Article type: *Concepts, Reviews and Syntheses*

Leaf isoprene emission as a trait that mediates the growth-defense tradeoff in the face of climate stress

Russell K. Monson[1], Sarathi M. Weraduwage[2,3], Maaria Rosenkranz[4], Jörg-Peter Schnitzler[4], Thomas D. Sharkey[2,3,5]

[1] Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309 USA

[2] Department of Biochemistry and Molecular Biology, MSU-DOE Plant Research Laboratory, East Lansing, MI 48824 USA

[3] Department of Energy, Office of Science, Office of Biological and Environmental Research, Great Lakes Bioenergy Research Center, East Lansing, MI 48824 USA

[4] Research Unit Environmental Simulation, Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, 85764 Neuherberg, Germany

[5] Plant Resilience Institute, Michigan State University, East Lansing, MI 48824 USA

Author declaration statement RKM conceived of the manuscript. All authors contributed to the research and analysis described in the manuscript. All authors contributed to the writing and editing of the manuscript.

Corresponding author: Russell Monson; russell.monson@colorado.edu

Abstract (244 words)

Plant isoprene emissions are known to contribute to abiotic stress tolerance, especially during episodes of high temperature and drought, and during cellular oxidative stress. Recent studies have shown that genetic transformations to add or remove isoprene emissions cause a cascade of cellular modifications that include known signaling pathways, and interact to remodel adaptive growth-defense tradeoffs. The most compelling evidence for isoprene signaling is found in the shikimate and phenylpropanoid pathways, which produce salicylic acid, alkaloids, tannins, anthocyanins, flavonols and other flavonoids; all of which have roles in stress tolerance and plant defense. Isoprene also influences key gene expression patterns in the terpenoid biosynthetic pathways, and the jasmonic acid, gibberellic acid and cytokinin signaling networks that have important roles in controlling inducible defense responses and influencing plant growth and development, particularly following defoliation. In this synthesis paper, using past studies of transgenic poplar, tobacco and *Arabidopsis*, we present the evidence for isoprene acting as a metabolite that coordinates aspects of cellular signaling, resulting in enhanced chemical defense during periods of climate stress, while minimizing costs to growth. This perspective represents a major shift in our thinking away from direct effects of isoprene, for example, by changing membrane properties or quenching ROS, to indirect effects, through changes in gene expression and protein abundances. Recognition of isoprene's role in the growth-defense tradeoff provides new perspectives on evolution of the trait, its contribution to plant adaptation and resilience, and the ecological niches in which it is most effective.

Keywords ozone • green leaf volatiles • carotenoids • chlorophyll • thermotolerance • drought • photosynthetic capacity • phytohormones • ROS • growth differentiation balance • lignin

Introduction

Biogenic isoprene (2-methyl-1,3-butadiene) is a light-dependent, volatile, hemiterpene emitted from the chloroplasts of many plants, including numerous woody species, and even some ferns and mosses (Harley et al. 1999, Hanson et al. 1999; Monson et al. 2013). The molecule has drawn the attention of plant physiologists, ecologists and atmospheric chemists because: (1) it appears to be an important trait that protects the photosynthetic apparatus of plants in the face of climate stress (Sharkey et al. 2008; Loreto and Schnitzler 2010); (2) it has a role in structuring tritrophic interactions among plants, herbivores and their parasites (Loivamäki et al. 2008); and (3) it has a role in controlling the oxidative state of the troposphere (Monson and Holland 2001, Monson 2002; Pike and Young 2009). One of the most debated issues concerning the topic of isoprene emissions is why plants produce it (Sharkey and Singsaas 1995; Monson et al. 2013; Sharkey 2013; Dani et al. 2014; Loreto and Fineschi 2015). In this synthesis, we take up this issue with a focus on recent data showing that isoprene participates in several cellular signaling networks and has a role in the coordination of growth-defense trait tradeoffs.

For nearly thirty years, evidence has accumulated that isoprene protects the photosynthetic apparatus of plants from abiotic stress, such as that caused by high temperature and drought (Sharkey and Singsaas 1995; Sharkey and Yeh 2001; Ryan et al. 2014; Fini et al. 2017; Taylor et al. 2019). Many of these studies relied on the elimination of isoprene emission through the introduction of chemical inhibitors or genetic modification, and they focused on the thermal tolerance of photosynthesis (Sharkey et al. 2001; Velikova and Loreto 2005; Behnke et al. 2007; Sasaki et al. 2007). Other research, using similar approaches, showed that isoprene is effective against cellular oxidative stresses that occur during drought and high light episodes, or ozone exposure (Loreto and Velikova 2001; Affek and Yakir 2002; Velikova et al. 2004; Vickers et al.

2009b; Behnke et al. 2009; 2010b; Pollastri et al. 2014). When considered as a whole, the past body of research focuses on properties of the isoprene molecule in an isolated protective role; one in which unsaturated hydrocarbon bonds stabilize protein-lipid and protein-protein interactions in chloroplast thylakoids and/or react directly with reactive oxygen species (ROS) (Sharkey et al. 2008; Siwko et al. 2007; Vickers et al. 2009a; Velikova et al. 2011; Parveen et al. 2019a).

Even in the face of these numerous reports, however, some past studies failed to observe an effect of isoprene applied as a short-term, single treatment on thermotolerance in isolated leaf discs (Logan and Monson 1999), or on the permeability and stability of isolated thylakoids and liposome membranes (Logan et al. 1999). Recently, Harvey et al. (2015) concluded that the partitioning of gaseous isoprene into phospholipid membranes, at realistic intra-leaf concentrations, was two orders of magnitude lower than levels thought to be effective in thermal protection. Furthermore, they found that even extremely high isoprene concentrations failed to affect the viscosity of phosphatidylcholine liposome membranes. These observations provide opposing evidence to the past theories of isoprene acting alone to stabilize membrane hydrophobic interactions. At the same time, a different set of observations began to emerge that further challenged the adequacy of the traditional theories. Using transgenic technologies, the capacity for isoprene emissions was introduced into otherwise non-emitting species, generating novel phenotypic effects that could not be explained by the conventional theories. For example, increased growth rates occurred in some species (Loivamäki et al. 2007; Sasaki et al. 2007; Vickers et al. 2009b; Zuo et al. 2019), but they decreased in others, even under non-stressful conditions (Zuo et al. 2019). These studies suggested that a complete understanding of isoprene's effects would require a broader theoretical scope. Despite these challenges, the overall body of

work from nearly three decades of research has provided unequivocal evidence, and general acceptance, that isoprene emission does indeed represent a trait with positive adaptive value in plants, especially with regard to enhancing photosynthesis in the face of stress. The newer questions that have been raised, are: what is the broader adaptive scope of the trait and how does stress tolerance fit into that broader scope?

