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Biochemical Control on the CO_2 Response of Leaf Isoprene Emisson: an Alternative View of Sanadze's Double Carboxylation Scheme

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(Presented by Academy Member T. Beridze)

ABSTRACT. Sanadze's double carboxylation scheme was originally proposed to explain aspects of biochemical control over the isoprene emission rate from photosynthesizing leaves. The scheme was based on two chloroplastic carboxylation reactions, including that by RuBP carboxylase, the initial carboxylation reaction in C_3 photosynthesis, and that by an unknown carboxylase with the proposed function of providing substrate to a chloroplastic version of the mevalonic acid pathway. Since the development of Sanadze's original scheme, discoveries have made it clear that chloroplastic isoprenoid biosynthesis occurs through a pathway other than that involving mevalonic acid, and that the substrate for isoprenoid biosynthesis originates in part from phosphoenolpyruvate (PEP) transported from the cytosol. We have developed a biochemical scheme to accommodate these observations and, at the same time, explain the response of isoprene emission rate to changes in atmospheric CO_2 concentration. Like Sanadze's original scheme, our scheme also depends on control by two carboxylases, one of which is RuBP carboxylase. However, unlike Sanadze's original scheme, the second carboxylase in our scheme is cytosolic in its location and is well known as PEP carboxylase. In this paper, we provide a brief review of this alternative 'double carboxylation' scheme, including the development of a biochemical model, based on control by PEP carboxylase, to explain the CO_2 response of isoprene emission rate. We also present new data on application of the model to describe the response of isoprene emission rate in poplar and aspen leaves to light, temperature and CO_2 concentration.

This paper is dedicated to the 80th birthday celebration of Guivi Sanadze whose research into the biochemical nature of isoprene emission inspired all of us to view photosynthetic carbon flow in a new light.
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Key words: *isoprene, double carboxylation, PEP carboxylase.*

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Introduction

In 1964, Guivi Sanadze published an intriguing observation (Sanadze 1964) showing that the rate of isoprene emission from the leaves of poplar trees decreased as the atmospheric CO_2 concentration increased. The intriguing aspect of this observation was that it was not, at first examination, consistent with evidence that accumulated shortly thereafter, from other experiments conducted in the Sanadze Laboratory, showing that the biosynthesis of isoprene was biochemically coupled to photosynthetic CO_2 assimilation (Sanadze 1966, Sanadze and Kursanov 1966, Sanadze and Dzhaiani 1972). How is it that the biosynthesis rate of a compound that depends on photosynthetic CO_2 assimilation decreases when the rate of CO_2 assimilation increases? Two of us (Russ Monson and Ray Fall) were first introduced to this enigma in 1988 when we conducted studies of isoprene emission from aspen leaves (*Populus tremuloides*), which confirmed Sanadze's observation of reduced emission rate in the presence of elevated CO_2 (Monson and Fall 1989). In January 1990, Monson and Fall traveled to Asilomar, California to attend a conference on trace gas emissions from plants and discuss the CO_2-isoprene effect with Professor Sanadze. Professor Sanadze presented a hypothesis to explain his results that depended on a double carboxylation scheme in the chloroplast (Figure 1) coupled with 61 competition between the two carboxylase-driven pathways for photosynthetically-produced reductant and ATP (Sanadze 1991, 2004). One of the carboxylases involved in the response was proposed to be Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBP carboxylase), the enzyme that catalyzes the initial assimilation of CO_2 in the reductive pentose phosphate pathway in the chloroplast. The second carboxylase was unidentified, but was hypothesized to have a role in the production of acetyl-CoA in a chloroplastic version of the mevalonic acid (MVA) pathway. To date, a second chloroplastic carboxylase has not been identified and evidence has accumulated that the chloroplastic mevalonic acid pathway is not the principal source of isoprene biosynthesis (Lichtenthaler et al. 1997). These discoveries, or lack thereof, have created doubt that Sanadze's original double carboxylation scheme exists as originally proposed (Sharkey et al. 71 2008), and thus we have been forced to search for alternatives to explain the CO_2 effect.

In 2000, Monson and Fall attended the First Gordon Conference on Biogenic Hydrocarbons and the Atmosphere in Ventura, California. At that conference, the issue of the CO_2-isoprene effect and its biochemical foundations rose once again in the discussions of several attendees. In some of those discussions the fact was also raised that Ulf-Ingo Flügge and co-workers in Germany had discovered a protein transporter that exchanges cytosolic phosphoenolpyruvate (PEP) for plastidic inorganic phosphate (Pi), and through this transporter, the cytosol supplies the chloroplast with a source of pyruvate to be used in the biosynthesis of isoprenoid compounds (Flügge 1999). Chloroplasts have an incomplete sequence of glycolytic-like enzymes (Dennis and Miernyk 1982), requiring them to import pyruvate equivalents as PEP. Monson and Fall began to merge these discoveries with the CO_2-isoprene effect, wondering if the inhibition of isoprene emission at elevated atmospheric CO_2 concentrations might be related to decreased activity of the PEP-Pi translocator. Upon return to their laboratories in Boulder, Monson and Fall initiated discussions with Todd Rosenstiel, a graduate student working with both professors as advisors. Within weeks of those discussions, Rosenstiel had conducted an experiment using isolated protoplasts of poplar leaves, to show that as CO_2 concentration in the surrounding medium increased, the activity of cytosolic PEP carboxylase also increased, and the rate of isoprene biosynthesis decreased. These experiments led to publication of an alternative mechanism to explain the CO_2-isoprene effect in which increased CO_2 concentration forced higher rates of cytosolic PEP consumption through PEP carboxylase, and thus lower rates of PEP transport into the

