Chapter 5
Molecular and Pathway Controls on Biogenic Volatile Organic Compound Emissions

Ziru Li and Thomas D. Sharkey

Abstract Plants make a number of volatile organic compounds (BVOCs), many of which are emitted in a light- and temperature-dependent manner. The vast majority of these BVOCs are isoprenoids including isoprene, monoterpenes, and sesquiterpenes. The total BVOC flux into the atmosphere is on the order of a petagram (10^{15} g) and has multiple effects on atmospheric chemistry. Understanding the biochemical and molecular regulation of BVOC emissions allows us to build prediction models that better reflect the underlying physiological and biochemical processes. In this chapter we review the enzymes and pathways involved in the biosynthesis of various BVOCs that originate from plants, using isoprene as a model. The biochemical and molecular control of BVOC emission in response to short-term environment drivers such as temperature, light, CO_2, and O_2, and long-term factors such as circadian, seasonal, and developmental effects are discussed. An emerging theme in the regulation of isoprene emission is that the enzyme isoprene synthase controls the basal emission rate in the long term, while the responses of isoprene emission to short-term factors are regulated by levels of the substrate (dimethylallyl diphosphate), which is in turn determined by upstream enzymes. In addition, we propose a new hypothesis to explain the high-CO_2 suppression of isoprene emission. At high CO_2 concentrations, a high cytosolic inorganic phosphate (P_i) gradient needed to transport triose phosphates out of the chloroplasts could work against the transport of phosphoenol pyruvate into the chloroplasts. This altered partitioning of phosphoenol pyruvate would then reduce the supply of pyruvate into the MEP pathway. Much work is still needed to understand the CO_2 response of BVOC emissions but we expect to see significant progress in the near future.

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5.1 Introduction

Understanding biological controls on biogenic volatile organic compound (BVOC) emissions is one of the key topics in contemporary plant biology dealing with plant abiotic and biotic stress resistance (Sharkey et al. 2008; Vickers et al. 2009a; Harrison et al. 2013). Furthermore, importance of BVOC emissions in atmospheric reactivity and regional and larger scale Earth processes (Ashworth et al. 2013; Kulmala et al. 2013 in this volume) underscores the relevance of accurate mechanistic description of BVOC emissions. Monoterpenes and isoprene make up the largest group, in terms of mass, of BVOC. A mechanistic description of biochemical and molecular regulation of monoterpene and isoprene emission allows us to build bottom-up models that test our understanding of biochemical regulations in different environments. These mechanistic models may be better at predicting changes in BVOC emissions under very different scenarios such as predicting isoprene emissions from the past (Possell et al. 2005; Schurgers et al. 2009) and predicting the effects of climate change on isoprene emission (Arneth et al. 2007; Young et al. 2009). A better understanding of the regulation of BVOC emissions also opens up the possibility for engineering low-emitting species (Behnke et al. 2011; Rosenkranz and Schnitzler 2013 in this volume), which in the long term may alleviate the impact of global climate change on BVOC emissions.

Many of the isoprenoids are synthesized in the chloroplast, and (along with other secondary metabolic pathways) are downstream to metabolites in central carbon metabolism, such as phosphoenol pyruvate (PEP) and glyceraldehyde 3-phosphate (GAP). Isoprenoid biosynthesis also shares a similar light response with photosynthetic carbon assimilation suggesting a link of isoprenoid synthesis to energetic cofactors produced in the light. Mechanistic models that have been proposed to date generally link isoprene emission capacity or rate to some parameter associated with photosynthesis (Niinemets et al. 1999; Martin et al. 2000; Zimmer et al. 2000, 2003; Arneth et al. 2007). While this is a good step in the direction of adding mechanistic understanding to empirical models, these models still are limited by the lack of full mechanistic understanding of the regulation of key biochemical pathways responsible for isoprenoid synthesis (Monson et al. 2012; Grote et al. 2013; Monson 2013). Thus, there is opportunity for another big step toward understanding properties of the enzymes of the major pathways supplying substrates and the enzymes that convert these substrates to BVOC. This chapter will cover recent advances in understanding biochemical and molecular regulations of the enzymes involved in isoprenoid BVOC emissions from trees. Emission mechanisms of other BVOCs including methanol, ethanol, acetic acid and green leaf volatiles and modelling and engineering of BVOC emissions is covered in several other chapters of the book (Ashworth et al. 2013; Grote et al. 2013; Guenther 2013; Harley 2013; Kreuzwieser and Rennenberg 2013; Rajabi Memari et al. 2013; Monson 2013; Rosenkranz and Schnitzler 2013). Here we present an in depth look at biochemical and molecular controls of isoprene emission and suggest the primary

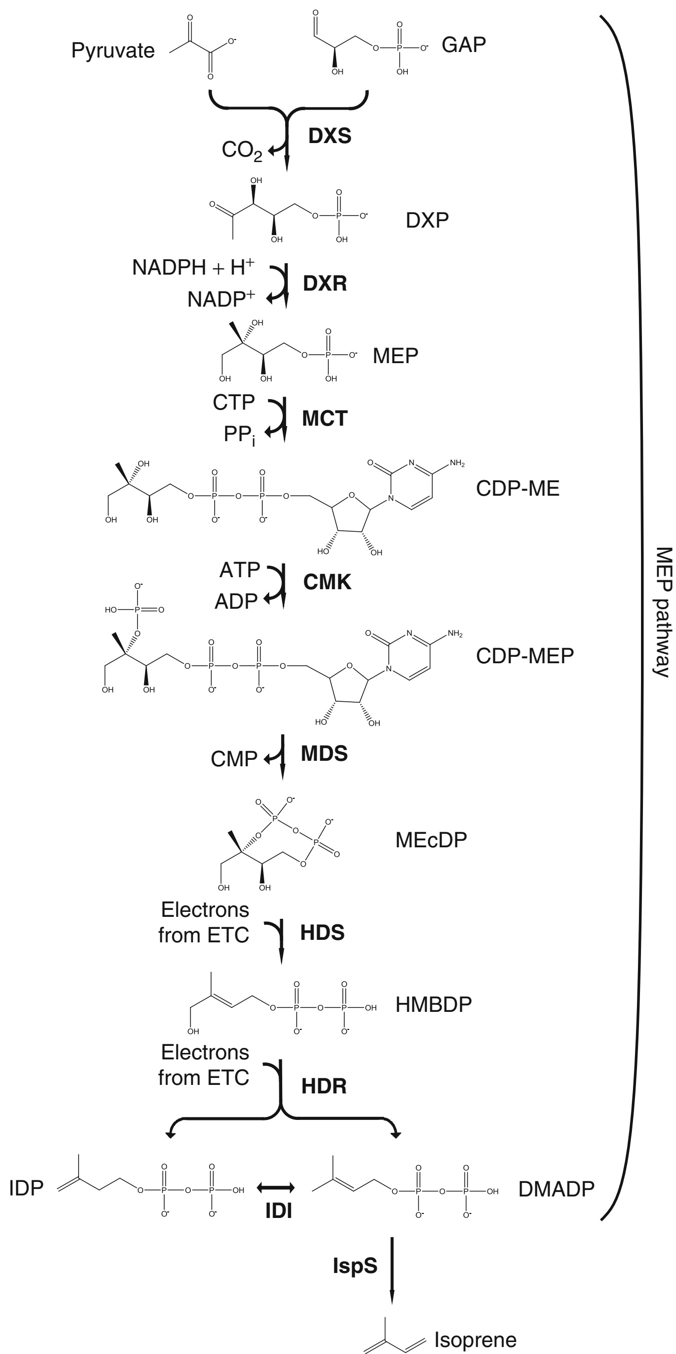
areas for future experimental research to fill the gaps in knowledge and aid towards development of fully mechanistic emission algorithms for plants stress studies and for biosphere models.

5.2 What Volatile Isoprenoids Are Emitted?

Many compounds made by plants are volatile. In some cases, the volatility is not a critical characteristic and in other cases the compound may be made non-enzymatically (e.g., methane). Other BVOC may be made enzymatically but their function may not be well known and the expected regulation unclear, for example methanol and acetaldehyde. However, trees and other plants use enzymes to make many compounds, especially isoprenoids, specifically because they are volatile. Because of the roles isoprenoids play in response to abiotic stress (chapters by Fineschi et al. 2013; Possell and Loreto 2013 in this volume) and biotic interactions (Holopainen et al. 2013; Trowbridge and Stoy 2013), specific genes are expressed to ensure the compounds are present when needed, but that carbon is not lost unnecessarily when the volatile compounds are not needed. Some of the more commonly emitted isoprenoids and their genetic and biochemical basis are covered below.