In recent work, observations have been made that address these questions and provide new hypotheses with regard to isoprene's mode of action. Rather than acting alone, isoprene is more likely to interact with several other metabolites known to protect the photosynthetic apparatus during stress (Loreto and Schnitzler 2010; Behnke et al. 2010a; Tattini et al. 2014; Velikova et al. 2014; Brunetti et al. 2015; Tattini et al. 2015; Vanzo et al. 2016; Marino et al. 2017; Lantz et al. 2019; Zuo et al. 2019; Parveen et al. 2019b; Monson et al. 2020; Liu et al. 2020). Much of the new evidence has been obtained employing plants modified in their isoprene emission capability; natural isoprene-emitters (IE) that were transformed to be non-emitters (NE) by knocking-down expression of the isoprene synthase gene (*ISPS*) (Behnke et al. 2007), and conversely, NE plants that were converted to IE plants by introducing *ISPS* (Loivamäki et al. 2007; Sasaki et al. 2007; Vickers et al. 2009b; Zuo et al. 2019). A picture is emerging of isoprene acting within a broad cellular network to both contribute to abiotic stress tolerance and organize aspects of growth-defense trait tradeoffs. In this synthesis, we bring together these emerging concepts on cellular signaling, growth and abiotic stress tolerance, and through an integration with known relations between metabolite production and plant defense, provide a new hypothesis on isoprene's potential role in mediating the growth-defense tradeoff. In essence, we make the case that isoprene has evolved in certain plant lineages as a means to stage an effective form of chemical defense, with minimal costs to growth, in the face of climate stress.

The growth defense tradeoff

Plant growth rate, whether considered in physiological or evolutionary terms, reflects resource limitations and the combined contributions and interactions of multiple traits as they acquire and use limited resources. Patterns in resource use and allocation can be described according to economic tradeoffs (Mooney 1972; Chapin 1980; Bloom et al. 1985; Reich 2014); whereby, utilization of limiting resources for one function must come at a cost to their utilization for a competing function. The realization of common currencies and interdependencies, within the context of plant resource use, lies at the foundation of growth-defense tradeoff theory – the mandated relation by which allocation to growth in a resource-limited environment occurs at a cost to defense, and vice versa (Stamp 2003; Schuman and Baldwin 2016; Züst and Agrawal 2017). Recognition of the utility of an economic framework, within which to describe growth, defense and fitness, has led to a broad foundation on which to build concepts of adaptation, trophic interactions and evolutionary compromises.

The principal ecological theories underlying growth-defense tradeoffs contain some aspect of cost and benefit. Bryant et al. (1983) conducted studies in boreal ecosystems and observed that plants native to environments with low nutrient availability, such as black spruce (*Picea mariana*) and various graminoid species, had slow growth rates, utilized carbon-based defenses and had high levels of defense at all life-cycle phases. Plants native to sites with higher nutrient levels, such as quaking aspen (*Populus tremuloides*) and several dicot herbaceous species, exhibited faster growth rates, had high levels of defense only as juveniles and, in many species, relied more often on nitrogen-based defenses. These observations were synthesized to produce the Carbon-Nutrient Balance (CNB) hypothesis, which has been shown to be most useful in addressing physiological plasticity, and the movement of carbon or nitrogen in excess of that

134 required for growth, to defense (Lerdau and Coley 2002; Stamp 2003). Coley et al. (1985)
135 expanded the principals of the CNB hypothesis to account for genetically fixed G-D tradeoffs
136 and interspecific differences in adaptation. In formulating the Resource Availability Hypothesis
137 (RAH), they presented the case that the G-D tradeoff reflects selection across a broad range of
138 traits in plants native to environments with different levels of resource availability. According to
139 the RAH, slower growth rates, but higher levels of constitutive defense, occur in plants native to
140 resource-limited environments, compared to plants native to resource-rich environments, and
141 these patterns of trait covariance maximize fitness. In the Growth-Differentiation Balance
142 Hypothesis (GDBH), Herms and Mattson (1992) developed the physiological case for a
143 mutually-exclusive allocation of resources to one or the other function – growth versus
144 differentiation (including defense). The GDBH included the tenets of the CNB hypothesis, but
145 expanded the arguments to include ontogenetic constraints and it framed growth-differentiation
146 tradeoffs in terms of cellular processes. Herms and Mattson (1992) applied their physiological
147 hypotheses to an evolutionary optimality model in an effort to link the processes of cellular
148 tradeoffs with natural selection, and therefore integrate the RAH and GDBH. The Optimal
149 Defense Theory (ODT), which predates the tradeoff theories discussed above, emerged from
150 studies showing that plants deploy their chemical defenses in ways that maximize their
151 effectiveness against herbivores (McKey 1974; Rhoades 1979). These observations led to a
152 general theory that plants have evolved patterns of defense in direct proportion to levels of
153 herbivory and impacts on fitness. The ODT differs from the RAH and GDBH in that the
154 principal determinant in natural selection is the balance between demand and fitness cost, rather
155 than growth and fitness cost.

Tradeoff theories that focus on selection and genetic correlations among traits, such as the RAH, GDBH and ODT, have generally worked well to predict patterns of growth and defense at higher taxonomic levels (Endara and Coley 2011; Schuman and Baldwin 2016; Züst and Agrawal 2017). However, they often fail in predicting tradeoffs within species (Agrawal 2020). With regard to intraspecific processes, it is common to find increases in both growth and defense as resource availability increases (van Nordwijck and de Jong 1986; Agrawal 2020). This is due to phenotypic plasticity – those individuals within a species with greater access to limiting resources will allocate greater amounts of those resources to both growth and defense, compared to individuals with lesser access to resources. Thus, in comparing individuals within a species, a transition from high-to-low resource availability may not result in the prioritization of defense over growth, as predicted by the optimization theories. This crucial concept is key to understanding the selective forces at play when a novel trait, such as isoprene emission, is introduced into a species. If that trait facilitates greater resource acquisition, and has the potential to direct the allocation of those resources to both growth and defense, then the selective value of the trait to both processes could increase.

In the past decade, progress has been made, using multi-omic approaches, applied primarily to *Arabidopsis*, within the context of growth-defense tradeoffs (D'Auria and Gershenzon 2005; Campos et al. 2016; Züst and Agrawal 2017). At the molecular scale, growth-defense tradeoffs are orchestrated through cellular signaling networks and enacted, to a large extent, through interactions among genetic transcription factors (TFs) (Karasov et al. 2017). These interactions control gene expression in the pathways of both constitutive and induced defenses, and are coupled through pathway crosstalk to the production and sensitivities of growth regulators, such

as salicylic acid, jasmonic acid, gibberellins, cytokinins, ethylene and abscisic acid (Howe et al. 2018; Guo et al. 2018a; Koo et al. 2020).

Transcriptional control of gene expression determines the enzymatic potential for increases or decreases in metabolite production (Schuman and Baldwin 2016; Züst and Agrawal 2017), and along with enzyme kinetics and substrate availability establishes the antagonistic push or pull 'forces' that determine whole-plant growth-defense allocation patterns. Transcriptional control can also provide resilience in the face of allocation commitments in a variable environment. In the theoretical condition of a zero-sum constraint, there is no margin for error in the face of environmental fluctuation, and mistakes in allocation strategy can lead to reduced fitness. Transcriptional control provides a means for selection to favor phenotypes that operate conservatively, below the zero-sum constraint, and retain a resource margin that can be used for adjustments in the growth-defense balance, leading to increased plant resilience in the face of episodic stress (Guo et al 2018b).