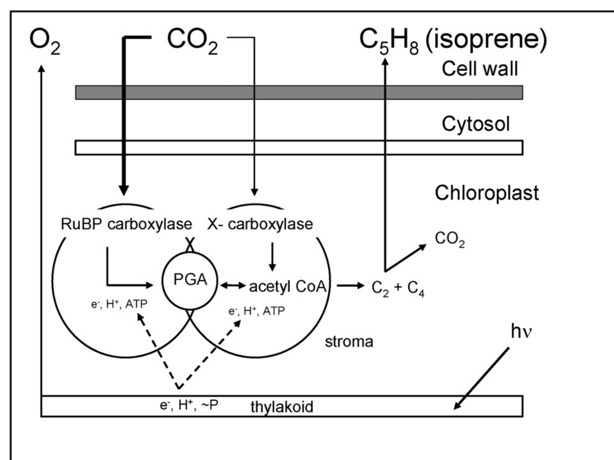

Fig. 1. Simplified version of Sanadze's double carboxylation scheme showing only carbon flows. Two chloroplastic carboxylases are proposed to control the production of the two carbon compound, acetyl CoA, which is proposed to combine with a 4-C substrate to form isoprene. The two carboxylases are functionally linked through the phosphoglyceric acid (PGA) pool in the stroma. PGA is produced through a reaction catalyzed by RuBP carboxylase, and the size of the PGA pool exerts feedback on RuBP carboxylase activity. PGA is also consumed by the mevalonic acid pathway, which works in concert with a second, yet-to-be-identified carboxylase (X-carboxylase) to produce acetyl CoA. (Redrawn from Sanadze 1991, 2004).

chloroplast (Rosenstiel et al. 2003). In essence, there were indeed two carboxylations involved in the CO_2-isoprene effect, but one of the carboxylations was catalyzed by cytosolic PEP carboxylase, and the underlying effect on isoprene biosynthesis was not competition for photosynthetically-produced reductant and ATP, but rather for PEP substrate. The original role of RuBP carboxylase proposed by Sanadze continued to be supported. In a serendipitous case of coincidence, echoes from Sanadze's double carboxylation scheme had come back into the isoprene literature.

The new double carboxylation hypothesis and its relevance to the CO_2-isoprene effect

The double carboxylation scheme that we have proposed in several publications subsequent to the 2000 experiments (Rosenstiel et al. 2003, 2004, Monson et al. 2007, Wilkinson et al. 2009) is founded on substrate competition between the cytosol and chloroplast, with the competition controlled by the activity of the PEP-Pi chloroplast translocator (hereafter called PPT) and the availability of cytosolic PEP (Figure 2). Of particular importance to our hypothesis is a central role for phosphoenolpyruvate carboxylase (PEPC) in controlling the flow of carbon to chloroplastic isoprenoid metabolism (as well as biosynthesis of fatty acids, aromatic amino acids and secondary compounds). PEPC, along with RuBP carboxylase (RuBPC) represent the two carboxylations that we propose as fundamental controls over the isoprene emission rate and its response to changes in CO_2 concentration. In C_4 plants, PEPC serves a key role in the assimilation of atmospheric CO_2 and the channeling of metabolites through the C4 photosynthetic pathway (Chollet et al. 1996). The role of PEPC in the leaves of C_3 plants is less certain, but it may contribute to control of cellular pH and/or provide oxaloacetate (OAA) to support mitochondrial respiration and the OAA-malate shuttle, which transfers reducing equivalents from the chloroplast to the cytosol to support NO_3^- reduction (Scheibe 1990, Scheibe 2004).

In the new double carboxylation hypothesis, isoprene emission rate is controlled by the availability of two possible substrates: glyceraldehyde 3-phosphate (G3P) and pyruvate (Pyr). In responding to changes in atmospheric CO_2 concentration, the availability of G3P for isoprene biosynthesis is most directly controlled by the activity of RuBPC, whereas the availability of chloroplastic Pyr is most directly controlled by the activity of PEPC. Ultimately, the availability of both substrates depends on the assimilation of atmospheric CO_2 by

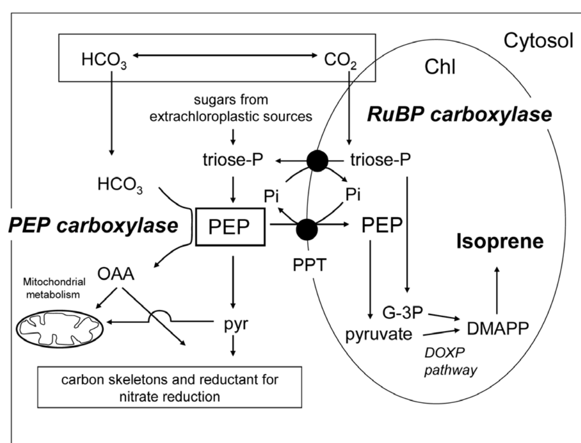

Fig. 2. Metabolic scheme to describe the roles of two carboxylases – RuBP carboxylase and PEP carboxylase – in regulating the flow of carbon substrates to isoprene biosynthesis. The cytosolic compound, phosphoenolpyryuvate (PEP), is hypothesized to be a key metabolite in the response of isoprene emissions to elevated CO_2. As atmospheric CO_2 concentration increases, the rate of glyceraldehyde 3-phosphate (G3P) production will increase due to increased RuBP carboxylase activity, and the rate of PEP production will similarly increase due to increased glycolytic activity in the cytosol. However, the concomitant increase in PEP carboxylase activity will shift the channeling of PEP to favor cytosolic, and potentially mitochondrial, processes, rather than chloroplastic processes such as isoprene biosynthesis.