5.2.1 Hemiterpenes

The most prominent hemiterpenes (C_5) emitted from trees are isoprene (2-methyl-1,3-butadiene) and methylbutenol (2-methyl-3-buten-2-ol, or MBO). Biogenic isoprene emission is the largest non-methane hydrocarbon flux into the atmosphere and this large flux of volatile organic carbon has a profound effect on atmospheric chemistry. For this reason, isoprene research has been active in the past and will be a major focus in this review. Isoprene is mainly given off by broadleaved trees such as aspens, oaks and eucalypts, as well as many legumes. MBO is only produced in gymnosperms that make little isoprene. Both isoprene and MBO are produced from dimethylallyl diphosphate (DMADP). DMADP with its isomer isopentenyl diphosphate (IDP) are the functional isoprene units in plants and animals and the building blocks of higher-order isoprenoids. DMADP and IDP in plants can be synthesized through two pathways – a mevalonic acid (MVA) pathway that resides exclusively in the cytosol and a methylerythritol phosphate pathway (MEP pathway, also called the non-mevalonate pathway) in the plastids (Fig. 5.1). The relative sizes of cytosolic and plastidic pools of DMADP and IDP can vary between species and depend on the assay method used. Isoprene and MBO emitted from plants are exclusively synthesized from the plastidic DMADP pool produced through the MEP pathway.


Pyruvate
GAP
DXS
CO_2
DXP
NADPH + H^+
$NADP^+$
DXR
MEP
CTP
PP_i
MCT
CDP-ME
ATP
ADP
CMK
CDP-MEP
CMP
MDS
MEcDP
Electrons from ETC
HDS
HMBDP
Electrons from ETC
HDR
IDP
IDI
DMADP
IspS
Isoprene
MEP pathway

5.2.2 Monoterpenes

The most abundant class of emitted hydrocarbons, in terms of the number of compounds, are the monoterpenes. Among the well-known compounds are pinene, limonene, and cineole (the scent of eucalypts). Each of these can exist as a variety of isomers, for example, pinene can be α or β (location of a double bond) and + or – (stereochemistry of asymmetric carbon atoms) (Fig. 5.2). Monoterpenes are much more varied than hemiterpenes. Monoterpenes can also be cyclic (pinene, limonene etc.) or acyclic (myrcene, ocimene etc.). One of the more common oxygenated, cyclic monoterpenes is cineole. Oxygenated acyclic monoterpenes include geraniol (primary alcohol) and linalool (tertiary alcohol).

Monoterpenes also can be stored in structures such as resin ducts, trichomes (hairs on the leaf surface), and glands in leaf tissue (especially in eucalypts). Other monoterpenes are not stored but released as soon as they are made and some plants will make both monoterpenes that are mostly stored and monoterpenes not stored. Storage in specialized structures significantly affects the emission characteristics. Emission of non-stored monoterpenes (and the never-stored hemiterpenes) is light-dependent and the effect of temperature is on the metabolism producing the compounds (see Harley 2013 for storage effects in "non-storing" emitters). For stored monoterpenes, the effect of light will be secondary, operating through heating effects, and physics of diffusion plays a dominant role in the rate of emission of these monoterpenes.

5.2.3 Sesquiterpenes

In terms of the amount of volatile carbon released, sesquiterpenes likely represent a much smaller source than isoprene or monoterpenes. This is in part because sesquiterpenes are much less volatile, but the lack of observation of sesquiterpene emission above tree canopies could also be influenced by their very short lifetime in the atmosphere (Jardine et al. 2011). Sesquiterpene synthesis differs significantly from isoprene and monoterpene synthesis. Sesquiterpenes are made by the cytosolic mevalonic acid pathway, which produces the same precursors, IDP and DMADP (Fig. 5.3). The next step is adding two IDP molecules to a DMADP to make farnesyl diphosphate. Farnesyl diphosphate is then used by sesquiterpene synthase enzymes to make sesquiterpenes.

Fig. 5.1 The MEP pathway. The MEP pathway provides substrates for all isoprenoids inside plastids including the volatile isoprenoids isoprene and monoterpenes. The enzymes and metabolites are defined in Table 5.1. ATP and CTP are regenerated by photophosphorylation and ferredoxin can contribute electrons from the photosynthetic electron transport chain (ETC) without first passing through NADPH. The requirement for ATP, CTP, and reducing power connects isoprene and monoterpene synthesis to photosynthesis and is the basis for several models of emission rates

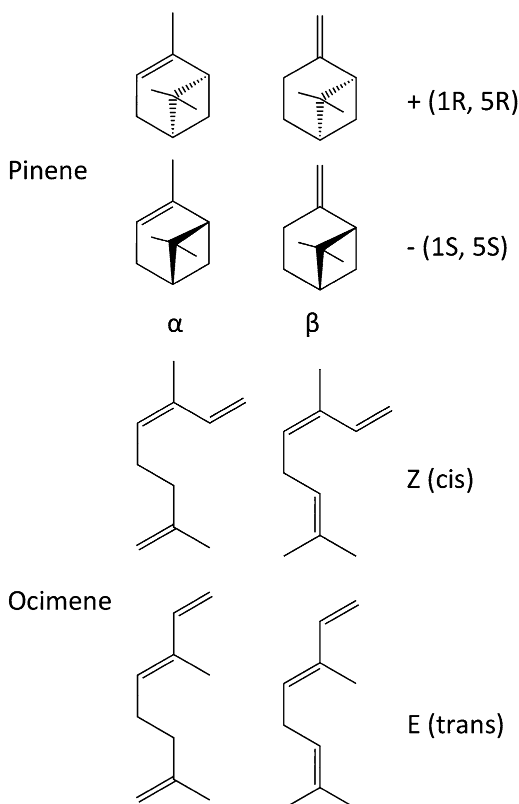


Fig. 5.2 Terpenes exist in many similar forms. Pinenes have two asymmetric carbon atoms and the double bond can be in either of two locations, resulting in different chiral (R/S or +/− or D/L) isomers. The acyclic ocimenes have a double bond that can be in either of two places and the orientation around another double bond can vary, resulting in different geometric (cis/trans or Z/E) isomers

5.3 Molecular and Pathway Controls of Volatile Isoprenoid Synthesis

5.3.1 The MEP Pathway

The existence of a mevalonic acid-independent pathway for isoprenoid synthesis was not recognized until the early 1990s. Observed labelling patterns in certain isoprenoids did not match predictions of a mevalonic acid pathway origin in ^{13}C-glucose- and ^{13}C-acetate-feeding experiments, leading to experiments that resulted in the discovery of the MEP pathway. The MEP pathway was shown to be the primary way that isoprenoids are produced in bacteria and in plastids of plants (Lichtenthaler et al. 1997; Putra et al. 1998). This connects volatile emissions of isoprene and monoterpenes with many other metabolic pathways including the synthesis of nonvolatile compounds related to abiotic stress such as carotenoids and abscisic acid.