Isoprene as a broad modulator of gene expression and pathway interactions

Much of the evidence for a broader cellular role for isoprene has come through the application of multi-omic observations of isoprene-altered phenotypes, using poplar (primarily the hybrid *Populus* x *canescens*), tobacco (*Nicotiana tabacum* L.) and *Arabidopsis thaliana*. Each species offers advantages, and together they provide insight across a range of phenotypes and growth forms reflecting different evolutionary histories. Poplar is a woody, IE species, providing an opportunity to study molecular and biochemical interactions when isoprene is present as a native trait. *Arabidopsis* and tobacco are herbaceous, NE species. While *Arabidopsis* follows a monocarpic annual life cycle, tobacco is a polycarpic perennial plant in its native or invasive forms (see Ren and Timko 2001; Jassbi et al. 2017), though it is often cultivated on an

annual rotation. The inclusion of *Arabidopsis* and tobacco as non-emitting species in the wild type, allowed us to study how the introduction of isoprene interacts with 'naïve' metabolism, similar to what happens following the initial evolutionary introduction of this trait into a population. In the *Arabidopsis* system we used transcriptomic results from: (1) a fumigation treatment intended to explore the direct, targeted role of isoprene as a signal, and (2) transgenic transfer of *ISPS* to explore the role of isoprene as an integrated, permanent component of the plant genotype. In the following sections, we consider patterns of cellular adjustment in key pathways and signaling networks in all three of these species focusing on those most relevant to the growth-defense tradeoff.

Isoprene and the shikimate/phenylpropanoid pathways

In early work, RNA interference (RNAi) was used to achieve translational inhibition of *ISPS* in poplar trees (Behnke et al. 2007). Transcriptomic and metabolomic analyses showed that RNAi silencing of *ISPS* also reduced gene expression in the shikimate and associated phenylpropanoid pathways, indicating a crucial role for isoprene as a positive regulator of pathway expression patterns (Behnke et al. 2010a). In a recent proteome study, also using RNAi poplar lines, growth in an experimental plantation in Arizona confirmed that transgenic suppression of *ISPS* caused reductions in multiple proteins associated with both pathways (Monson et al. 2020). When combined, these studies showed that at least fifteen genes/proteins in the shikimate and phenylpropanoid pathways were expressed at higher levels, and one protein was expressed at a lower level in IE poplar trees, compared to transgenic NE poplar (Fig. 1).

There is uncertainty about the significance of changes in one isoform of cinnamyl alcohol dehydrogenase (CAD9; Fig. 1), which participates in the final step in the synthesis of lignin monomers, and was present at 50% lower levels in IE trees, in the study of Monson et al. (2020).

The gene, *CAD9*, belongs to a family of 15 genes in poplar, and specifically to a sub-group of leaf *CAD* genes with primary roles in plant defense (Barakat et al. 2009). At this time, the potential advantage of 50% less CAD9 protein in the presence of isoprene, is not clear. One hypothesis is that it expression of the gene for this protein is suppressed in the presences of isoprene and that this adjustment balances resource flow from one type of less advantageous defense metabolite (e.g., lignins) to a different, more effective, defense metabolite (e.g., phenolic glycosides). Transcripts for a second member of the same gene family (*CAD1*) were observed to be higher in IE poplar, compared to NE poplar in the study of Behnke et al. (2010a). CAD1 is associated with monolignol production in xylem tissues, where it forms a multi-protein complex with cinnamoyl CoA-reductase (CCR) (Yan et al. 2019). In past studies using RNAi to minimize CCR in poplar, a 50% reduction in lignin production, along with CAD1, was observed (Li et al. 2014), and temperature-sensitive dwarfed growth has been observed in alfalfa above 30 °C in *CAD1* mutants (Zhao et al. 2013). Thus, we hypothesize that there is an upregulation of *CAD1* in the presence of isoprene, and that this might improve growth, especially in warm habitats or in the face of sustained high temperature stress.

Increases in phenylpropanoid pathway gene expression have also been observed in tobacco and *Arabidopsis* (Tattini et al. 2014; Zuo et al. 2019). In wild-type *Arabidopsis* exposed to isoprene (Harvey and Sharkey, 2016), expression of genes encoding the first six enzymes of the phenylpropanoid pathway, along with the later-pathway enzyme, flavonol 3-O-glucosyl transferase (3GT), were upregulated. The first step of the pathway is catalyzed by phenylalanine ammonia-lyase (PAL), which is a family of four isoforms coded by genes, *PAL1 – PAL4* (Raes et al. 2003). *PAL1* and *PAL2* encode the phenylpropanoid forms in *Arabidopsis* (Fraser and Chapelle 2011), and these are two of the forms with increased expression in *Arabidopsis* exposed

to isoprene, along with *PAL4* (Harvey and Sharkey 2016); *PAL1* expression was also increased in transformed IE tobacco (Zuo et al. 2019). In transformed IE tobacco and *Arabidopsis*, and wild-type *Arabidopsis* fumigated with isoprene, expression of *calcium dependent protein kinase 1* (*CPK1*) that phosphorylates and activates PAL protein, increased, while in the two *Arabidopsis* systems, the expression of the *Kelch repeat F-box 20 protein* (*KFB20*), which mediates PAL degradation, decreased (Harvey and Sharkey 2016; Zuo et al. 2019). Thus, there is strong validation of isoprene effects that enhance phenylpropanoid expression in species beyond poplar, indicating generality in this signaling system.

Isoprene and the terpenoid pathway

Isoprene is produced from dimethylallyl diphosphate (DMADP), a product of the methylerythritol 4-phosphate (MEP) pathway in chloroplasts. MEP pathway flux begins with the substrates glyceraldehyde 3-phosphate (GAP) and pyruvate (Pyr) from the reductive pentose phosphate (RPP) pathway (the photosynthetic Calvin-Benson cycle). The amount of DMADP available for isoprene production is not only sensitive to upstream flux through the MEP pathway, but also its downstream utilization for the production of higher terpenoids, such as the carotenoids, gibberellic acid, cytokinins, tocopherols, the phytyl tail of chlorophylls, abscisic acid and an array of monoterpenes and sesquiterpenes.

The presence of isoprene in cells controls metabolite flows through alternative branches of the terpenoid pathway, and there are clear interspecific differences. In IE poplar and tobacco, in contrast to the situation for the shikimate and phenylpropanoid pathways, the presence of isoprene caused reductions in multiple steps in the expression of terpene biosynthetic genes and proteins (Fig. 2; Monson et al. 2020; Zuo et al. 2019). In *Arabidopsis* wild-type plants exposed to

isoprene, increases, rather than decreases in transcript abundances were observed, especially concerning genes related to carotenoid biosynthesis.

One novel observation in poplar involves a set of genes encoding rubber elongation factors (*REF*s; see #1 in Figure 2). The abundance of two different REF proteins was decreased in the presence of isoprene (Monson et al. 2020). REF proteins are typically found in rubber-producing plants, such as rubber tree (*Hevea brasiliensis*) and guayule (*Parthenium argentatum*) (Lau et al. 2016). Rubber elongation is catalyzed by membrane-bound complexes containing *cis*-prenyltransferase (CPT) enzymes. At least five different *CPT* genes have been reported in *Populus trichocarpa*, in which they have roles in the synthesis of tetra- (C_{40}), di- (C_{20}) and sesquiterpenes (C_{15}). It is possible that the REF proteins detected in poplar are part of a larger family of proteins with prenyltransferase roles (i.e., lipid-droplet associated proteins, LDAPs; Gidda et al. 2013), including those associated with the cellular compartmentation and storage of isoprenoid compounds. In leaves, LDAPs may be involved with cellular energy balance, cellular signaling and plant stress responses (van der Schoot et al. 2011, Walther and Farese, 2012). Thus, in poplar, isoprene may contribute to coordination between photosynthetic capacity and lipid energy storage and/or lipid-based stress responses. The channeling of photosynthate to growth during periods of high photosynthetic capacity (and high rates of isoprene synthesis), but storage or stress tolerance during periods of low photosynthetic capacity (and low rates of isoprene synthesis), would be consistent with observations of *REF* gene suppression in the presence of isoprene. The situation appears to be different in wild-type *Arabidopsis*, as *REF* gene expression is increased in response to fumigation with isoprene (Harvey and Sharkey, 2016).