RuBPC; that is, the export of G3P from the chloroplast and its glycolytic conversion to PEP, is ultimately dependent on RuBPC activity. The PEP produced through glycolysis then forms a key metabolite pool from which both chloroplastic and cytosolic processes must compete for substrates. In this regard, the transport of PEP into the chloroplast is subject to competitive inhibition due to increased activity from cytosolic PEPC as CO_2 concentration increases; as CO_2 concentration increases, PEPC activity also increases, shifting the competition for PEP to favor cytosolic metabolism. Thus, it is ultimately the activity of two carboxylases that control the channeling of substrate to isoprene biosynthesis and its response to CO_2 concentration.

Several lines of evidence support this new double carboxylation scheme. First, when inhibitors of PEPC are added to excised poplar leaves, the rate of isoprene emission increases (Rosenstiel et al. 2003). Second, in poplar trees grown in different CO_2 concentrations there exists a negative correlation between the isoprene emission rate and PEPC activity (Loreto et al. 133 2007). Third, when poplar trees are grown in conditions that force the utilization of NO_3^- versus NH_4^+ as the primary nitrogen source, leaf PEPC activity increases, leaf dimethylallyl diphosphate (DMAPP) content (the substrate for isoprene biosynthesis) decreases, and isoprene emission rate also decreases (Rosenstiel et al. 2004).

The double carboxylation hypothesis and models of isoprene emission rate

Numerous studies have now confirmed the negative response of isoprene emission rate to elevated atmospheric CO_2 concentration, as originally observed by Sanadze (Monson and Fall 1989, Loreto and Sharkey 1990, Rosenstiel et al. 2003, Centirtto et al. 2004, Raparini et al. 2004, Possell et al. 2005, Pegoraro et al. 2005, Monson et al. 2007, Calfapietra et al. 2008, Wilkinson et al. 2009). A biochemically-based model describing the CO_2 effect has now been constructed based on the new double carboxylation hypothesis (Wilkinson et al. 2009), and this model has been used to examine the effects of future increases in atmospheric CO_2 concentration on global isoprene emissions (Heald et al. 2009). Thus, the original observations by Sanadze, over 40 years ago are now finding there way into the global change biology research arena, providing a truly relevant link between cellular biochemistry and global biogeochemistry.

Wilkinson et al. (2009) used the double carboxylation hypothesis as the basis for a new model of isoprene emission rate. Here, we review the fundamental logic underlying the model. The model was constructed so as to be driven by tradeoffs between two controlling processes: (1) at low atmospheric CO_2 concentration isoprene emission rate is uncoupled from instantaneous photosynthetic G3P production, and is controlled instead by the mobilization of G3P from stored carbohydrate reserves; (2) at high CO_2 concentration, isoprene emission rate is progressively, but indirectly, controlled by increases in the activity of PEPC. In the model, the tradeoff between these mutually exclusive controls is dictated by the prevailing intercellular CO_2 concentration (C_i). Thus, the velocity of the reaction catalyzed by PEPC increases as C_i increases, and within a relatively narrow range of increasing C_i, the isoprene emission rate is forced to a transition from G3P limitation to Pyr limitation (Figure 3). Above the transition range, isoprene emission rate is controlled by the Michaelis-Menten type response of PEPC to increases in CO_2 concentration. Below the transition range, the isoprene emission rate is controlled by the rate of G3P mobilization from carbohydrate reserves. Mathematically, we described the switch in controlling functions with a Heaviside function $H(x)$ conditioned on C_i:

$$H(x) = \begin{Bmatrix} f_1(x) \ \ if \ \ C_i < x_1 \\ f_2(x) \ \ if \ \ C_i \geq x_1 \end{Bmatrix} \quad (1)$$

where C_i acts as a toggle allowing $H(x)$ to switch between the two functions. We can express H(x) as a single function:

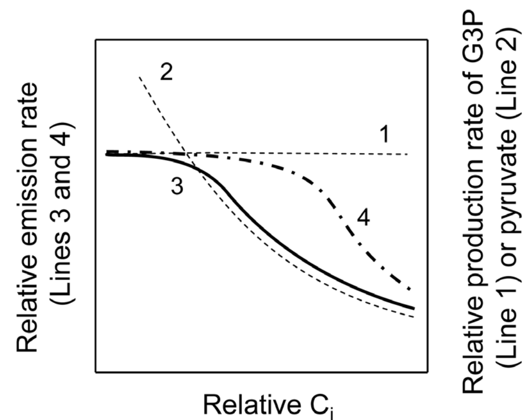

Fig. 3. Conceptual relations among the supply of G3P from stored reserves (Line 1), which is assumed to be constant in the face of changing C_i); the supply of pyruvate provided by transport of PEP into the chloroplast from the cytosol (Line 2) and controlled by the Michaelis-Menten type response of PEP carboxylase to increased Ci; a normalized response of Is to changes in C_i similar to what was observed for trees grown at 400 μmol mol^{-1} CO_2 (Line 3); and a normalized response of Is to changes in C_i similar to what was observed for trees grown at 800 ìmol mol^{-1} CO_2 (Line 4). (From: Wilkinson et al. 2009).