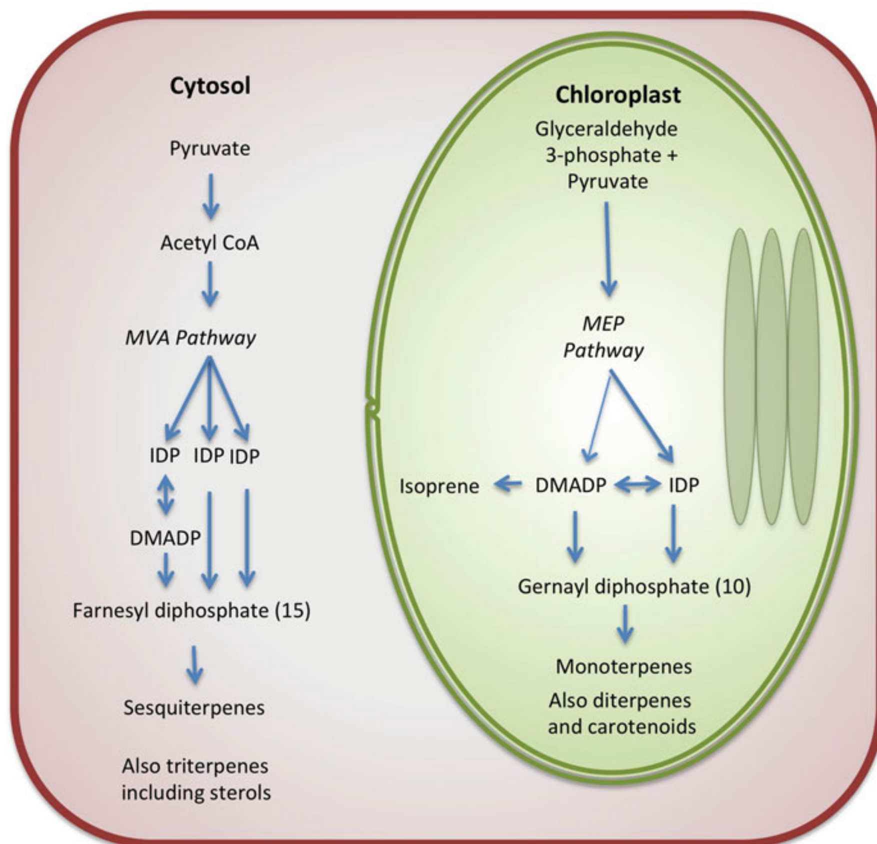


Fig. 5.3 Isoprenoid biogenic volatile organic compounds are made either in the cytosol by mevalonate (MVA) pathway (sesquiterpenes) or in the plastids by 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway (hemi- and monoterpenes). In the cytosol, the mevalonic acid (MVA) pathway converts acetyl-CoA to isopentenyl diphosphate (IDP). Some IDP is converted to dimethylallyl diphosphate (DMADP) and one DMADP plus two IDP are combined to make the sesquiterpene precursor farnesyl diphosphate. Most monoterpenes and isoprene are made inside the chloroplast where the MEP pathway converts glyceraldehyde 3-phosphate and pyruvate into IDP and DMADP for the production of isoprene (from DMADP) and monoterpenes (from the precursor geranyl diphosphate)

An important step in understanding the new isoprenoid synthesis pathway was the discovery that labeled 1-deoxy-D-xylulose was rearranged to a branched chain (Arigoni et al. 1997). The first metabolite in the MEP pathway was shown to be the 1-deoxy-D-xylulose 5-phosphate (DXP) originating from glyceraldehyde 3-phosphate and pyruvate (Rohmer et al. 1996). The first enzyme in the pathway in plants was shown to be responsible for a lethal mutation known as *cla1* (Mandel et al. 1996). The second enzyme was discovered as the target of fosmidomycin

(Takahashi et al. 1998), an antibiotic that has proven to be useful in studying the function of isoprene in trees.

Since then, great strides have been made toward elucidating the steps and enzymes in this pathway, using a combination of reverse genetic and comparative genomic approaches. The entire MEP pathway was elucidated by 2003 (Fig. 5.1 and Table 5.1). The first enzyme in the pathway, DXP synthase (DXS), forms DXP from D-glyceraldehyde 3-phosphate and pyruvate. One CO_2 molecule is lost in the forward reaction towards DXP formation. DXP produced by DXS is also a substrate in thiamine and pyridoxal synthesis. DXP reductoisomerase (DXR) then catalyzes the formation of 2-*C*-methyl-D-erythritol phosphate (MEP), the first committed metabolite that also gives this pathway its name. The next enzyme, MEP cytidyltransferase (MCT) transfers a cytidyl moiety from CTP to form 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol (CDPME) with the release of a pyrophosphate. This compound is phosphorylated by CDPME kinase (CMK) to produce CDPME 2-phosphate (CDPMEP), which is then cyclized to 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (MEcDP) by MEcDP synthase (MDS) with the loss of the cytidyl group. Finally, 4-hydroxy-3-methylbut-2-enyl diphosphate (HMBDP) is produced by HMBDP synthase in the penultimate step in the pathway, and is then converted to DMADP and IDP by HMBDP reductase (HDR) in an approximately 1:5 ratio (Rohdich et al. 2002). Under steady-state conditions the equilibrium ratio between DMADP and IDP is approximately 2:1, and the isomerization is accelerated *in vivo* by an IDP isomerase (IDI) that is present in both the cytosol and the chloroplast. Modelling of this pathway requires information on the kinetic constants for all of the enzymes but in many cases these are only known from bacterial enzymes (Table 5.2).

5.3.2 *Isoprenoid Synthases*

Most hemiterpenes and monoterpenes emitted to the atmosphere are made by proteins coded by genes in the terpene synthase (Tps) family. The Tps family can be traced back evolutionarily to a gene in the moss *Physcomitrella patens* (Fig. 5.4) (Tholl 2006; Chen et al. 2011). This gene codes for kaurene synthase, an important step in the synthesis of the plant hormone gibberellin. The Tps genes have evolved to provide many important terpenoids in most lands plants (Trapp and Croteau 2001). Two of the main sections of this family are responsible for the majority of hydrocarbons emitted by trees (Bohlmann et al. 1998; Rajabi Memari et al. 2013; Rosenkranz and Schnitzler 2013).

5.3.2.1 Isoprene Synthase

Isoprene synthesis from DMADP is catalyzed by the enzyme isoprene synthase (IspS) (the abbreviation IspS will be used italicized when describing the gene and

Table 5.1 Nomenclature of enzymes in the MEP pathway with corresponding genes in the bacterium *Escherichia coli* and in the annual vascular plant *Arabidopsis thaliana*

Step	Enzyme name	EC number	Abbreviation	Other abbreviations	*E. coli* gene	*A. thaliana* gene
1	1-Deoxy-D-xylulose 5-phosphate synthase	2.2.1.7	DXS		*dxs*	*DXS* (At4g15560)
2	1-Deoxy-D-xylulose 5-phosphate reductoisomerase	1.1.1.267	DXR		*ispC* (*yaeM*, *dxr*)	*DXR* (At5g62790)
3	2-*C*-Methyl-D-erythritol 4-phosphate cytidylyltransferase	2.7.7.60	MCT	MECT, CMS	*ispD* (*ygbP*)	*MCT* (At2g02500)
4	4-(Cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol kinase	2.7.1.148	CMK	CMEK	*ispE* (*ychB*)	*CMK* (At2g26930)
5	2-*C*-Methyl-D-erythritol 2,4-cyclodiphosphate synthase	4.6.1.12	MDS	MECPS, MECS, MCS	*ispF* (*ygbB*)	*MDS* (At1g63970)
6	4-Hydroxy-3-methylbut-2-enyl diphosphate synthase	1.17.7.1	HDS		*ispG* (*gcpE*)	*HDS* (At5g60600)
7	4-Hydroxy-3-methylbut-2-enyl diphosphate reductase	1.17.1.2	HDR	IDS	*ispH* (*lytB*)	*HDR* (At4g34350)

Modified from Phillips et al. (2008)

Table 5.2 Available estimates of kinetic parameters of MEP pathway enzymes

Enzymes	Substrate	K_m (mM)	k_{cat} (s^{-1})	References
DXS	GAP	0.12*	14*	Kuzuyama et al. (2000), Hahn et al. (2001), Bailey et al. (2002), Eubanks and Poulter (2003), Lee et al. (2007), and Brammer and Meyers (2009)
	Pyruvate	0.096*		
DXR	DXP	0.14	4.4	Engprasert et al. (2005), Rohdich et al. (2006), Jawaid et al. (2009), and Takenoya et al. (2010)
	NADPH	0.056		
MCT	MEP	0.50	26	Rohdich et al. (2000)
	CTP	0.11		
CMK	CDPME	0.14*	–	Bernal et al. (2005) and Sgraja et al. (2008)
	ATP	0.32*		
MDS	CDPMEP	0.48	2.5	Geist et al. (2010)
HDS[a]	MEcDP	0.56	0.4*	Kollas et al. (2002), Seemann et al. (2005, 2006), and Zepeck et al. (2005)
HDR[a]	HMBDP	0.31*	3.7	Altincicek et al. (2002); Gräwert et al. (2004)
IDI	IDP	0.0057	0.69*	Spurgeon et al. (1984), Jones et al. (1985), Dogbo and Camara (1987), and Lützow and Beyer (1988)
IspS	DMADP	2.5	1.8	Silver and Fall (1995), Wildermuth and Fall (1996), Schnitzler et al. (2005), Wiberley et al. (2008) and Rasulov et al. (2009a, b)

If kinetic data from a plant enzyme is available, then only data for the plant enzymes are used, otherwise values are derived from bacterial enzymes and are denoted by an asterisk. The median values of reported estimates for each enzyme are listed

[a]The HDS enzyme in *Arabidopsis* can obtain electrons directly from photosynthesis, possibly via ferredoxin (Seemann et al. 2006). HDR displays activity in presence of ferredoxin/ferredoxin-NADP$^+$/NADPH system, but its electron source in plants is less clear (Rohdich et al. 2002). No kinetic data has been reported yet for the second substrate (the electron donor) for either of the two enzymes

not italicized when referring to the protein), a close relative to other monoterpene and diterpene synthases and a member of the Tps-b family (Miller et al. 2001).