In IE poplar, we observed a reduction in the amount of protein for tocopherol cyclase (VTE1) (Monson et al. 2020; see #2 in Figure 2). The observation of reduced VTE1 levels is

292 consistent with past studies that showed reduced levels of the associated metabolite, α-
293 tocopherol, in IE poplar leaves (Behnke et al. 2009), and heat-stressed Holm oak (*Quercus ilex*)
294 leaves fumigated with isoprene (Peñuelas et al. 2005). α-tocopherol is an important antioxidant
295 that is active in reducing cellular ROS during physiological stress. In wild-type *Arabidopsis*
296 plants exposed to isoprene, *VTE1* gene transcripts were increased, as well as those for tocopherol
297 methyltransferase (*TMT*) (Harvey and Sharkey 2016), once again, showing the tendency for
298 interspecific differences in terpenoid pathway effects, especially between poplar and
299 *Arabidopsis*.

300 The proteins for z-carotene desaturase (ZDS1), carotene ε monooxygnase (CYP97C1), and
301 zeaxanthin epoxidase (ZEP), which catalyze key steps in the flow of GGPP toward carotenoid
302 and ABA biosynthesis, were at lower abundances in IE poplar lines, compared to NE lines
303 (Monson et al. 2020; see #3 in Fig. 2). These patterns were similar in the transcriptomic studies
304 in tobacco, in which transcript numbers for genes of several enzymes involved in the channeling
305 of GGPP to carotenoid synthesis were reduced, including those for phytoene synthase (*PSY*),
306 phytoene desaturase (*PSD*), z-carotene desaturase (*ZDS1*), carotenoid isomerase (*CRTISO*), β-
307 cyclase (*LCY1*) and zeaxanthin epoxidase (*ZEP*; Zuo et al. 2019). A few past studies on empty-
308 vector IE and transgenic NE poplar trees have shown variable results on leaf concentrations of
309 carotenoids and the non-stressed deepoxidation state. Behnke et al. (2010b) observed no
310 significant differences in carotenoid amount or epoxidation status in non-stressed IE and NE
311 poplars. However, in a different study, Behnke et al. (2009) observed reduced amounts of
312 zeaxanthin, but a higher deepoxidation ratio in IE poplars, which is consistent with the proteomic
313 analyses that show reduced expression in *ZDS1* (decreasing the pool of zeaxanthin) and *ZEP*
314 (increasing the deepoxidation ratio) (Fig. 2).

315 In contrast to the poplar and tobacco systems, transcripts of most carotenoid-related
316 enzymes were significantly increased in wild-type *Arabidopsis* exposed to isoprene (Harvey and
317 Sharkey 2016; Fig. 2). In addition to upregulation in many of the same proteins that were
318 described above, transcripts for the gene encoding β-carotene hydroxylase (*crtZ*) were increased
319 in wild-type *Arabidopsis* exposed to isoprene. The protein, crtZ, converts β-carotene to
320 zeaxanthin and can control the size of the xanthophyll cycle pool, and overexpression of *crtZ* in
321 *Arabidopsis* has been linked to high-light and high-temperature stress tolerance (Davison et al.
322 2002). Finally, expression of the gene for the enzyme violaxanthin deepoxidase (*VDE*), which
323 converts violaxanthin to zeaxanthin in the flexible, photoprotective part of the carotenoid cycle,
324 was increased in wild-type *Arabidopsis* exposed to isoprene (Harvey and Sharkey 2016; Zuo et
325 al. 2019).
326 A second component of the antioxidant system in plants, ascorbate, also appears to be
327 differentially expressed in response to isoprene in poplar versus *Arabidopsis*.
328 In poplar, the enzyme ascorbate oxidase (AAO), which leads to a lower ascorbate content in
329 leaves, is present at a 44% higher level in IE lines, compared to NE lines (Monson et al. 2020).
330 This observation is consistent with past observations showing lower ascorbate contents in non-
331 stressed IE poplar (Behnke et al 2009). In wild-type *Arabidopsis* exposed to isoprene (Harvey
332 and Sharkey 2016; Zuo et al. 2019), two genes for the protein GDP-galactose phosphorylase
333 (*VTC2* and *5*), which has been shown to be the only significant pathway in *Arabidopsis* that
334 produces ascorbate (Dowdle et al. 2007), exhibited increased expression.
335 In poplar, there were no isoprene-associated shifts in gene expression or protein contents
336 related to the synthesis of abscisic acid (ABA) (Behnke et al. 2010a; Monson et al. 2020).
337 However, in transformed *Arabidopsis* and wild-type *Arabidopsis* exposed to isoprene, expression

of the chloroplast gene that encodes an isoform of 9-cis-epoxycarotenoid dioxygenase (NCED), which converts violaxanthin to ABA, was upregulated (Fig. 2). Two isoforms, NCED3 and NCED5, have been shown to have important roles in plant growth and drought tolerance (Frey et al. 2012). In both *Arabidopsis* systems and transgenic tobacco, transcription of *ATAF1*, a key TF that positively regulates *NCED3* (Jensen et al. 2013), was also increased. This observation was of particular interest for transgenic tobacco, given that the general expression of carotenoid genes was downregulated by the introduction of isoprene in this species. The results suggest that isoprene-mediated upregulation of the ABA component of the carotenoid pathway is controlled independently from factors that control the remainder of the pathway.

Finally, in poplar, it was observed that isoprene synthesis reduces production of the enzyme that converts *cis*-zeatin, the low-activity form of cytokinin, to a stabilized, metabolically-inert pool (Monson et al. 2020). This action sustains a pool of *cis*-zeatin for isomerization to *trans*-zeatin, the most active form of cytokinin (see #4 in Fig. 2). Signaling through *trans*-zeatin has been implicated in controls over plant re-growth and shifts in carbohydrate source-sink balance that enhance photosynthetic capacity following partial plant defoliation (Roitsch and Ehneß 2000; Glanz-Idan et al. 2020).

There was a clear pattern of species differences in the effects of isoprene on gene expression in the terpenoid pathway. Poplar and tobacco tend to downregulate several pathway steps in the presence of isoprene, whereas *Arabidopsis* upregulates them (in both the fumigation and transgenic treatments). It is most likely that these interspecific differences are due to past selection for different adaptive priorities in plant responses to abiotic stress. In poplar and tobacco, selection to increase allocation to shikimate and phenylpropanoid production, at the expense of carotenoid production, might have occurred in response to high levels of herbivory

during past selection episodes. [Although tobacco, *Nicotiana tabacum* L., is cultivated on an annual rotation, it grows naturally as a perennial and was likely derived as a natural amphidiploid hybrid with genetic contributions from three perennial ancestors (Ren and Timko 2001). The native perennial nature of tobacco likely explains its tendency to allocate a relatively high amount of resource to the production of defensive metabolites.] In contrast, in *Arabidopsis*, an annual plant with relatively high growth rates and native affinities for open habitats with well-drained soils, photoprotection, administered through an effective antioxidant system, might have carried a higher selective value, at the expense of a well-provisioned chemical defense system. Given these pre-existing differences in phenotype, the introduction of isoprene, either through evolution as in the case of poplar, or through transgenic introduction as in tobacco and *Arabidopsis*, would be differentially integrated into existing signaling systems. Thus, the pre-existing metabolic phenotype might be as important as the properties of isoprene itself in determining its role as a signal modulator.