$$f(x) = f_1(x)u_{x1} + f_2(x)u_{x2} \quad (2)$$

which forces control of the dependent variable (isoprene emission rate) to f_1 below the critical switch (designated as $ux1$), and to the sum of f_1 and f_2 at or above the critical switch (designated as u_{x2}). In order to move Equation 2 to the specific case of CO_2 control over isoprene emission, we can write:

$$C_{ci} = I_{s\max} u_{ci1} - \left[\frac{I_{s\max} C_i}{C_{i50} + C_i} \right] u_{ci2} \quad (3)$$

Ismax represents the maximum isoprene emission rate and C_{i50} is a coefficient analogous to the Km of Michaelis-Menten enzyme kinetics models. Equation (3) forces a switch in the CO_2 response such that below a critical C_i value, the Ismax is limited by the rate of G3P production, but at or above the critical C_i value, the response is driven by the activity of PEPC according to Michaelis-Menten kinetics.

In order to facilitate its general use, we designed an analytical form of Equation (3) that does not depend on step-wise triggers, but rather is driven by continuous dependence of isoprene emission rate on C_i:

$$C_{ci} = I_{s\max} - \left[\frac{I_{s\max} (C_i)^h}{(C^*)^h + (C_i)^h} \right] \quad (4)$$

Term 1 Term 2

Term1 Term 2 where C_{ci} is the CO_2 scaling term, or CO_2 activity factor. In Equation 4, h is a unitless tunable coefficient that forces Term 2 to be 'penalized' exponentially at low C_i but 'amplified' exponentially at high C_i. The net result of h is to force the function into sigmoidal form, which matches the form of many of the CO_2 response curves observed in past studies (e.g., Wilkinson 186 et al. 2009). We have re-defined C_{i50} as C^* such that C^* becomes a more generalized C_i scalar, rather than a strict analogue to the Michaelis constant, Km. By introducing C^*, we can scale Equation 4 differentially to account for growth-CO_2 effects on the switch between the two competing metabolic controls; as growth CO_2 increases, C^* should increase, shifting the penalty phase of Term 2 to higher C_i domains.

Equation 4 was developed to match the form of the scaling factors developed originally by Guenther et al. (1991, 1993) and commonly referred to as the 'Guenther algorithms'. The intent of the light and temperature scaling factors (C_L and C_T, respectively) is to adjust a normalized (basal) emission rate (Isb) (sometimes called the 'emission factor') to incident light intensity and leaf temperature. In combination with the previously-defined scaling factors, the new scaling factor, C_{Ci}, can be used to adjust Isb (which is typically determined at a leaf temperature of 30 °C, an incident PPFD of 1000 μmol m^{-2} s^{-1} and, in this case, the C_i that occurs at an ambient atmospheric CO_2 concentration of 400 μmol mol^{-1} to instantaneous combinations of these factors that differ from the 'basal' state:

$$I_s = I_{sb}(C_T * C_L * C_{Ci}) \qquad (5)$$

Application of the light, temperature and CO2 scaling models to observed isoprene emission rates

We have tested the model described in Equation 4 against observations of isoprene emission rate at different C_i values in poplar leaves. Here we present the results of two different experiments: (1) we made observations on three-year old poplar trees (*Populus deltoides*) and aspen trees (*Populus tremuloides*) which had leafed out in controlled-environment growth chambers at 400 or 800 μmol mol^{-1} atmospheric CO_2 concentration; (2) we made observations on three-year old aspen trees grown in controlled-environment growth chambers at 400 or 800 μmol mol^{-1} atmospheric CO_2 concentration with an additional watering treatment (drought versus no-drought). In the first experiment we aimed to test the model presented above against observations of the CO_2 dependence of isoprene emissions for trees grown in high or low CO_2. In the second experiment, we aimed to test the ability of the temperature and light-dependent algorithms used to describe isoprene emission rate (i.e., Guenther 1991, 1993) for trees grown at different atmospheric CO_2 concentrations and for trees exposed to different drought treatments. Expressed another way, in the second experiment we tested whether the effect of different CO_2 concentrations in a leaf during growth, either due to different atmospheric CO_2 concentrations or due to lower intercellular CO_2 concentrations caused by drought and concomitant stomatal closure, influenced our ability to model the light and temperature dependencies of isoprene emission rate.

Methods

Three-year old trees were grown in controlled-environment growth chambers (Conviron, model PGR 15, Winnipeg, Canada). Twelve trees were placed in an 'ambient' CO_2 chamber (400 ± 10 μmol mol^{-1}) and 12 trees were placed in an 'elevated' CO_2 chamber (800 ± 15 μmol 226 mol^{-1}) for eight weeks prior to measurement. All trees were defoliated before being placed in the chambers so that new leaves developed in the treatment [CO_2]. On a weekly basis we moved the trees in the chambers to minimize potential artifacts due to systematic environmental gradients. The trees were grown in 10-l pots containing commercial potting soil, and fertilized regularly with half-strength Scotts' solution (21:18:18; Scotts-Sierra Horticultural products Company, Maryville, Ohio, USA). The photoperiod was 14 h with PPFD of 700 μmol m^{-2} s^{-1} measured near the top of the tree crown. Day/night air temperatures were kept at 25/15 °C. In order to impose a drought treatment on trees in the second experiment, we initially watered the pots to field capacity, and then re-weighed them daily to estimate rates of water loss. For the drought treatment, we replaced only 75% of the water lost each day, and for the well-watered controls we replaced 100% of the water. The progressively diminishing water supply in the drought-treated plants caused a slow water stress to occur over the span of 2-3 weeks.