IspS in major emitting species has a plastid-targeting sequence and is localized to the chloroplasts. Due to its high volatility, isoprene emitted from plants does not build to substantial amounts within the leaf, but instead passes through two membrane systems (the chloroplast membranes and plasma membrane) and is released into the atmosphere. High K_m values have been reported for isoprene synthase (0.5–8 mM) suggesting that substrate concentration can play an important role in regulation of isoprene emission. Reported values for k_{cat} for isoprene synthase range from 0.03 to 0.26 s^{-1} (Gray et al. 2011). The isoprene synthases that have been sequenced up to now belong to a single clade within the Tps-b group

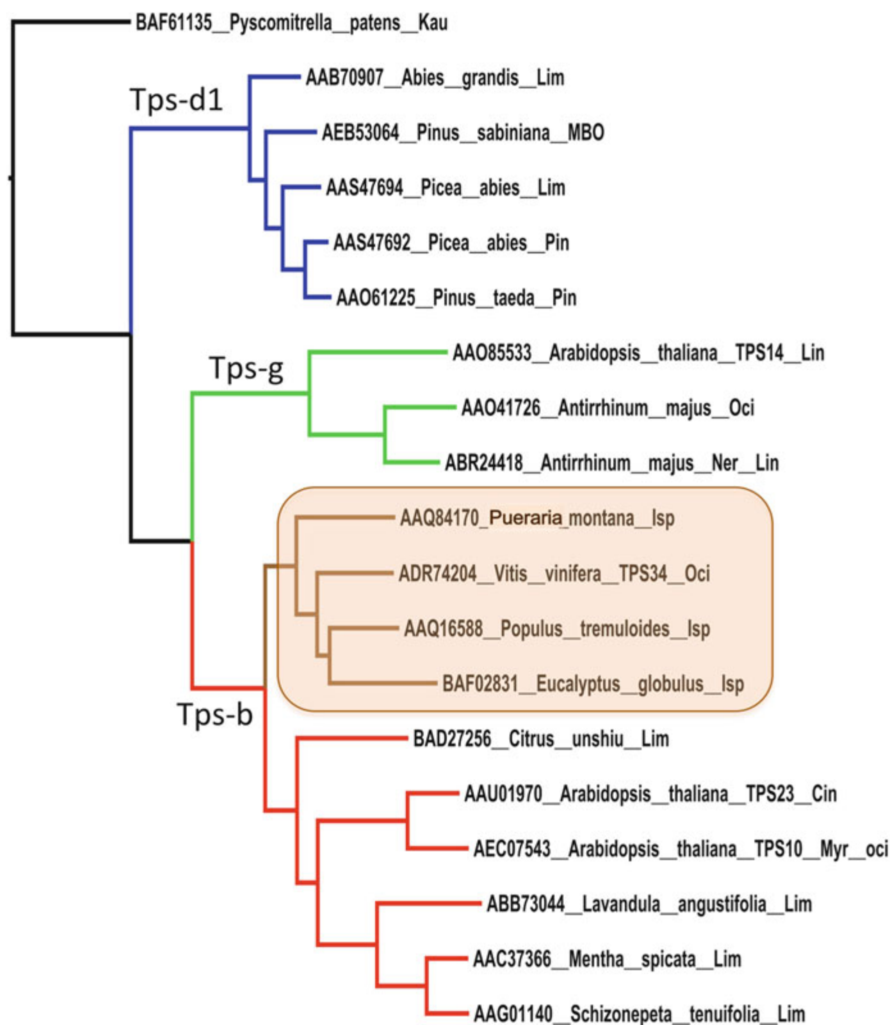


Fig. 5.4 Phylogenetic tree of a subset of genes making enzymes important for hydrocarbon emissions from trees, terpene synthases (Tps). The amino acid sequences of these genes were obtained from NCBI. The genes were selected to show the relationship of the Tps genes between gymnosperms (Tps-d) and angiosperms (Tps-g and Tps-b). Abbreviations for gene products are Kau, kaurene; Lim, limonene; MBO, methyl butenol; Pin, pinene; Lin, linalool; Oci, ocimene; Ner, nerolidol; Isp, isoprene; Cin, cineole. The *brown box* shows the ocimene/isoprene synthase clade, *blue* indicates gymnosperms, *green* is Tps-g genes and *red* Tps-b genes of angiosperms. The tree was constructed based on a Bayesian analysis (Mr. Bayes 3.2) as described by Sharkey et al. (2012)

of terpene synthases (Fig. 5.4 and Sharkey et al. 2013). In addition to isoprene synthases, this clade has genes that code for *E*-β-ocimene synthases but no other monoterpenes. This clade occurs only in the rosid group of angiosperms. It is likely that emission from non-rosid angiosperms, for example palm and bamboo, results from a different gene type and that this other isoprene synthase arose independently. It is now believed that *IspS* evolved ca. 100 million years ago in multiple lineages and is a trait that has been gained and lost multiple times, similarly to the evolutionary history of C_4 photosynthesis (Monson et al. 2013; Sharkey et al. 2013).

5.3.2.2 Methylbutenol Synthase

Enzymatic production of MBO from DMADP was first demonstrated in pine needles by Fisher et al. (2000). The MBO synthase cloned from *Pinus sabiniana* produces multiple products, primarily making MBO, but also producing isoprene in trace amounts (Fisher et al. 2000; Gray et al. 2011). This propensity to make MBO is enhanced in vivo by the K^+ dependence of this enzyme, where it has been shown that MBO production increases and isoprene production decreases with increasing K^+ concentrations. At physiological concentrations of K^+ in leaves, MBO synthase produces very little or no isoprene which can explain why no isoprene emission is observed from pine trees. The K_m of MBO synthase (10–20 mM) is high, although comparable to those of angiosperm isoprene synthases, while k_{cat} is comparable to monoterpene and sesquiterpene synthases. The MBO synthase evolved independently from angiosperm isoprene synthases and falls into the Tps-d1 group.

5.3.2.3 Monoterpene Synthases

The monoterpenes are made by terpene synthases from geranyl diphosphate [with a few exceptions, for example phellandrene made in tomato trichomes from neryl diphosphate, an isomer of geranyl diphosphate (Schilmiller et al. 2009)]. Like isoprene, the precursor for monoterpenes is made by the MEP pathway inside chloroplasts. The IDP and DMADP made by the MEP pathway are combined head to tail by geranyl diphosphate synthase resulting in a new allylic diphosphate molecule, geranyl diphosphate.

The gymnosperms have Tps-d genes and the Tps-d1 sub-group has most of the genes responsible for volatiles made by gymnosperm trees (Martin et al. 2004; Rajabi Memari et al. 2013; Rosenkranz and Schnitzler 2013). In the angiosperms a different group of genes are responsible for hemi- and monoterpene synthase enzymes. Tps-g genes (denoted by green in Fig. 5.4) are related to Tps-b but are more rare [with new genomes being analysed the number of known Tps-g genes is increasing (Martin et al. 2010)]. Tps-g proteins always make acyclic monoterpenes such as ocimene and linalool. The ocimene synthases are often relatively unspecific,

making especially myrcene in addition to ocimene. Within the Tps-b genes is a clade made up of ocimene synthases and isoprene synthases (denoted by brown in Fig. 5.4). The ocimene synthases of the ocimene/isoprene synthase clade are specific for ocimene synthase and do not make other monoterpenes. Ocimene synthases in other sections of the Tps-b often make ocimene and myrcene and sometimes other monoterpenes as well.

