of combined changes in $c_i$ and PAR (Fig. 7D), without the need for any additional function.

**Responses to leaf temperature**

The temperature dependence of isoprene emission differs from that of photosynthesis. Temperature optima for carbon assimilation are usually $\leq 30$ °C in C$_3$ plants, while isoprene emission peaks at $\approx 40$ °C (Guenther et al., 1993; Niinemets et al., 1999; Sharkey and Yeh, 2001; Pacífico et al., 2009). An increase of $\text{Iso}/A$ with temperature is usually observed (Sharkey and Loreto, 1993; Harley et al., 1996; Sharkey et al., 1996; Niinemets et al., 1999; Sharkey and Yeh, 2001). The optimum for isoprene emissions rarely exceeds 40 °C, so the temperature dependence of isoprene emission cannot be fully explained by the temperature dependence of isoprene synthase, which is maximally active between 45 and 48 °C (Monson et al., 1992; Lehning et al., 1999; Niinemets et al., 1999; Rasulov et al., 2010). The decrease in isoprene emissions above 40 °C has long been considered to be linked to the behaviour of the photosynthetic electron transport rate (Guenther et al., 1991; Niinemets et al., 1999). Rasulov et al. (2010) found that this decrease is associated with decline in the DMADP pool size and the energetic status of the leaf.

In G93 the temperature dependency of isoprene emission is fixed with a temperature optimum around 38 °C. In the Niinemets model it is assumed to be primarily controlled by IspS activity, with the fraction of electrons used for isoprene production exponentially increasing with temperature. The temperature optimum for isoprene emissions in the Niinemets model is thus close to the optimum for IspS. Some global-scale studies have set an upper limit for the increase of $\text{Iso}/A$ with temperature, thereby reducing the temperature optimum to a value closer to 38 °C (Pacífico et al., 2011) (Supplementary Data A.2).

Our model is based on the hypothesis that the production of DMADP depends on photosynthetic electron flux and variations in electron availability for functions other than carbon assimilation. Thus, our modelled optima for isoprene emissions are primarily driven by the behaviour of the light-limited electron flux. Figures 9 and 10 illustrate how a temperature response arises in our model. Carbon assimilation follows the lower of the temperature response curves of the Rubisco and light-limited assimilation rates. Rubisco activity usually has a higher temperature optimum than electron transport (Crafts-Brandner and Salvucci, 2000; Medlyn et al., 2002; Cen and Sage, 2005; Kattge and Knorr, 2007). At high PAR an excess of NADPH can arise for temperatures below the optimum for electron transport ($J$), so isoprene emissions increase. Above this optimum ($T_{\text{opt}}$), $J$ still increases even if assimilation is reduced, due to the higher affinity of Rubisco for O$_2$ at high temperatures. Both $J$ and $(J - J_c)$ decrease for temperatures higher than $T_{\text{opt}}$ (as illustrated in Fig. 9B). Our model thereby predicts a temperature optimum of isoprene emissions that is lower than the temperature optimum of the electron transport rate. Beyond this optimum, our model predicts a drop in the availability of reducing power, leading to a decrease of DMADP and consequently isoprene emissions. At low PAR (light-limited condition), however, $(J - J_c)$ decreases with increasing temperature, compensating for the increase of $J$. Predicted emissions are thus almost insensitive to temperature or even decrease with temperature.

**Loreto and Sharkey (1990)** measured changes in isoprene emission with changing $c_i$ at different PAR fluxes in *Quercus rubra*. Both $\text{IsolA}/A_{\text{net}}$ and isoprene emission are shown (Fig. 7C, D) to increase with PAR, consistent with a dependence on NADPH availability, at all values of $c_i$. We compared the responses of G93 and the Niinemets model to $c_i$ at different PAR fluxes, together with our model (Fig. 8). Note that both G93 and the Niinemets model are applied here in their original formulations (see Supplementary Data for details), and therefore do not include additional parameterizations of the CO$_2$ effect. A number of studies have used these same models with additional empirical functions, introduced specifically to account for the observed CO$_2$ inhibition (Arnth et al., 2007b; Heald et al., 2009; Pacífico et al., 2011). G93 in its original formulation simulates no change at all in isoprene emission with changes in $c_i$, although it has isoprene emission depending on PAR (Fig. 8B). The Niinemets model in its original formulation also simulates increasing isoprene emission with PAR, but here the modelled emissions increase with increasing $c_i$, due to the fact that this model tightly links isoprene emission to light-limited assimilation (Fig. 8C). Thus, additional functions are required in both models to account for the observed effects of varying $c_i$.
formation on the temperature responses of enzyme activity may need to be considered, as well as this shift (Figs 9C and 10C). So we suggest that temperature account the temperature response of IspS, we can reproduce an increase in IspS activity (Rasulov et al., 2010). This behaviour is not realistic, so the model may be overestimating the effect of (J – Jₐ) at low PAR.