Isoprene and the oxylipin pathways

The oxylipin pathway is initiated in the chloroplast and produces C-6 aldehydes, alcohols and esters, known as green leaf volatiles (GLVs) (Hatanaka et al. 1987), and the jasmonic acid (JA) pathway precursor, 13(S)-hydroperoxy-octadecatrienoic acid (13-HPOT). Chloroplast-derived oxylipins are produced from C18-polyunsaturated fatty acids (Matsui 2006), which are freed from membranes by a family of phospholipases in response to herbivory, pathogen infection or abiotic stress (Ameye et al. 2018; Liu et al. 2020). Once freed, the fatty acids are oxidized by a family of lipoxygenase (LOX) enzymes, which control the channeling of oxylipins into several wound- and defense-associated pathways, some of which involve other organelles, such as peroxisomes (Feussner and Wasternack 2002; Koo 2018).

384 The presence of isoprene increases expression in several *LOX* genes, and thus the potential
385 for GLV production, in all of the experimental systems used in this analysis (Behnke et al.
386 2010a; Harvey and Sharkey, 2016; Zuo et al. 2019; Monson et al. 2020) (Fig. 3). Beyond this
387 initial step, however, isoprene effects on oxylipin processes among species begin to diverge,
388 especially with respect to JA signaling.
389 The active form of JA occurs when it forms a macromolecular complex with the amino
390 acid, isoleucine (Ile). A principal receptor of the JA-Ile conjugate is the Coronatine Insensitive 1
391 (COI1) F-box protein, which is part of an E3 ubiquitin ligase complex. When JA-Ile reaches a
392 threshold level, COI1 associates with JA-Ile to form a multi-protein transcriptional modifier
393 (Ruan et l. 2019). The JA-Ile-COI1 complex targets a large family of proteins known as
394 Jasmonate ZIM (JAZ), which generally function as negative transcriptional regulators (Fig. 3). In
395 the absence of JA-Ile-COI1, JAZ proteins bind to TFs with a high degree of specificity, blocking
396 their ability to participate in the formation of transcription initiation complexes. The COI1-JAZ
397 interaction triggers ubiquitin-dependent degradation of the JAZ proteins, freeing the TFs and
398 activating transcription.
399 From the past studies in *Arabidopsis*, there are antagonistic interactions involving proteins
400 in the JAZ and DELLA families, which are associated with the JA and gibberellic acid (GA)
401 pathways, respectively. JA synthesis leads to an enhancement in DELLA transcription and
402 degradation of JAZ proteins, which leads to decreased growth and increased defense,
403 respectively – in essence, providing a molecular context for the growth-defense tradeoff; in this
404 case, favoring defense (Wild et al. 2012; Campos et al. 2016). GA synthesis leads to degradation
405 of DELLA proteins and an enhancement of GA-activator signaling, which enhances growth and,
406 at the same time, increases the number of free JAZ proteins, which suppress JA-associated

407 defense signaling (Campos et al. 2016). Once again, these molecular interactions enable the
408 growth-defense tradeoff; but, in this case, favoring growth. Antagonism between JA and GA
409 signaling in determining the growth-defense tradeoff is also influenced by extrinsic
410 environmental cues involving spectral shifts in incident light (Ballarè 2014), and it can be
411 uncoupled by phytochrome b gene mutations (Campos et al. 2016).
412 The expression of three genes important for JA synthesis were increased by isoprene in
413 *Arabidopsis* and tobacco. For example, in addition to LOX, 12-oxo-phytodienoic acid reductase
414 (*OPR*) was enhanced in transgenic IE *Arabidopsis* and IE tobacco, while 3-oxo-2-(2'-[Z]-
415 pentenyl) cyclopentane-1-octanoate CoA ligase (*OPC*-8:CoA ligase) increased in IE tobacco. In
416 addition, expression of the gene for MYB59 transcription factor was reduced in both the
417 fumigated and transgenic *Arabidopsis* systems in response to isoprene (Harvey and Sharkey
418 2016; Zuo et al. 2019). MYB59 suppresses expression of certain genes important for oxylipin
419 biosynthesis (e.g., the gene for allene oxide synthase, *CYP74A,* and *OPR3*), as well as the gene
420 for the MYC2 TF that regulates JA signaling (Boter et al. 2004). Expression of the gene for
421 jasmonate O-methyltransferase (*JMT1*) was increased in wild-type *Arabidopsis* exposed to
422 isoprene. JMT catalyzes the reaction leading to methyl jasmonate (Me-JA), a volatile
423 hydrocarbon that can function as a long distance chemical signal with significant effects on JA
424 related gene expression (Benevenuto et al. 2019). Both Me-JA and isoprene have been shown to
425 be produced in response to wounding (Loreto and Sharkey 1993; Lantz et al. 2019; Benevenuto
426 et al. 2019); a response that seemed out of place with the past literature emphasizing a role for
427 isoprene in abiotic stress tolerance, but that now makes more sense within the context of a multi-
428 pathway signaling network. In addition to an isoprene-induced increase in *JMT1* expression in
429 wild-type *Arabidopsis*, two negative regulators of *JMT1* expression (*BBD1* and *BBD2*; Seo et al.

2013) exhibited reduced transcript levels in transgenic IE *Arabidopsis* and wild-type *Arabidopsis* exposed to isoprene (Zuo et al. 2019). In IE tobacco, there was evidence for reduced jasmonate-amido synthase (*JAR1*), expression which catalyzes the synthesis of JA-Ile. Thus, based on transcriptomic data, it seems that isoprene favors production of volatile Me-JA over JA-Ile, at least in *Arabidopsis* and tobacco. We are just beginning to sort the ways that isoprene interacts with JA and its derivatives in determining growth-defense tradeoffs; though, it is clear that much of the insight concerning molecular control over the tradeoff is to be found in JA and GA pathway interactions (see Züst and Agrawal 2017).

Unlike the case for IE tobacco, in IE poplar lines Monson *et al*. (2020) observed a 40% decrease of the JA pathway protein, 3-oxo-2-(2'-[Z]-pentenyl) cyclopentane-1-octanoate CoA ligase (OPC-8:CoA ligase), compared to the transgenic NE lines (Fig. 3). Such a large downregulation of this 'gatekeeper' enzyme would likely lead to substantial decreases in the capacity for JA signaling. In poplar, there might be advantages to suppressing JA signaling in the presence of isoprene. Two of the JA signaling processes that are activated when JAZ proteins are degraded involve upregulation of the phenylpropanoid pathway (Zhou et al. 2017) and downregulation of the salicylic acid (SA) pathway (Campos et al. 2014). In poplar, suppression of JA-Ile formation might provide a means to sustain JAZ-mediated suppression of phenylpropanoid biosynthesis, thus avoiding conflicts between JA- and isoprene-regulated controls in the same pathway. The ability to isolate control over phenylpropanoid biosynthesis from the several other growth and defense interactions affected by JA signaling might also be especially important for poplar. For example, SA signaling has an important role in plant defense that is distinct from, and in several cases antagonistic to, JA signaling (Durrant and Dong 2004). A suppression of JA signaling allows for sustained JAZ-mediated suppression of those TFs that

might otherwise negatively influence SA signaling (Fig. 3). Sustained SA activity might be an important source of defense signaling in poplars, especially concerning the production of phenylpropanoid-associated compounds, such as proanthocyanidins (condensed tannins) and their monomeric catechin constituents (Ullah et al. 2019).