Leaf gas exchange measurements were conducted using a portable photosynthesis system and 'broadleaf' cuvette (model 6400, LiCor, Inc., Lincoln, Nebraska, USA). In Experiment #1, the cuvette was coupled to a chemiluminescence fast isoprene sensor (model FIS; Hills Scientific, Boulder, Colorado, USA). In experiment #2, the cuvette was coupled to a proton transfer reaction-mass spectrometry instrument (PTR-MS; Ionicon GmbH., Innsbruck, Austria); both of these instruments were used to measure isoprene concentration in the cuvette air. Air delivered to the portable photosynthesis system was scrubbed of ambient VOCs and

ozone using a clean air generator (model, Aadco, Inc., Ohio, USA). The FIS was calibrated each day by serial dilution of a 6 ppmv isoprene gas standard. Calibration curves were conducted at five isoprene concentrations from 0-400 ppbv. The PTR-MS instrument was operated at 125 Townsend (1.25×10^{-17} V cm^{-1}) to reduce compound fragmentation.

Results

In both the high- and low-CO_2 grown aspen trees, the isoprene emission rate (Is) decreased as C_i increased (Figure 4A). When normalized to the isoprene emission rate observed at C_i = 400 μmol mol^{-1}, the sensitivity of Is to increases in C_i was shown to be less in the trees grown at an elevated [CO_2]. Equation 4 provided a relatively good fit to the observations for both sets of trees. The shape of the observed CO_2 response is slightly sigmoidal, which is clearly seen in the results from the trees grown at elevated CO_2. Equation 4, while reflecting a sigmoidal response function overall, does not quite capture the sigmoidal nature of the response for the trees grown at normal, ambient [CO_2]. Nonetheless, the fit is relatively good. These data have been presented in a previous paper (Wilkinson et al. 2009), and are only repeated here to show the good match that we achieved between the model that we developed and our observations.

We have expanded these previous observations to include a second poplar species, *Populus deltoides*; but, in this case only for trees grown at normal ambient CO_2 (400 μmol mol^{-1}) (Figures 4B and C). As seen with the aspen leaves, cottonwood leaves exhibited reduced isoprene emission rates when exposed to increased atmospheric CO_2 concentrations. The model parameters required to accurately represent the observed response were different than those obtained from aspen leaves; this is seen in the poor match of the modelled response shown in the grey line of Figure 4B, compared to the actual observations. When we adjusted the model parameters to achieve the best fit to the *P. deltoides* data, we obtained a good fit (the black solid 2 line in Figure 4B). As in previous studies, the negative response of Is to C_i contrasts that for net CO_2 assimilation rate, which increases as C_i increases (Figure 4C).

In conducting the second experiment, we made observations to see if the growth of trees at different CO_2 concentrations or in different soil water regimes (which influences the intercellular CO_2 concentration) produces novel emergent responses that might impose a need for different forms of the light and temperature response

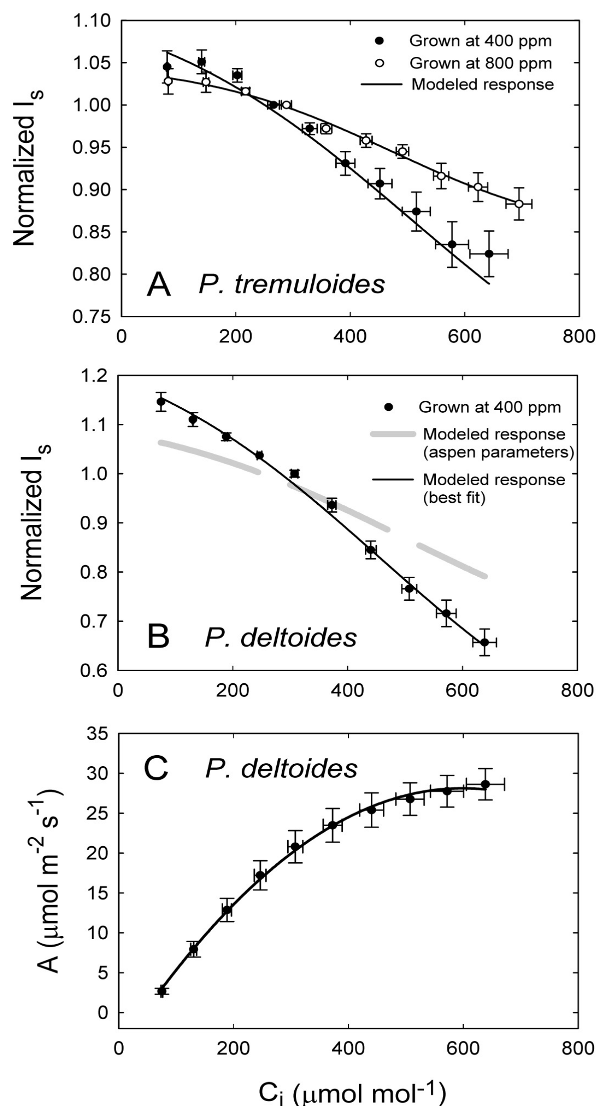

Fig. 4. Observations and modeled relationships between intercellular CO_2 concentration (C_i) and normalized isoprene emission rate (Is; normalized to an emission rate of 1.0 at the Ci that occurs when atmospheric CO_2 concentration is 400 μmol mol^{-1}), and CO_2 assimilation rate (A).