Usually enzymes are stereospecific, but Tps enzymes frequently make a variety of products including different isomers. Isomers can be defined by location of double bonds (typically labeled α or β) and can also be related to the orientation around double bonds (E, or cis versus Z or trans) or the orientation at asymmetric carbon atoms (chirality). Four isomers of pinene (bicyclic) and ocimene (acyclic) are shown in Fig. 5.2. The lack of strict isomeric specificity in many Tps enzymes is unusual as is the formation of multiple products from one enzyme.

It is believed that the lack of specificity in Tps enzymes results from the reaction mechanism. For hemi- and monoterpenes an allylic diphosphate precursor first loses its diphosphate to make a carbocation. The presence of a positive charge on a molecule with double bonds makes a highly unstable reaction intermediate that is quenched by water to make oxygenated terpenes or abstraction of a proton to make non-oxygenated terpenes. The specific compound formed and its isomeric conformation will depend on which proton is abstracted and the conformation of the active site. It has become possible to predict how to interconvert product specificity by changing very few amino acids (Katoh et al. 2004; Hyatt and Croteau 2005; Kampranis et al. 2007). Because product specificity is so variable, terpene synthases tend to group by phylogenetic considerations more than by the products they make. For example, the two *Arabidopsis thaliana* genes in Fig. 5.4 (and the other five Tps-b genes of *Arabidopsis*, data not shown) group together while monoterpene synthase genes of spices (bottom three species in Fig. 5.4) form a single group (even when over 70 Tps-b genes are included in the analysis, Sharkey et al. 2013). The products shown in Fig. 5.4 vary in location on this phylogenetic tree, for example limonene is found at the top and bottom. On the other hand, enzymes with very different products from closely related species tend to be closely grouped.

5.3.2.4 Sesquiterpene Synthases

Sesquiterpene synthases are the enzymes forming specific sesquiterpenes from farnesyl diphosphate. They are typically Tps-a in the case of angiosperms and typically Tps-d2 and d3 in the case of gymnosperms. The division between chloroplast (C5, C10, C20, C40) and cytosolic (C15, C30) terpene synthase activities is reasonably strict but it is not clear why this should be. Like monoterpene synthases, sesquiterpene synthase product specificity can be manipulated by changing a limited number of amino acid residues (Yoshikuni et al. 2006; O'Maille et al. 2008).

To ensure that the Tps enzymes are in the right compartment a transit sequence of about 50 amino acids is added to the hemi- and monoterpene gene sequences that targets them to the chloroplast and is cleaved once the gene product is inside

the chloroplast. Almost all Tps-b genes have a transit sequence but Tps03 of *Arabidopsis thaliana* does not (Huang et al. 2010). Tps03 is nearly identical to Tps02, which does have a transit sequence. Both enzymes can make ocimene or farnesene depending on the substrate provided. It appears that *Arabidopsis* uses the presence or absence of a transit sequence to cause one enzyme to make ocimene (in the chloroplast) while the other makes farnesene (in the cytosol). The advantage of separating sesquiterpene synthesis from hemi- and monoterpene synthesis is not clear.

5.4 Environmental Regulation of Monoterpene and Isoprene Emissions

Monoterpenes and sesquiterpenes are often stored in storage bodies (e.g., resin ducts or trichomes) and can accumulate to significant levels within plant tissues. However, relatively little is known about the biochemical and genetic controls of the accumulation of compounds in these structures and release from these structures is more a matter of physics, and less biology. There are some projects designed to elucidate how trichome biochemistry, for example, is controlled and it has been observed that in some cases, gene expression is very tightly controlled so that it occurs only in the tips of trichomes (Schilmiller et al. 2008, 2010). Compounds that are soluble can accumulate inside leaf tissue which can cause effects of stomatal opening on emission rates in addition to the effects of biochemistry (Copolovici and Niinemets 2005). On the other hand, isoprene has a relatively high Henry's law constant (a measure of the partitioning between air and water) and so is emitted essentially as soon as it is made by isoprene synthase. Initially the lack of stomatal effects on isoprene emission rate or kinetics was interpreted to mean that isoprene diffuses through the leaf epidermis instead of through stomata (Monson and Fall 1989). However, it was subsequently shown that changes in stomatal conductance caused compensating changes in isoprene concentration inside the leaf making isoprene emission independent of stomata (Sharkey 1991; Fall and Monson 1992).

The rate of emission of isoprene is therefore much more dependent on the regulation of enzymes involved and more responsive to rapid changes in environmental variables. In particular, isoprene emission rates are characterized by rapid fluctuations in natural environments, presumably driven by changes in leaf temperature due to rapid conductive heat exchange from the surrounding air currents. Both isoprene and MBO emissions are characterized by strong light and temperature dependencies (Monson and Fall 1989; Harley et al. 1998; Gray et al. 2005). Isoprene scales positively with light, and increases with increasing temperature until ~40–45 °C, at which temperature emission rates fall sharply. Isoprene emission in many species decreases with increasing CO_2 concentrations, and the cause of this high- CO_2 suppression effect is unclear. The following sections will discuss and summarize present knowledge regarding regulation of BVOC emissions from a biochemical and molecular biology perspective, using studies of isoprene emission as an example.

5.4.1 Short-Term Effects

Isoprene emission (and emission of some monoterpenes) responds very quickly to changes in the environment, for example changes in light, temperature, and CO_2. Short-term effects are generally interpreted in terms of changes in metabolite pool sizes and availability of energetic intermediates such as ATP and NADPH. Over time, the biochemical control of the short-term temperature response has become more clear. The short-term light response has been assumed to be related to photosynthetic electron transport. Additional short-term effects of CO_2 and O_2 are now recognized but the underlying mechanisms are still being actively studied.

5.4.1.1 Temperature

Isoprene emission varies strongly with leaf temperature (Sanadze and Kalandaze 1966). The response to leaf temperature is extremely rapid (Singsaas and Sharkey 1998). Unlike photosynthesis which has a maximum at 25–30 °C, isoprene emission exhibits a strong temperature dependence up to 40–45 °C, a temperature which is often deleterious to photosynthesis (Sharkey 2005). The reported temperature maxima for isoprene emission differ by as much as by 8 °C, and this is to a large extent due to differences in the measurement methodologies (Monson et al. 1992; Singsaas and Sharkey 2000). Isoprene emission at temperatures above 40 °C is not sustainable for an extended period of time (usually less than 20 min), due to a shortage of substrates (Li et al. 2011). Therefore, depending on how fast leaf temperature was elevated, and whether the same leaf or different leaves were used for different temperature points, the temperature responses will vary. The relatively large pools of MEP pathway intermediates support very high rates of isoprene synthesis for short periods during heat flecks; when leaves return to lower temperature, these pools can refill to be ready for the next heat fleck (Singsaas and Sharkey 1998).

It was known for a long time that IspS activity increases greatly with temperature and the link between emission rate and isoprene synthase activity has been postulated from very early on (Monson et al. 1992). It was also noticed that the optimum for IspS is higher than that of isoprene emission, and the possibility of a substrate-side limitation was raised (Lehning et al. 1999). Rasulov et al. (2009a) developed a method for estimating DMADP levels *in vivo* by measuring post-illumination isoprene emission. In addition, we recently presented evidence that a post-illumination isoprene burst is a good approximation for other intermediate metabolites in the MEP pathway (Li and Sharkey 2013). Using these techniques it has been shown that the temperature at which DMADP accumulates the most is ~35 °C, and this is the same for intermediate metabolites in the MEP pathway (Rasulov et al. 2010; Li et al. 2011; Rasulov et al. 2011). The optimum temperature for IspS on the other hand is ~50 °C with an activation energy of approximately 40–50 kJ mol^{-1} (Monson et al. 1992; Lehning et al. 1999; Rasulov et al. 2010; Li et al. 2011).