In closing this section, it is also worth noting the potential interactions between isoprene and oxylipin synthesis and signaling in nature, especially involving signals that affect MEP pathway activity and, ultimately, the production of isoprene itself. It has been reported that transcription of 1-deoxy-D-xylulose 5-phosphate synthase 2 (*DXS*), of the MEP pathway, increases in response to exogenous JA and exposure to mechanical stress or wounding (Tretner et al. 2008). A second study showed an increase in *DXS* expression, but a decrease in *ISPS* expression and isoprene emission, in *Ficus septica* treated with JA (Parveen et al. 2019b). Collectively, we can assume that isoprene and JA levels are modulated in native emitters (or when plants are engineered to emit isoprene) to optimize responses to herbivory and other stresses. Enhancement of *DXS* expression and suppression of *ISPS* expression by JA likely enables MEP pathway metabolites to be diverted from isoprene production and channeled into the synthesis of other MEP pathway-derived secondary metabolites, many of which are involved in defense roles (e.g., the higher-order terpenes). Parveen et al. (2019b) also found cis-elements on the *Ficus septica ISPS* promotor that makes it responsive to regulation by MYC2, a TF protein under the control of Me-JA synthesis. This establishes the condition for JA-associated feedback to isoprene signaling, and represents an example of potential crosstalk between JA and isoprene pathway signaling. A recent analysis of sequence motifs in the promoter of *ISPS* from poplar revealed several elements that are likely responsive to multiple signaling pathways,

including those associated with Me-JA, gibberellins, auxins, SA and ABA (see Fig. S3 in Miloradovic van Doorn et al. 2020).

Isoprene as a mediator of the growth-defense tradeoff in the face of climate stress

Given the several studies that show isoprene effects on gene expression and cellular signaling, there is need for a theory that provides a broader adaptive scope for the trait. The multiple interactions among signaling pathways are complex and variable across species, which make it difficult to identify the primary costs and benefits that have shaped the trait. However, there are some clear influences that lead us to a starting point. In every species and experimental system examined to date, the presence of isoprene causes large, positive effects on gene expression in the shikimate and phenylpropanoid pathways. Phenolic compounds and phenylpropanoids serve multiple adaptive roles in plants, including those associated with growth (e.g., auxins), defense (alkaloids, salicylic acid, phenolic glycosides, tannins, flavonols), and abiotic stress tolerance (anthocyanins, flavonoids). The universal trend toward isoprene-induced upregulation of shikimate and phenylpropanoid production, shows the central importance of these pathways to integrated, multi-trait adaptive responses that drive the growth-defense tradeoff within the constraint of abiotic stress tolerance.

While there are clear interspecific similarities in the way that isoprene influences expression in the shikimate and phenylpropanoid pathways, there are differences in its role within the terpenoid and JA pathways. In a comparative analysis of isoprene-associated gene expression in *Arabidopsis* and tobacco, Zuo et al. (2019) concluded that isoprene shifts expression in favor of defense-associated metabolites in tobacco, but growth-associated metabolites in *Arabidopsis*. In this study, we noted that poplar was observed to downregulate the potential for carotenoid and α-tocopherol synthesis, as well as the prenyl-transferase activity

498 associated with REF proteins, while in *Arabidopsis* expression for all three were increased.
499 These differences are likely to reflect the different selection regimes experienced by the species.
500 Interestingly, *Arabidopsis* and tobacco were capable of responding to the introduction of
501 isoprene, despite no past history of selection for phenotypes expressing the trait. This indicates
502 that isoprene is a trait that, once evolved, can rapidly become incorporated into naïve
503 phenotypes. Many of the regulatory sequence motifs in the *ISPS* promoter of IE poplar are
504 shared with the promoter of *1,8-cineole synthase* (*AtTPS-Cin*) from NE *Arabidopsis*, the gene for
505 a terpene synthase that is closely-related to *ISPS* (see Fig. S3 in Miloradovic van Doorn et al.
506 2020). These include regulatory elements that respond to ABA, MeJA, SA and binding with the
507 MYB transcription factor family. Thus, at least one terpene synthase, unrelated to isoprene
508 biosynthesis, exists in *Arabidopsis* with pre-existing metabolic connections to several cellular
509 signaling pathways. These factors might facilitate rapid (generationally-speaking) directional
510 selection during the evolution of isoprene signaling within naïve lineages, and provide high
511 selective value to the trait early during its appearance.
512 The adaptive value of isoprene is likely also linked to its association with photosynthesis.
513 From the earliest days of isoprene research, it has been recognized that variances in emission rate
514 and photosynthesis are correlated across many determining variables (Sanadze et al. 1972;
515 Rasmussen and Jones 1973; Tingey et al. 1979). Subsequent research showed numerous
516 biochemical dependencies of isoprene synthesis on photosynthate and photoreductant (Sharkey
517 and Yeh 2001; Sharkey and Monson 2017; Sharkey et al. 2020). These dependencies provided
518 explanations for: (1) the positive correlations of isoprene emission and photosynthetic capacity
519 (Monson and Fall 1989; Loreto and Sharkey 1990), (2) the role of increased resources, such as
520 nitrogen, in supporting higher isoprene emission rates (Harley et al. 1994; Litvak et al. 1996;

521 Fernandez-Martinez et al. 2018), and (3) the observations that within a phylogenetic clade,
522 isoprene emissions are found in species with higher photosynthetic capacities, higher growth
523 rates, and niche affinities that favor sunny habitats (Harley et al. 1999; Dani et al. 2014; Loreto
524 and Fineschi 2015). The many metabolic and ecological associations between isoprene and
525 photosynthesis likely elevate the selection differential for isoprene. (Selection differential is the
526 difference between the average value to fitness of a quantitative trait in an entire population and
527 the average value in those individuals selected to reproduce and form the next generation). With
528 the new insight into isoprene's role in regulating gene expression, we can expand the scope of
529 isoprene's trait value to include plant defense. Metabolically, isoprene sits in a position wedged
530 between the processes that determine the supply of photosynthetic substrates and the demand of
531 secondary metabolite products – the two economic determinants underlying most contemporary
532 growth-defense theories.

533 Relying on these concepts, we offer a new formal theory for the adaptive role of isoprene
534 emission (Fig. 4). Within the context of traditional theories (e.g., the GDBH), the growth-defense
535 tradeoff can be represented as a negative correlation constrained at an upper limit by
536 photosynthetic capacity (e.g., Züst and Argawal 2017). The upper limit is reduced in the face of
537 stress, providing the realized limit for operation of the tradeoff (shown as the dashed line in Fig.
538 4). The observed tradeoff for non-isoprene emitting plants (NE) must occur within the space
539 below the realized limit. We propose that the evolution of isoprene emission (IE) will provide the
540 advantage of better tolerating the stresses that determine the realized limit, allowing plants to
541 accommodate allocations to defense with less cost to growth.

542 This view of isoprene emissions is compatible with previous observations and theories
543 proposing a positive effect on abiotic stress tolerance (e.g., Loreto and Schnitzler 2010). It is also

compatible with past reports that isoprene protects photosynthesis against the impact of oxidative stress (e.g., Agati et al. 2013). The new theory that we offer only diverges from past perspectives in positioning the adaptive role of isoprene as a direct contributor to multiple signaling networks, affecting broad patterns of gene expression, rather than the role of isoprene as a single and isolated actor. Further, our theory expands the role of isoprene from one of stress tolerance, alone, to one of stress tolerance within the context of the growth-defense tradeoff.