A. The relationships for leaves of aspen (*Populus tremuloides*) from trees grown at 400 μmol mol^{-1} or 800 μmol mol^{-1}. Points represent the means obtained from 10 different trees ± S.E. The solid lines are modeled responses using Ismax = 1.072 or 1.046 nmol m^{-2} s^{-1} (for growth at 400 or 800 μmol mol^{-1}, respectively), C* = 1218 or 2025 μmol mol^{-1} (for growth at 400 or 800 μmol mol^{-1}, respectively) and *h* = 1.7 or 1.54 (dimensionless scaling factor) (for growth at 400 or 800 μmol mol^{-1}, respectively).

B. The relationships for leaves of poplar (*Populus deltoides*) from trees grown at a [CO_2] of 400 μmol mol^{-1}. Points represent the mean obtained from 9 different trees ± S.E. The grey solid line represents the modeled response using the same parameters obtained for aspen leaves grown at 400 μmol mol^{-1}. The solid black line represents the modeled response using the best fit parameters for this species: Ismax = 1.34 nmol m^{-2} s^{-1}, C* = 1000 μmol mol^{-1} and *h* = 1.7.

C. The relationship between net CO_2 assimilation rate and C_i for poplar (*Populus deltoides*) trees grown at 400 μmol mol^{-1} (the same leaves used in the relationship plotted in panel B).

models described by the 'Guenther algorithms' (Figure 5). Growth of the trees at elevated CO_2 resulted in lower isoprene emission rates at any given photosynthetic photon flux density (PPFD) or leaf temperature. Growth with less water availability caused a slight increase in Is at any given PPFD or leaf temperature. In the past it was hypothesized that this is due in part to reduced C_i during growth in the face of drought (e.g., Pegoraro et al. 2005). In our experiment, the observed C_i in drought-treated plants was 494 μmol mol^{-1}, compared to 555 μmol mol^{-1} for well-watered plants grown in elevated CO_2, and 269 μmol mol^{-1} versus 279 μmol mol^{-1} for drought-treated and well-watered plants grown in normal ambient CO_2, respectively. When Is is normalized to values at PPFD of 1000 μmol m^{-2} s^{-1} and leaf temperature of 30 °C within a treatment, the responses to PPFD and temperature collapse to a common function, whether trees were grown in different CO_2 regimes or different soil water regimes. However, if normalized to a common PPFD and leaf temperature for trees in the normal ambient CO_2 treatment, the algorithms will not accurately estimate isoprene emission rates.

Discussion

The model presented in Equation 4 is robust in its ability to predict responses of the isoprene emission rate to elevated intercellular CO_2 concentration (Figure 4). In addition to the results presented here, we have tested the model in trees grown in field experiments and when grown at a range of elevated CO_2 concentrations (Wilkinson et al. 2009). In the latter study, the model was shown to not only predict the shape of the instantaneous C_i response, but also the longer-term response to growth in elevated CO_2. The latter response presumably involves adjustments in gene expression, in addition to the changes in instantaneous activity of enzymes