The increase in substrate availability combined with the increased IspS activity as temperature is increased up to 35 °C results in a very high temperature sensitivity of isoprene emission, exceeding the temperature sensitivity of isoprene synthase. Between 35 and 40 °C the substrate concentration declines, but IspS activity increases, giving an overall higher isoprene emission rate. Above 40–45 °C, the decline in substrate outweighs the stimulatory effect of temperature on IspS resulting in reduced isoprene emission as temperature goes above 40–45 °C. Empirical models fit equations thought to predict single enzyme responses to temperature (Guenther et al. 1993) but, while these work well, they do not have a mechanistic basis given that substrate concentration changes *and* effects of temperature on k_{cat} contribute, in varying proportions, to the overall temperature response of isoprene emission. It is now generally accepted that the response of isoprene emission to temperature results from the thermodynamic properties of the enzymes involved, and the control is shared between the enzyme IspS, and the MEP pathway enzymes that determine DMADP levels. While enzymes in the MEP pathway generally have a temperature optimum that is somewhat above the ambient temperature [e.g., 37 °C for DXR (Rohdich et al. 2006)], the temperature optimum for IspS is even higher; such that, isoprene emission is characterized by a marked temperature response (up to 40–45 °C), while synthesis of other downstream housekeeping isoprenoids, e.g., carotenoids and quinones, are presumably much less so. It might be interesting to speculate why this has evolved to be the case. Isoprene may play a role in protecting plants against moderate heat stress on hot summer days when leaf temperatures frequently reach but usually do not go much beyond 40 °C (Sharkey et al. 2008).

5.4.1.2 Light

Historically, two hypotheses had been put forward to explain the light response of isoprene emission: (1) changes in DMADP levels (Loreto and Sharkey 1993; Rosenstiel et al. 2002; Rasulov et al. 2009b) and (2) changes in IspS activation state (Wildermuth and Fall 1996; Fall and Wildermuth 1998; Sasaki et al. 2005). While transcription of *IspS* is light-dependent (Sasaki et al. 2005) and *IspS* appears to be under circadian regulation (discussed later), transcription and translation of genes typically take place on a longer timescale and cannot explain the instantaneous responses to light levels. Measurement of DMADP content by non-aqueous fractionation (Rosenstiel et al. 2002), post-illumination isoprene emission (Rasulov et al. 2009b) and mass spectrometry (Li and Sharkey 2013) showed that DMADP content varies, while calculated isoprene synthase activity stays roughly constant with varying light intensities. These pieces of evidence suggest substrate-level control of isoprene emission. The upstream enzymes in central carbon metabolism and the MEP pathway that determine DMADP levels both require a significant amount of ATP and NADPH, presumably provided by the light reactions of photosynthesis.

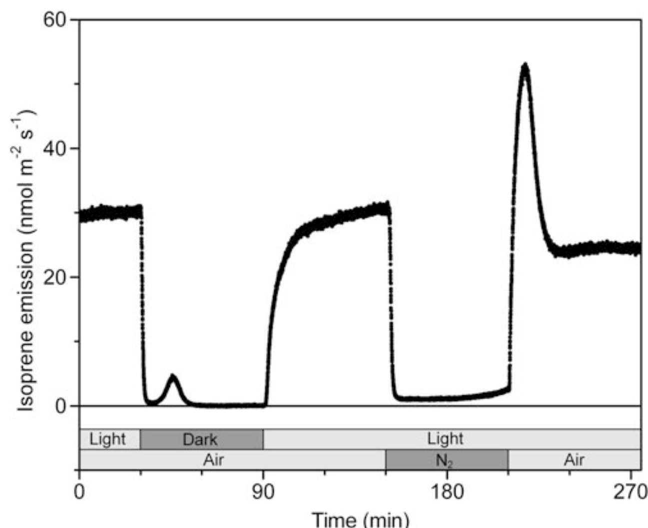


Fig. 5.5 Illustration of kinetic changes of isoprene emission from a leaf of hybrid poplar (*Populus tremula*) following light–dark transients and changes in ambient gas composition (Modified from Li and Sharkey 2013). When the light is turned off, isoprene emission from leaves falls rapidly but then shows a short post-illumination burst. When reilluminated, the leaf will resume making isoprene as much as before. When a leaf is subjected to O_2- and CO_2-free air (i.e., 100 % N_2) isoprene emission is rapidly inhibited but no subsequent burst is observed. Resupplying O_2 and CO_2 allows isoprene emission to go to very high rates but it does not fully recover

The question then becomes: which are the upstream steps that control light-dependent changes in DMADP? An important clue was gained from studies of isoprene emission during light-dark transients. When light is turned off on an emitting leaf, isoprene emission rapidly decreases to almost zero within 8–10 min (phase I) (Fig. 5.5). Emission level usually then starts to increase again in the dark, peaks at 20–25 min before dropping off again to zero in approximately 45 min (phase II). Timing of the so-called "post-illumination isoprene burst" is temperature-dependent, and the burst occurs sooner at higher temperatures. Measurement of MEP pathway metabolites during this period shows intermediate metabolites in the MEP pathway, primarily MEcDP, stays at approximately the same level during phase I when isoprene emission declined by >90 %. Later, MEcDP was converted to isoprene, forming the post-illumination burst. Therefore, the decline of isoprene emission during phase I can be explained by a rapid depletion of reducing power, inhibiting HDS (albeit incompletely). During photosynthesis, NADPH turns over faster than any other photosynthetic metabolite and can have a half-life of just 10 ms, compared to ATP with a half-life of 280 ms (Arrivault et al. 2009).

The inhibition of HDS is then reversed in the first part of phase II leading to an increase in emission levels. NADPH could be regenerated through the pentose phosphate pathway or plastidic glycolysis; alternatively, the switch of HDS from using ferredoxin to NADPH as a reducing power source may take time. What

causes the eventual decline in isoprene emission (later part of phase II) is less clear. NADPH presumably has already been regenerated as seen in the post-illumination isoprene burst, and it is also needed for anabolic cellular processes in the dark. At this time, all of the MEP pathway metabolites dropped to minimal levels (Li and Sharkey 2013). This suggests steps in the central metabolism upstream of DXS have been turned off, cutting off the carbon supply to the MEP pathway. GAP is likely to be the limiting substrate as GAP levels were quickly reduced upon darkness while levels of 3-phosphoglyceric acid (3-PGA), from which pyruvate is made, accumulates initially in darkness (Sharkey et al., 1986; Loreto and Sharkey 1993). We suggest that the darkness-induced reduction in GAP levels results from the loss of redox power to convert PGA to GAP rather than a simple consequence of reduced carbon assimilation, since substantial isoprene emission can be seen under photorespiratory conditions (e.g., CO_2-free air) where the carbon balance is more negative than the carbon balance in darkness. The tight physiological control in darkness decreased isoprene emission to essentially zero but when light is turned back on emission capacity is fully reversible. This is in sharp contrast to isoprene emission in N_2 (i.e., no O_2 and no CO_2), where the disruption of redox balance is non-physiological; despite a strong inhibition at HDS, a trace amount of isoprene is still emitted in N_2, and isoprene emission capacity is irreversibly damaged after the treatment (Fig. 5.5 and Li and Sharkey 2013).

5.4.1.3 CO_2 and O_2

Starting from CO_2-free air, isoprene emission often increases with increasing CO_2 concentrations until ~50 μmol mol^{-1} CO_2, or approximately the CO_2 compensation point of photosynthesis, where emission levels off and then sometimes decreases with increasing CO_2 concentration. The short-term decrease in isoprene emission with increasing CO_2 has been seen often but not universally. The CO_2 response is temperature-dependent, and the high CO_2 suppression effect goes away at higher temperatures (Rasulov et al. 2010). This interaction between temperature and CO_2 effects could be important for modelling considerations but has so far gained little recognition.

The suppression of isoprene at high CO_2 concentration is perplexing. Judging from the CO_2 response of photosynthesis, we would predict isoprene emission to increase, not decrease, with increasing CO_2. Glyceraldehyde 3-phosphate (an end-product of photosynthesis) is one of the two substrates for the MEP pathway, and $^{13}CO_2$-labelling studies have shown that under standard conditions a large proportion of isoprene emission comes from recent photosynthates (Delwiche and Sharkey 1993; Karl et al. 2002; Loreto et al. 2004). Interestingly, in CO_2-free air, a substantial amount of isoprene is emitted, at a rate that is comparable to emission at ambient CO_2 levels. Isoprene emission from leaves in CO_2-free air decreases slowly over time, but still does not reach zero after >10 h (Li, Z. and Sharkey, T.D. unpublished data). Carbon required for isoprene synthesis could obviously come from an alternative source (e.g., transitory starch).