The theory that we propose is also consistent with the perspective that the G-D tradeoff is dependent on the overall amount of resource available for G-D support (van Noordwijk and de Jong 1986; Agrawal 2020). The dependency of the G-D tradeoff, along with life-history tradeoffs, on the total amount of available resource has been explained by the simple fact that as organisms obtain more resources, either through changes in environmental conditions or the evolution of novel traits, they often allocate more resource to both G and D (van Noordwijk and de Jong 1986; Houle et al. 1991; Agrawal et al. 2010). From our theory, the evolution of isoprene emission, and its positive influence on the expression of selected defense compounds that also carry advantages in abiotic stress tolerance, will facilitate greater amounts of photosynthate that will be available for allocation to both G and D, compared to non-emitting phenotypes. This would drive positive selection for the trait in certain environments.

The mechanism(s) of isoprene in cellular signaling networks

Isoprene is relatively insoluble in water, and its solubility in lipids, while higher than that in water, is less than often assumed (Appendix 2 in Harvey et al. 2015). As a result, isoprene exists predominantly in the gas phase of the leaf and is quickly lost through diffusion. Isoprene's high volatility is difficult to reconcile with conventional cellular signaling mechanisms. Other signaling volatiles, such as Me-JA are converted to an active and relatively soluble form by

567 conjugation; for example, with isoleucine. To date, no conjugates involving isoprene have been
568 identified. The hydrophobic nature of isoprene, however, may confer signaling advantages,
569 including its ability to cross membranes and influence pathways that are distributed across
570 multiple organelles, and to access signaling components in the hydrophobic domain of
571 membrane lipid bilayers.

572 Conventional signal-receptor interactions are based on macromolecular shape
573 complementarity (Covarrubias et al. 2020). In the case of the plant immune signal, salicylic acid
574 (SA), SA-protein binding interactions, involving a set of cooperating transcriptional activators
575 (NPR1) and repressors (NPR3 and NPR4), occurs in a conventional manner and controls SA
576 signal reception (Wu et al. 2012; Ding et al. 2018). However, redox-driven interactions are also
577 common, especially in signaling networks based on reactive oxygen (ROS) and nitrogen species
578 (RNS) (Nathan 2003; Shetty et al. 2008; Vickers et al. 2009a). For ROS, signaling occurs
579 through the oxidation of key amino acids, such as cysteine, on the surface of transcription-
580 modifier proteins, leading to direct control over gene expression (Neill et al. 2002). In the case of
581 RNS, protein activities are modified by post-translational modification (PTM) involving nitric
582 oxide (NO), a process known as S-nitrosylation (Spadaro et al. 2010). S-nitrosylation most
583 commonly reduces enzyme activities in plants, leading to reduced rates of metabolite production
584 (Lindermayr et al. 2005). S-nitrosylation is known to regulate inducible responses to abiotic
585 stress (Corpas et al. 2011; Vanzo et al. 2014; Begara-Morales et al. 2018), and past studies in
586 poplar have shown that isoprene (through an unknown mechanism) modulates the H_2O_2-
587 dependent translational cascade and NO-dependent post-translational cascade (Vanzo et al
588 2016). Several past theories concerning the adaptive functions of isoprene have posited that
589 isoprene's potential role as an antioxidant molecule provides a mechanism for direct chemical

590 reactions that reduce ROS or RNS to a level that causes pathway modulation (Velikova et al.
591 2005; 2008; Vickers et al. 2009a; Behnke et al. 2010a). Such past theories of isoprene directly
592 affecting cellular redox signaling through its role as an antioxidant are too narrow to account for
593 the broad set of influences and diverse set of pathways indicated in the recent multi-omic
594 analyses.

595 Furthermore, there are reasons to question the feasibility of isoprene acting as an effective
596 antioxidant metabolite, even separate from its potential to directly trigger ROS and RNS
597 networks. Given that the concentration of isoprene is about 60 molecules per million (lipid
598 molecules), which is much lower than the cellular concentration of known antioxidant molecules
599 such as carotenoids (Harvey et al. 2015), it does not follow logically that isoprene can increase
600 the antioxidant margin of cellular protection to a significant advantage. Isoprene only contributes
601 ~0.1% the double bonds available for nucleophilic reactions, compared to carotenoids (Harvey et
602 al. 2015); and, while some past reports have included observations of isoprene's oxidation
603 products, such as methyl vinyl ketone and methacrolein, as leaf volatiles (Jardine et al. 2012),
604 these observations have not been widely confirmed. From these perspectives, isoprene does not
605 appear to be a plausible candidate to provide effective antioxidant protection, or to act as an
606 efficient redox signal compound, at least not with respect to direct alterations of ROS and RNS
607 concentrations. Harvey and Sharkey (2016) hypothesized that in *Arabidopsis* the presence of
608 isoprene causes an upregulation of key TFs involved in phenylpropanoid and carotenoid pathway
609 expression which, in turn, alter cellular ROS and RNS concentrations, as well as activating other
610 signal cascades. This perspective leads to the hypothesis of an indirect role between isoprene and
611 cellular oxidants (Fig. 5). The recent discovery of isoprene effects in roots, especially in the
612 vascular tissues, where ISPS is present but expressed at very low levels, supports a role for

613 indirect (through signaling) involvement of isoprene in ROS signaling (Miloradovic van Doorn
614 et al. 2020). The transgenic (RNAi) suppression of *ISPS* expression, even at the very low levels
615 present in roots, affected lateral root development in a manner consistent with ROS
616 accumulation. Furthermore, expression of *ISPS* in roots was increased in the presence of auxin,
617 suggesting a complex interaction with developmental processes; well beyond the more
618 commonly cited context of antioxidant activity. These observations are most compatible with the
619 model of isoprene acting as a signal molecule, not an antioxidant. The studies by Harvey and
620 Sharkey (2016) and Miloradovic van Doorn et al. (2020) provide good reason to recast the
621 discussion of isoprene effects, away from single molecule redox reactions, and toward one of
622 broad interactions involving numerous signaling pathways.

623 At this time, it is not clear as to how the dependency of isoprene emissions on diurnal and
624 seasonal environmental change fits into its role as a signaling metabolite. In some ways, isoprene
625 is similar to an inducible defense trait – its expression is promoted by high temperature, high
626 light, mechanical wounding and high nitrogen availability (Harley et al. 1999; Sharkey et al.
627 2008). Observations have also revealed higher emission rates during drought recovery periods
628 (Fortunati et al. 2008; Tattini et al. 2015; Velikova et al. 2016; 2018), and in the isoprene-
629 emitting species *Arundo donax* the increased emission rates were accompanied by increased
630 production of selected phenylpropanoid compounds (Velikova et al. 2016; Ahrar et al. 2017).
631 Generally, the seasonal environments that promote isoprene emission also provide reliable cues
632 of herbivory risk – warm, high light, mid-summer weather with high soil fertility, and/or
633 following the stressful conditions of a drought. The work by Tattini et al. (2015) showed that
634 metabolic adjustments that promote tolerance of abiotic stress extremes can also occur on the
635 scale of hours, and are timed for midday stress extremes; including isoprene emission. Thus, like

636 the cues for inducible defenses, high isoprene emission rates could condition plants to anticipate
637 future episodes of combined abiotic and biotic stress on quite short time scales, and thus provide
638 an effective form of phenotypic plasticity.