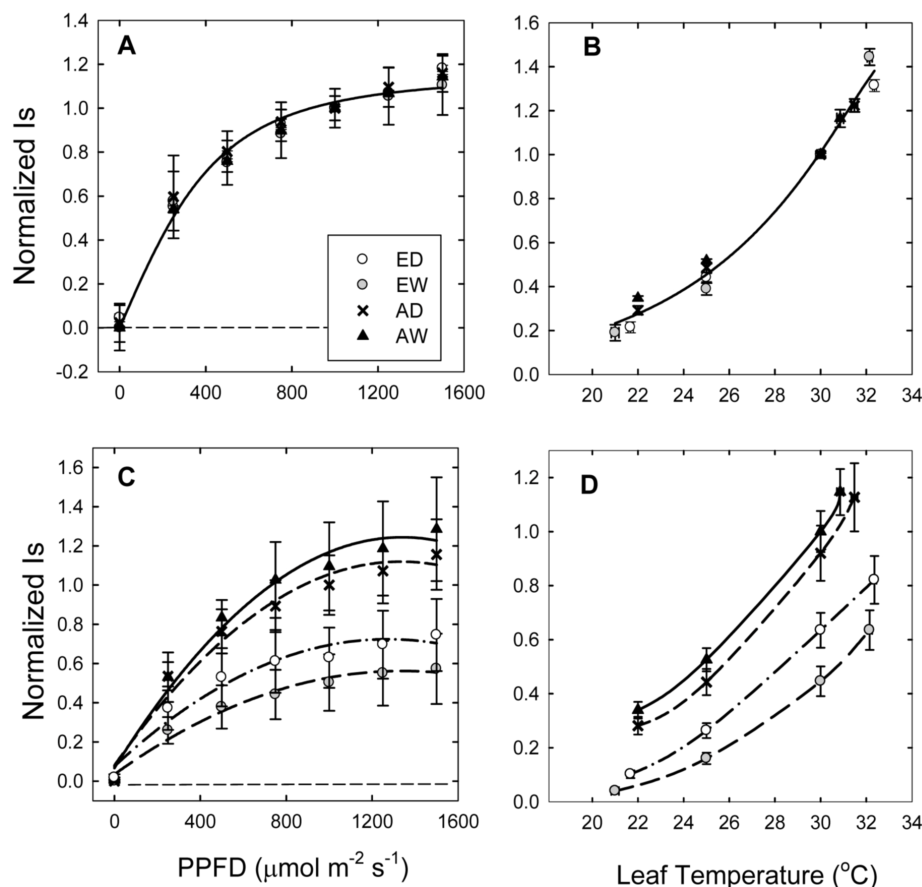

Fig. 5. A and B. Normalized isoprene emission rate as functions of photon flux density (PPFD) or leaf temperature for aspen (*P. tremuloides*) leaves grown in elevated CO_2 (800 μmol mol^{-1}) and drought (ED) or well-watered (EW) conditions, or grown in ambient CO_2 (400 μmol mol^{-1}) in drought (AD) or well-watered (AW) conditions. Emission rates were normalized to the rate observed at 1000 μmol m^{-2} s^{-1} PPFD and 30 °C leaf temperature for each treatment separately. The solid lines are the responses calculated from the 'Guenther algorithms' (Guenther et al. 1991, 1993).

C and D. Emission rates normalized to 1000 μmol m^{-2} s^{-1} and 30 °C for well watered trees grown at a CO_2 concentration of 400 μmol mol^{-1}. Panels A and B show that the 'Guenther algorithms' are robust when normalized within treatments. Panels C and D show that the algorithms will not accurately estimate isoprene emission rates if changes in C_i, brought about by different growth environments, are ignored.

and size of substrate pools that we assume to control the shorter-term instantaneous response.

It is possible that the short- and long-term responses of isoprene emission to elevated CO_2 are due to different mechanisms. For example, it is possible that growth of trees at elevated CO_2 causes decreased expression of the isoprene synthase gene. Scholefield et al. (2004) observed decreased activities of isoprene synthase in plants of a reed grass growing near a high CO_2 spring and Loreto et al. (2001) observed reduced activities of monoterpene synthase, which are similar in expression pattern to those of isoprene synthase, in oak trees grown at elevated CO_2. More recently, Calfapietra et al. (2007) showed no significant effect of growth at elevated CO_2 on isoprene synthase expression in aspen trees, but they also observed no significant effect on the overall isoprene emission rate, which contrasts with our past observations on the same trees (Monson et al. 2007). (It should be noted that Calfapietra et al., 2007, did indeed observe a trend toward decreased isoprene emission rates and isoprene synthase expression in aspen trees grown at elevated CO_2, but it was not strong enough to show statistical significance.). An alternative explanation for reduced isoprene emission rates in trees grown at elevated CO_2 is that substrate supply, rather than isoprene synthase activity, controls the response. While there is no direct evidence that substrate supply for isoprene biosynthesis is reduced when trees are grown in elevated CO_2, it has been shown that the activity of PEPC increases when trees are grown at elevated CO_2, potentially reducing the availability of pyruvate substrate for isoprene biosynthesis (Loreto et al. 2007). Additionally, Rontein et al. (2002) used cultured tomato cells grown with different supply rates of glucose to show that a build-up of leaf sugars also results in the up regulation of PEPC activity. These authors argued that flexibility in the expression of PEPC genes in response to cellular carbohydrate supply may be a general response in plants, and thus could generally be consistent with reduced substrate supply for isoprenoid biosynthesis in trees grown at elevated [CO_2]. At the present time, it is not possible to determine the exact cause for the decrease in isoprene emission rate when trees are grown at elevated CO_2. However, there is general support for the role of substrate supply as a control over isoprene emission rate in the short-term response to CO_2 (Rosenstiel et al. 2003). If the same type of control were consistently present in the long- and short-term responses, then the general fit of the model to both types of response could be easily explained. Further research will have to be conducted to better characterize the causes of these two types of responses.

Whatever, the exact cause, the form of Equation 4 makes clear that the responses of isoprene emission rate to CO_2 are best represented by a maximum possible emission rate at low CO_2 balanced against sigmoidally-shaped inhibition of that rate as CO_2 concentration increases. Sigmoidally-shaped responses are commonly observed in studies of enzyme-substrate interactions, and one common model of enzyme kinetics in the presence of allosteric regulators (the so-called Hill equation) is commonly used to describe inverse, sigmoidal responses of reaction velocity against substrate concentration. We have argued that the inverse, sigmoidal form of Equation 4 can also be explained by trade-offs in two processes known to affect substrate supply to isoprenoid biosynthesis (Figure 3); the supply of G3P from extrachloroplastic sources versus the supply of pyruvate.

We obtained evidence that growth of aspen trees at an elevated atmospheric CO_2 concentration, or under drought conditions that change the intercellular CO_2 concentration, does not affect the fundamental responses of isoprene emission to changes in PPFD or leaf temperature (Figure 5); the absolute rate of emission changes, but the relative responses to light and temperature do not. This observation simplifies the modelling of these instantaneous responses for projections of future climate with elevated atmospheric CO_2 concentrations and drier soils, as long as the algorithms are normalized to the basal isoprene emission observed within the elevated CO_2 or drought-stressed growth regime. If the CO_2 effect is ignored, however, the predicted instantaneous isoprene emission rate can be significantly in error as shown in Panels C and D of Figure 5.