Measurements of DMADP levels by non-aqueous fractionation showed that the CO_2 response of isoprene emission is regulated by substrate levels. Based on this experiment, Rosenstiel et al. (2003) proposed that CO_2 response of isoprene emission reflects a competition for PEP between PEP carboxylase in the cytosol and import into the chloroplast through the P_i/PEP transporter (PPT) for conversion to pyruvate. An alternative hypothesis is that energetic cofactors required for the MEP pathway, such as ATP and NADPH, are affected at high CO_2 conditions (Rasulov et al. 2009b). As CO_2 concentrations increase, photosynthesis switches from being limited by Rubisco to being limited by linear electron transport that generates ATP and NADPH (Farquhar et al. 1980). At higher CO_2 concentrations, photosynthesis can be also feedback-limited by inorganic phosphate levels which is determined by the speed of triose phosphate synthesis relative to its consumption by starch and sucrose synthesis (Sharkey 1985). This decrease in phosphate levels reduces ATP synthesis (Sharkey and Vanderveer 1989; Kiirats et al. 2009). However, it is important to note that cellular phosphate levels could have multiple regulatory roles.

Here we propose yet another explanation for the CO_2 inhibition of isoprene synthesis. We suggest that reduced plastidic phosphate levels at high CO_2 concentrations affect the equilibrium across the P_i/PEP antiporter on the chloroplast membrane, and reduce PEP concentration in the chloroplasts without a reduction in PEP levels in the cytosol. A P_i gradient from outside to inside the chloroplast is required to move triose phosphate out of chloroplasts for sucrose synthesis and for PGA export for PEP synthesis (Fig. 5.6). This would work against import of PEP and so could limit the supply of pyruvate for the MEP pathway. Sucrose synthesis has a very high temperature sensitivity and so the P_i gradient working against PEP import into the chloroplast might decline at high temperature (Sage and Sharkey 1987; Stitt and Grosse 1988). This would explain why CO_2 inhibition of isoprene emission often disappears at moderate to high temperature. One way to distinguish PEP carboxylase competition from reduced PEP import because of unfavorable PEP distribution within the cell is to measure PEP levels within the leaf as a function of CO_2. We know of no such measurements in isoprene-emitting species, but there is a report of PEP levels in *Arabidopsis* leaves as a function of CO_2 (Arrivault et al. 2009). These investigators found a strong increase in PEP as CO_2 was increased, consistent with the PEP distribution hypothesis and inconsistent with the PEP carboxylase competition hypothesis.

In addition to PEP import, a sodium-dependent pyruvate transporter has been reported (Furumoto et al. 2011). It is not clear whether this is active during the day or how changing CO_2 might change the distribution of pyruvate across the chloroplast envelope. Additional work, including determining partitioning of PEP and pyruvate between the cytosol and chloroplast in isoprene-emitting species will help resolve the biochemical basis of the CO_2 suppression of isoprene emission.

Under low O_2 conditions, isoprene emission typically increases. Lower levels of photorespiration could lead to an increased availability of energetic cofactors, increasing the capacity for isoprene synthesis. In the absence of both CO_2 and O_2 (N_2 conditions, no O_2 and no CO_2), isoprene emission is quickly abolished. Metabolic profiling of the MEP pathway showed that HMBDP synthase (HDS, step

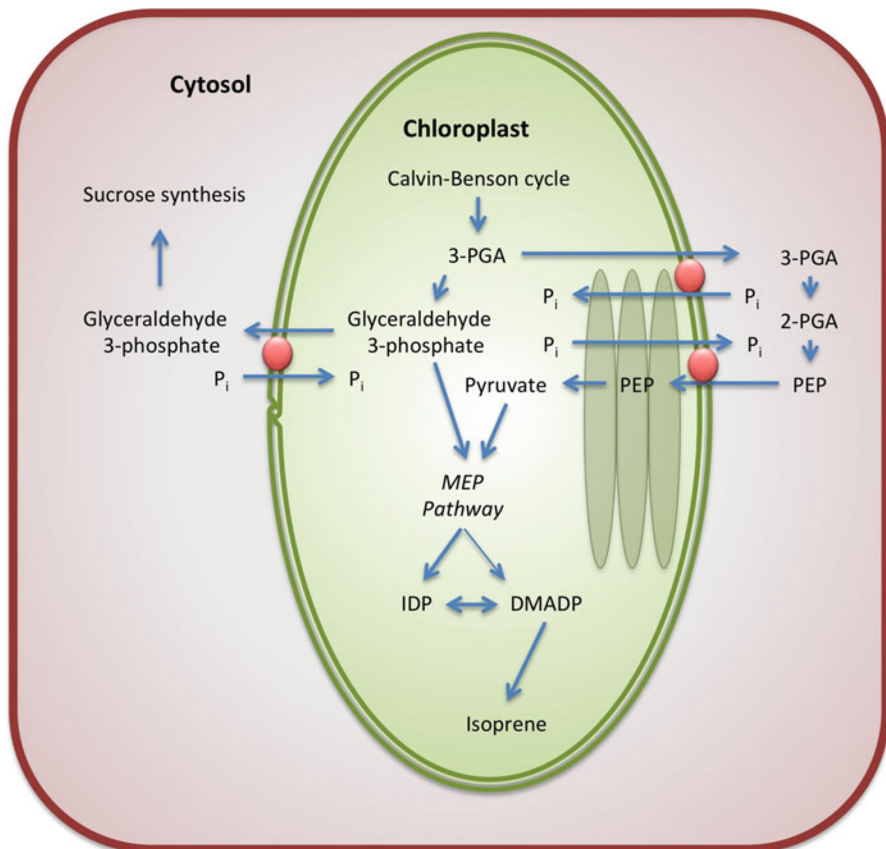


Fig. 5.6 Supply of carbon for isoprene synthesis from photosynthesis. The primary product of photosynthesis, glyceraldehyde 3-phosphate, can be used directly from the Calvin-Benson cycle, but chloroplasts generally do not have phosphoglucomutase to convert 3-phosphoglyceric acid (3-PGA) to 2-PGA. This may help metabolism in the chloroplasts to go in the direction of sugar synthesis. Glyceraldehyde 3-phosphate and 3-PGA can be exported from the chloroplast by exchange for phosphate on the triose phosphate/phosphate antiporter. PEP can be taken up by a PEP/phosphate transporter. However, the phosphate gradient that must be high in the cytosol to favor glyceraldehyde 3-phosphate export, will make PEP import difficult

6 of the MEP pathway) is strongly inhibited under this condition causing a 30-fold increase in substrate levels. Switching off both CO_2 and O_2 would inhibit both the carboxylation and oxygenation reactions of Rubisco, in essence turning off the Calvin-Benson cycle, which would disrupt cellular redox balance. The plant HDS is an oxygen-sensitive enzyme (Seemann et al. 2002) and the altered cellular redox potential may lead to increased enzyme turnover (Rivasseau et al. 2009).

5.4.2 Long-Term Effects

In contrast to the short-term responses dominated by biochemical regulations, longer-term effects are increasingly recognized and these are generally related to molecular controls such as changes in gene expression and even the presence or absence of specific genes. For example, species differences in whether or not a plant emits isoprene may result primarily from the presence or absence of a functional isoprene synthase. Transformation of *Arabidopsis* (Sharkey et al. 2005; Sasaki et al. 2007) or tobacco (Vickers et al. 2009b) with an isoprene synthase gene can cause a species that normally does not emit isoprene to begin emitting isoprene. The emission rate measured or corrected to 30 °C and photon flux density of 1,000 μmol m^{-2} s^{-1} (to control for short-term effects) varies over the course of a day, over the course of a season, in response to weather, and by leaf location within a canopy. Overall, we note that it is difficult to deconvolute ontogenetic and environmental effects on isoprene emission and the observed responses may often actually reflect combined effects of environmental variations and leaf age.

5.4.2.1 Circadian Effects on Isoprene Emission Capacity

There have been several reports of circadian changes in isoprene emission capacity (Funk et al. 2003; Wilkinson et al. 2006; Loivamäki et al. 2007; Wiberley et al. 2008, 2009). When measurements are made under ambient conditions, the circadian changes in emission can result from light or temperature changes. However, as early as in 1986, it was recognized that some of the circadian change in isoprene emission was beyond what could be accounted for by changes in light (Ohta 1986). In some plants, the circadian effect can be absent or modest (Lehning et al. 1999), but when measured at high temperature or photon flux density, the effect is greater (Wilkinson et al. 2006). When measured under constant light, isoprene emission capacity in poplar leaves exhibits an ultradian cycle with a 12 h period (Wiberley et al. 2009). Trees grown at 30 °C show a more pronounced circadian effect than trees grown at 20 °C (Wiberley et al. 2008). The amount of mRNA for *IspS* also varies over the course of the day but these variations are not reflected in measurable protein amounts (Wiberley et al. 2009). Several enzymes of the MEP pathway, especially DXS and HDR also show very large circadian patterns in mRNA accumulation (Wiberley et al. 2009), but again there is no evidence for significant changes in protein amount. The half-life of IspS protein was estimated to be 5.3 days for trees grown at 20 °C and 3.4 days for trees grown at 30 °C. Little is known about the relative availability of substrate through the day, so the importance of the MEP pathway versus isoprene synthase regulation for circadian effects is not yet known.