We infer that energetic control alone is insufficient to fully explain the observed temperature dependency of isoprene emission. In principle the activities of enzymes along the MEP pathway should also influence the production rate of DMADP, but very little is known about their temperature responses (Zimmer et al., 2000). Temperature optima for isoprene production are shifted toward higher temperature than T_{opt_J}, probably because a decrease in DMADP pool size is compensated for by an increase in IspS activity (Rasulov et al., 2010). Taking into account the temperature response of IspS, we can reproduce this shift (Figs 9C and 10C). So we suggest that temperature effects on enzyme activity may need to be considered, as well as temperature effects on electron availability.

A further limitation of our model is the paucity of available information on the temperature responses of J_{max} and V_{cmax} (Medlyn et al., 2002; Katge and Knorr, 2007). The experiments needed to quantify these responses are time-consuming, and in particular, few studies have included temperatures >40 °C. In general we would expect a decline in DMADP production for temperatures >40 °C due to thylakoid damage, while at temperatures above 45–48 °C irreversible damage to enzyme function will cause isoprene emission to cease.

Using data from Medlyn et al. (2000) and references therein, we checked variations with temperature of electron availability among isoprene emitting species at 1000 μmol m⁻² s⁻¹ PAR (Fig. 11). We also tested the influence of the temperature response parameterization of V_{cmax} by contrasting an Arrhenius function with a peak function (Supplementary Data), as described in Medlyn et al. (2000). The temperature optima for the selected species are all higher for V_{cmax} than J_{max} (Medlyn et al., 2002; Katge and Knorr, 2007). Consequently, we predicted a decline in DMADP pool size above T_{opt_J}, due to the decline of J being accompanied by a decline in (J – Jₐ), but the shape of the decline depended on the parameterization adopted.

**DISCUSSION**

We have used a conceptual model to ask whether variation in the availability of NADPH in the chloroplast can plausibly account for observed changes in isoprene emission with PAR, c₁ and leaf temperature. The answer is yes. By modelling isoprene emission as proportional to a simple metric of the excess or deficit of electrons relative to the demands of carbon assimilation, we have provided a unifying explanation for the lack of close coupling of isoprene emission with carbon assimilation, the disparities in...
carbon allocated to isoprene production, high isoprene emissions at low \( c_i \) and the shift of the temperature optimum for isoprene emission above that of carbon assimilation but below that of isoprene synthase.

To our knowledge, this is the first study that has attempted to model the flux of reducing power into the MEP production pathway based on the idea of a balance between electron supply and demand. Our hypothesis invokes mechanisms that are incompletely understood and thus is to some extent speculative. Nevertheless, it appears to have significant predictive power in explaining the already documented responses of isoprene emission to PAR, \( c_i \) and (with some caveats) temperature. Moreover, this hypothesis provides a parsimonious explanation for the response of isoprene emission to drought. Under moderate to mild drought where the photosynthetic apparatus is not damaged (Cornic and Briantais, 1991), carbon assimilation is first reduced by stomatal closure (and thus reduced \( c_i \)). Under higher drought severity, this reduction is greatly increased by decreased ATP in water-deficient leaves (Lawlor and Tezara, 2009), which reduces the photosynthetic metabolic potential (\( A_{\text{pot}} \)), even if \( c_i \) increases due to light respiration. The resulting oversupply of reducing power ensures that isoprene emissions continue at a high rate, although carbon assimilation is reduced (Niinemets, 2010). However, a decrease in ATP could also reduce isoprene emissions. Under extreme drought, however, damage to the photosynthesis apparatus eventually results in the cessation of both carbon assimilation and isoprene emission.

A strong diurnal cycle is observed in isoprene emission at canopy scales. Low emissions during early morning and late afternoon contrast with high emissions during the midday period (Hewitt et al., 2011; Keenan and Niinemets, 2012). Our hypothesis explains this as a consequence of higher PAR and \( c_i \), and lower \( c_i \) due to partial stomatal closure associated with higher evaporative demand in the midday period. This simple explanation does not require the intervention of a circadian clock, as had been proposed by Hewitt et al. (2011).