The differences in C_i between the drought-treated trees and well-watered trees, when grown in both elevated and ambient CO_2 concentrations, were small compared to the magnitude of the inhibition of normalized Is during drought. This is most easily seen in the results of the temperature dependence of Is for the well-watered or drought-treated trees grown in elevated CO_2 and scaled to the Is for well-watered trees grown at normal, ambient CO_2 (Figure 5D). (The differences in normalized Is between the well-watered or drought-treated trees grown in normal CO_2 were not significant; only those for the elevated CO_2-grown trees were statistically significant at $P < 0.05$.) In Figure 5D, it is clear that there is a ~20% increase in Is when trees were grown at elevated CO_2 with drought, compared to trees grown at elevated CO_2 without drought. This increase in Is was accompanied by an 11% decrease in C_i, from 555 μmol mol^{-1} to 494 μmol mol^{-1}. As shown in the results of Figure 4A, a decrease in C_i of this magnitude should only cause an increase in Is of 4-5% in well-watered trees. Thus, the

effects of the drought treatment in trees grown at elevated CO_2 (shown in Figure 5D) are either forcing greater sensitivity of Is to C_i, or are causing Is to increase by mechanisms other than an increase in C_i alone. The relative influences of C_i through the CO_2 effect, versus other biochemical and physiological influences on Is, during drought remains as an under-explored frontier in the isoprene research community.

Conclusions

Forty years after Guivi Sanadze's initial observations on the effect of atmospheric CO_2 concentration on isoprene emission rates, we are beginning to understand the basis for the response at a level that permits mechanistic (albeit with gaps) modelling. The relationships presented in Equation 4 appear to predict the CO_2 response across a range of species and for both the long- and short-term responses to CO_2. The basis for the form of Equation 4 is still uncertain, though it can be justified on past observations and discoveries that focus on the supply of pyruvate substrate to the chloroplast, and its subsequent conversion to DMAPP, the substrate for isoprene biosynthesis. The scheme that we present describing carbon flow in isoprene-emitting plants is similar in conception to that described almost twenty years ago by Sanadze (Sanadze 1991); both rely on the interactions of two carboxylases. One of the carboxylases, RuBP carboxylase, is similar to both schemes. Unlike Sanadze's original scheme, however, the second carboxylase in our scheme is proposed to be PEP carboxylase, a cytosolic enzyme. This is different than Sanadze's hypothetical X-carboxylase, a chloroplastic enzyme involved in a putative chloroplastic mevalonic acid pathway. There is considerable work ahead to further validate the role of PEP carboxylase in regulating the response of isoprene emission to CO_2. The pursuit of this goal is likely to continue into the next generation of isoprene researchers, as we hand the 'torch' to them with the same enthusiasm that Guivi Sanadze handed the 'torch' to us twenty years ago at the Asilomar Conference on Trace Gas Emissions. In that spirit, we offer him our heartfelt congratulations on the celebration of his 80th birthday.

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ბიოქიმია, მოლეკულური ბიოლოგია

ფოთლებიდან იზოპრენის გამოყოფის ბიოქიმიური კონტროლი CO_2-თან დამოკიდებულებაში: სანაძის ორმაგი კარბოქსილირების სქემის ალტერნატიული ხედვა

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სანაძის ორმაგი კარბოქსილირების სქემა თავდაპირველად წარმოდგენილი იყო ფოტოსინთეზირებადი ფოთლებიდან იზოპრენის გამოყოფის სიჩქარის ბიოქიმიური კონტროლის ასპექტების ასახსნელად. სქემა

ეყრდნობოდა ქლოროპლასტური კარბოქსილირების ორ რეაქციას: ერთი - RuBP კარბოქსილაზით, რომელიც წარმოადგენს ძირითად კარბოქსილირების რეაქციას C_3 ფოტოსინთეზში. მეორე – უცნობი კარბოქსილაზით, რომელიც სუბსტრატს მიაწვდიდა მევალონატის ბიოსინთეზის ქლოროპლასტურ გზას. შემდგომში, სანაძის ორიგინალური სქემის დამუშავების შედეგად აღმოჩნდა, რომ იზოპრენოიდების ქლოროპლასტური ბიოსინთეზი მიმდინარეობს მევალონატურისაგან განსხვავებული გზით და იზოპრენოიდის ბიოსინთეზის სუბსტრატი ნაწილობრივ წარმოიქმნება ფოსფოენოლპირუვატიდან (PEP), რომელიც ციტოზოლიდან გადმოადგილდება. ჩვენ განვავითარეთ იზოპრენის სინთეზის შესაძლო ბიოქიმიური სქემა, რომელიც შეესატყვისება ამ დაკვირვებებს და რომელშიც, ამავე დროს, ახსნილია იზოპრენის გამოყოფის სიჩქარის დამოკიდებულება ატმოსფერული CO_2-ის კონცენტრაციის ცვლილებაზე. სანაძის თავდაპირველი სქემის მსგავსად, წარმოდგენილი სქემაც დამოკიდებულია ორ კარბოქსილაზაზე, რომელთაგან ერთი არის RuBP კარბოქსილაზა, ხოლო მეორე კარბოქსილაზა, განსხვავებით სანაძის სქემისაგან, არის ციტოზოლური წარმოშობის და ცნობილია როგორც PEP კარბოქსილაზა. სტატიაში მოცემულია "ორმაგი კარბოქსილირების" ამ ალტერნატიული სქემის მოკლე მიმოხილვა, რომელიც შეიცავს PEP კარბოქსილაზაზე დაფუძნებული ბიოქიმიური მოდელის შემუშავებას, იზოპრენის გამოყოფის სიჩქარის CO_2-ზე დამოკიდებულების ახსნის მიზნით. მოცემულია აგრეთვე ორი სახეობის ვერხვიდან გამოყოფილი იზოპრენის სიჩქარის დამოკიდებულება სინათლის ინტენსივობაზე, ტემპერატურასა და CO_2-ის კონცენტრაციაზე.

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