5.4.2.2 Seasonality

Seasonal changes in isoprene emission capacity have been reported many times (Monson et al. 1994; Guenther 1997; Schnitzler et al. 1997; Goldstein et al. 1998; Fuentes and Wang 1999; Fuentes et al. 1999; Zhang et al. 2000; Pegoraro et al. 2007). There is some evidence for changes in mRNA levels for *IspS*, but with a strong effect of temperature interacting with seasonal effects (Mayrhofer et al. 2005).

5.4.2.3 Weather

The seasonal effects reflect two other effects, a weather effect and a developmental effect. The weather effect refers to the fact that a period of several warm days results in higher isoprene emission capacity than a period of several cool days. This effect has been seen many times (Sharkey et al. 1999; Geron et al. 2000; Pétron et al. 2001). This has also been seen for methylbutenol (Gray et al. 2006) even though the gene for methylbutenol synthase has a very different evolutionary history from known isoprene synthases (Gray et al. 2011). The time period over which temperature effects affect isoprene emission capacity has been found to be anywhere from 6 h to 15 days. This effect has been studied in trees and mosses (Hanson and Sharkey 2001a, b; Wiberley et al. 2008). There is evidence for changes in the amount of isoprene synthase enzyme that can account for some of the effect of weather on isoprene emission capacity (Wiberley et al. 2008).

5.4.2.4 Developmental Effects

Isoprene emission capacity develops more slowly during leaf development than does the capacity for photosynthesis and this effect is temperature-dependent (Grinspoon et al. 1991; Kuzma and Fall 1993; Sharkey and Loreto 1993; Harley et al. 1994). The extractable activity of isoprene synthase can account for this effect (Kuzma and Fall 1993). More recently it was shown that the temperature-dependent delay in the onset of isoprene emission capacity was regulated by expression of the isoprene synthase gene (Wiberley et al. 2005; Sharkey et al. 2008). It is also likely that as leaves senesce, isoprene synthase is degraded, resulting in time-dependent reduction of isoprene emission rates (Sun et al. 2012).

Seasonal effects are treated as a separate phenomenon but they may reflect a combination of weather and developmental effects (Grote et al. 2013 for further discussion of difficulties in describing seasonality in models). In any case, empirical models include algorithms for approximating the changes in isoprene emission capacity through the season and this has improved model performance.

5.4.2.5 Canopy Location

Leaves at the top of a canopy will be hotter during the day, colder at night, and exposed to much more light than leaves in the middle of a canopy. It has been found that leaves at the top of a canopy emit significantly more isoprene than leaves at the bottom of a canopy (Sharkey et al. 1996; Niinemets et al. 2010). The analysis of Niinemets et al. (2010) showed that isoprene emission capacity was well-correlated with light availability, but it is also possible that temperature differences of leaves at different locations in the canopy contribute to the differences in isoprene emission capacity at different locations in a canopy.

5.4.2.6 Ozone and CO_2

Trees grown in high [CO_2] or high ozone reduced isoprene emission capacity (Calfapietra et al. 2007). Elevated [CO_2] caused a very slight reduction in message level for *IspS* and slightly more reduction in protein level, though neither was statistically significant. In elevated ozone both message level and protein level were significantly reduced and the presence or absence of elevated [CO_2] had no further effect.

The effect of elevated [CO_2] on the long-term capacity for isoprene emission has sometimes been interpreted in the same way as the short-term effects but Sun et al. (2012b) challenged this view. They found that substrate (DMADP) was less available, but there was more activity of isoprene synthase at elevated [CO_2]. Possell and Hewitt (2011) found less DMADP and isoprene synthase activity in plants grown in elevated [CO_2] but their DMADP measurements were made by acidifying whole leaves. This technique measures whole-leaf DMADP, while only DMADP inside chloroplasts is readily available for isoprene production and acidification was found to significantly overestimate DMADP levels when compared to measurements made using mass spectrometry (Weise et al. 2013).

5.4.3 Isoprene Synthase Gene Expression

Much of the changes in long-term emission capacity are related to changes in the expression of the isoprene synthase gene. Given that the typical substrate levels in leaves are in the range of the K_m, both IspS capacity and DMADP supply rate will affect the overall rate, and DMADP supply is likely the most important factor for short-term changes in rate of emission. Only one report has suggested strong post-translational regulation of isoprene synthase (Lehning et al. 2001), but in most studies this has not been invoked. Gene expression can be controlled by many factors, but studies of the effect of the DNA immediately upstream of the coding sequence (the promoter region) are just beginning. The promoter region of

grey poplar (*Populus x canescens*) *IspS* was sequenced and examined for motifs known to influence gene expression (Loivamäki et al. 2007). A motif known to confer circadian regulation was found. Similar circadian elements were reported from *Populus trichocarpa* in *IspS*, *DXS*, *CMS*, *MCS*, and *HDS* by Wiberley et al. (2009). Heat shock promoter elements were found in *IspS*, *DXS*, and *HDR* as well. The grey poplar promoter was tested by fusing it to a reporter gene and expressing it in *Arabidopsis* (Cinege et al. 2009). The promoter caused gene expression primarily in leaves and had properties that would explain a number of properties of *IspS* expression.

Many genes have a motif consisting of TATA that serves to start the process of transcribing the DNA into RNA for subsequent protein production. In grey poplar (*Populus x canescens*) this TATA box was proposed to reside about 100 base pairs upstream of the protein coding start site, but a more likely TATA box is found 1270 base pairs upstream (Sharkey, T.D., unpublished data). On the other hand, only 250 base pairs of upstream sequence is available for kudzu (*Pueraria lobata*) *IspS* and a possible TATA box is found near the beginning of this region. It was possible to express this kudzu gene in *Arabidopsis* using this short promoter, providing evidence that the TATA box is functional (or that some other transcriptional start motif is used in the case of kudzu). Soybean (*Glycine max*) has two genes nearly identical to the kudzu *IspS*, but soybean does not emit isoprene. The promoter regions of the two soybean genes show significant alteration and lack the possible TATA box found in kudzu (Sharkey, T.D., unpublished data). There are also no reported ESTs for these genes suggesting that these are pseudogenes that are no longer expressed. These provide insight into how plants have lost the trait of isoprene emission. The analysis of the promoters is now much easier because of large-scale sequencing projects. Important gene expression controls can be studied and new insights are likely to come in the near future (Rajabi Memari et al. 2013; Rosenkranz and Schnitzler 2013 for further discussion).

5.5 Conclusions and Future Perspectives

Significant advances are being made on the front of understanding biochemical and molecular regulation of BVOC emissions. The elucidation of MEP pathway enzymes in the early 2000s, the new techniques developed in measuring trace BVOC levels (e.g., fast isoprene sensor, proton-transfer reaction time-of-flight mass spectrometer) and the advent of the omics era all contributed to the increasing repertoire of knowledge about biochemical and molecular regulation of BVOC emissions from trees. In particular, the potential for using the MEP pathway in bacteria as chemical factories for producing commercially profitable compounds, as well as targeting the bacterial MEP pathway in drug development, has sparked tremendous interest and studies to elucidate the control mechanisms of the MEP pathway. However, caution should be taken as we extrapolate existing knowledge about the MEP pathway in microorganisms to understand BVOC regulation in

trees, as the two systems may not be entirely identical. A notable example, as mentioned above, is HDS. The plant HDS enzyme accepts electrons directly from photosynthesis (ferredoxin rather than NADPH) and may be an important regulatory step in nature. The bacterial homolog, IspG, on the other hand, uses NADPH as the source of reducing power. The MEP pathway metabolic profile of *E. coli* also appears to be distinct from that of plant extract (Weise, S.E., Li, Z., Sharkey, T.D., unpublished data). Nevertheless, significant additional progress in understanding molecular and biochemical control of BVOC emission from trees is likely in the near future.

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