**Fig. 7.** Observed changes in the ratio of isoprene emission to net carbon assimilation with changes in (A) leaf-internal CO2 concentration \( (c_i) \), (B) electron excess \( (J–J_v) \) (data from Possell and Hewitt, 2011). Observed changes with \( c_i \) of (C) the ratio of isoprene emission to carbon assimilation and (D) isoprene emission, for three PAR fluxes (\( \mu \text{mol m}^2 \text{s}^{-1} \)). Data digitized from Loreto and Sharkey (1990).
Note that we are not advocating a function of isoprene emissions as an 'electron sink' as was earlier proposed (e.g. Logan et al., 2000). It is clear from the findings of Li and Sharkey (2012) that the quantity of electrons used in isoprene synthesis is far too small for this function to be plausible. The low affinity of IspS for DMADP already argues strongly against this notion (Silver and Fall, 1995; Schnitzler et al., 1997). Our model implies that the allocation of reducing power to this pathway occurs under those conditions when electron availability is in excess, which fortuitously occurs during stress events when isoprene biosynthesis and emission is advantageous to the plant (e.g. Sharkey et al., 2001; Vickers et al., 2009).

Our results provide an alternative, robust approach to modelling isoprene emissions for global change applications. But more work is needed before implementing the model in a global context. Particular attention should be given to the influence of enzymatic activity on temperature responses of the modelled rates of isoprene. The values of the parameters $a$ and $b$ (eqn 1), and their potential species and environmental dependencies, also call for further investigation at several scales:

1. For leaves, by setting up experiments that could test interactions among the short-term responses of isoprene emission to different environmental drivers, and associated variations in the excess of electrons (i.e. isoprene/assimilation responses to $c_i$ at different PAR fluxes, together with isoprene/assimilation response to PAR at different $c_i$); and the influence of growth conditions on the parameters. Note that, as most of the process-based models are closely linked to photosynthesis models, information on the values of $V_{c_{\text{max}}}$ and $J_{\text{max}}$ associated with the isoprene standard emission rate would be valuable.

2. For ecosystems, by upscaling the model from the leaf scale to the canopy, with particular attention to the response of Isol $A_{\text{gross}}$. This step would require a representation of the canopy structure and vertical mixing as well as the canopy chemistry accounting for isoprene oxidation, deposition and OH regeneration.

3. At the global scale, with the possibility of using remotely sensed formaldehyde column concentrations to better constrain model parameters for different plant function types and environments. Formaldehyde is a product of isoprene oxidation. As it is observed by satellite, with global coverage, numerous studies have used formaldehyde data to investigate isoprene emission at larger scales (Palmer et al., 2003, 2006; Barkley et al., 2008; Stavrakou et al., 2009; Fortems-Chiney et al., 2012).

**Fig. 8.** Modelled responses of isoprene emission versus $c_i$ for three PAR fluxes (80, 180 and 700 $\mu$mol m$^{-2}$ s$^{-1}$). (A) our model, (B) G93 and (C) the Niinemets model. Parameters values as in Fig. 2. Emissions were normalized to a standard emission rate at $T = 30^\circ$C, $c_i = 273$ $\mu$mol mol$^{-1}$, PAR = 1000 $\mu$mol m$^{-2}$ s$^{-1}$.
A comprehensive approach to isoprene modelling would also have to account for longer-term acclimation over a time scale of weeks to months, including responses to antecedent temperatures (Guenther et al., 2006), phenological stages, and differences between the short-term and acclimated responses to CO₂ (Sun et al., 2012), which are presumably mediated by transcriptional control of the MEP pathway enzymes. It would be of particular interest to examine whether these acclimatory changes in isoprene emission are correlated with acclimatory changes in reducing power. However, some acclimatory shifts are unlikely to be explained by a model based on reducing power alone. For example, growth at higher temperatures leads to increased emission rates measured at a common temperature (Pe´ tron et al., 2001; Niinemets et al., 2010), whereas reducing power at a given temperature tends to be reduced by high growth temperatures due to a decline in the \( J_{\text{max}}/V_{\text{cmax}} \) ratio (Hikosaka et al., 1999; Onoda et al., 2005). Longer-term responses of isoprene emission to changes in growth temperature are therefore...
presumably governed by other factors, including transcriptional control of the MEP pathway enzymes.

Conclusions

A simple model of the biochemistry and physiology of isoprene emissions has been developed and used to test the hypothesis that the reducing power available to the synthesis pathway for isoprene varies according to demands of carbon assimilation. The model explains the observed response of isoprene production to environment and the coupling/decoupling between carbon assimilation and isoprene emission. The model has the potential to improve global-scale modelling of vegetation isoprene emissions, as well as emissions of isoprenoids that do not originate from storages.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of details of the following. The G93 algorithm for prediction of isoprene emission from vegetation; the Niinemets model based on quantifying the NADPH cost for isoprene synthesis; and the model for photosynthetic carbon assimilation based on the Farquhar model.

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