639 **Conclusions**

640 The explanation of the effects of isoprene on plants proposed here represents a major shift
641 in our thinking away from direct effects of isoprene, for example, by changing membrane
642 properties or quenching ROS, to indirect effects, through changes in gene expression and protein
643 abundances. The presence of isoprene affects a number of transcription factors important in
644 signaling processes involved in shikimate, phenylpropanoid, terpenoid and oxylipin synthesis,
645 and in the production of numerous compounds involved in plant growth, defense, and abiotic
646 stress tolerance. This suggests that isoprene can affect the outcome of several regulatory
647 cascades. We presume that in some conditions the altered regulatory landscape must be
648 deleterious, in order to account for the frequent losses of the isoprene emission trait. The
649 research challenges that lie ahead include developing an improved context for how the multiple
650 effects of isoprene are adjusted in the face of interactive influences involving growth, herbivory
651 and threats from extreme climate stress, to optimize fitness. Improved knowledge of how
652 environmental variation influences the processes that control trait tradeoffs, including those
653 influenced by isoprene, will not only lead to more accurate ecological theories concerning plant
654 adaptation, but will also facilitate better strategies for the development of sustainable agriculture.
655 With the rapidly expanding opportunities for genetic modification using CRISPR-Cas9
656 technology, the possibility exists to develop finely-targeted strategies to mediate the growth-
657 defense tradeoff to simultaneously improve yield through the combined effects of higher rates of

658 biomass increase and lower rates of biomass loss. Isoprene might have an important role in the
659 design of such strategies.

660

661 **Acknowledgements** RKM is thankful for the helpful discussions with Professor Manuel Lerdau
662 (University of Virginia) on several concepts concerning cellular signaling and plant defense
663 theory that are presented in the manuscript. RKM acknowledges support from the Ecosystems
664 Program in the Division of Environmental Biology (NSF Award 1754430) and TDS
665 acknowledges support from the IOS-Integrative Ecological Physiology program (NSF award
666 2022495).

667

668 **Data accessibility** This is a synthesis study such that all data was drawn from the existing
669 publications cited in the text; including electronic supplementary materials.

670

671

672 **References**

673 Affek HP, Yakir D (2002). Protection by isoprene against singlet oxygen in leaves. Plant Physiol
674 129:269–277. https://doi.org/10.1104/pp.010909

675 Agati G, Brunetti C, DiFerdinando M, et al. (2013) Functional roles of flavonoids in photo-
676 protection: new evidence, lessons from the past. Plant Physiol Biochem 72:35–45.
677 https://doi.org/10.1016/j.plaphy.2013.03.014

678 Agrawal AA (2020). A scale-dependent framework for trade-offs, syndromes, and specialization
679 in organismal biology. Ecology 101:e02924. https://doi.org/10.1002/ecy.2924

680 Ameye M, Allmann S, Verwaeren J, et al. (2018) Green leaf volatile production by plants: a
681 meta-analysis. New Phytol 220:666-683. https://doi.org/10.1111/nph.14671

682 Ahrar M, Doneva D, Tattini M, Brunetti C, et al. (2017) Phenotypic differences determine
683 drought stress responses in ecotypes of *Arundo donax* adapted to different environments. J Exp
684 Bot 68:2439-2451. https://doi.org/10.1093/jxb/erx125

685 Ballare CL (2014) Light regulation of plant defense. Ann Rev Plant Biol 65:335-363.
686 https://doi.org/10.1146/annurev-arplant-050213-040145

687 Barakat A, Bagniewska-Zadworna A, Choi A, et al. (2009) The cinnamyl alcohol dehydrogenase
688 gene family in *Populus*: phylogeny, organization, and expression. BMC Plant Biol 9:26.
689 https://doi.org/10.1186/1471-2229-9-26

690 Begara-Morales JC, Chaki M, Valderrama R, et al. (2018) Nitric oxide buffering and conditional
691 nitric oxide release in stress response. J Exp Bot 69:3425-3438.
692 https://doi.org/10.1093/jxb/ery072

693 Behnke K, Ehlting B, Teuber M, et al. (2007). Transgenic, non-isoprene emitting poplars don't
694 like it hot. Plant J 51:485–499. https://doi.org/10.1111/j.1365-313X.2007.03157.x

695 Behnke K, Kleist E, Uerlings R, et al. (2009) RNAi mediated suppression of isoprene
696 biosynthesis impacts ozone tolerance. Tree Physiol 29:725–736.
697 https://doi.org/10.1093/treephys/tpp009

698 Behnke K, Kaiser A, Zimmer I, et al. (2010a) RNAi-mediated suppression of isoprene emission
699 in poplar transiently impacts phenolic metabolism under high temperature and high light
700 intensities: a transcriptomic and metabolomic analysis. Plant Mol Biol 74: 61-75.
701 https://doi.org/10.1007/s11103-010-9654-z
702 Behnke K. Loivamaki M, Zimmer I, et al. (2010b) Isoprene emission protects photosynthesis in
703 sunfleck exposed grey poplar. Photosyn Res 104:5–17. https://doi.org/10.1007/s11120-010-
704 9528-x

705 Benevenuto RF, Seldal T, Hegland SJ, et al. (2019) Transcriptional profiling of methyl
706 jasmonate-induced defense responses in bilberry (*Vaccinium myrtillus* L.). BMC Plant Biol
707 19:70. https://doi.org/10.1186/s12870-019-1650-0

708 Berenbaum MR (1995) The chemistry of defense – theory and practice. Proc Natl Acad Sci
709 (USA) 92:2-8. https://doi.org/10.1073/pnas.92.1.2

710 Bloom AJ, Chapin FS, Mooney HA (1985) Resource limitation in plants—an economic analogy.
711 Ann Rev Ecol Syst 16:363–392. https://doi.org/10.1146/annurev.es.16.110185.002051

712 Boter M, Ruíz-Rivero O, Abdeen A, Prat S (2004) Conserved MYC transcription factors play a
713 key role in jasmonate signaling both in tomato and *Arabidopsis*. Genes Dev 18:1577-1591.
714 https://doi.org/10.1101/gad.297704

715 Brunetti C, Guidi L, Sebastiani F, Tattini M (2015) Isoprenoids and phenylpropanoids are key
716 components of the antioxidant defense system of plants facing severe excess light stress. Environ
717 Exp Bot 119:54-62. https://doi.org/10.1016/j.envexpbot.2015.04.007

718 Bryant, JP, Chapin FS III, Klein DR (1983) Carbon nutrient balance of boreal plants in relation
719 to vertebrate herbivory. Oikos 40:357-368. https://doi.org/10.2307/3544308

720 Campos ML Yoshida, Y Major, IT et al. (2016) Rewiring of jasmonate and phytochrome B
721 signalling uncouples plant growth-defense tradeoffs. Nature Comm 7:12570.
722 https://doi.org/10.1038/ncomms12570

723 Chapin FS III (1980) The mineral nutrition of wild plants. Ann Rev Ecol Sys 11:233–260.
724 https://doi.org/10.1146/annurev.es.11.110180.001313

725 Coley PD, Bryant JP, Chapin, FS (1985) Resource availability and plant antiherbivore defense.
726 Science 230:895-899. https://doi.org/10.1126/science.230.4728.895

727 Corpas FJ, Leterrier M, Valderrama R, et al. (2011) Nitric oxide imbalance provokes a
728 nitrosative response in plants under abiotic stress. Plant Sci 181:604–611.
729 https://doi.org/10.1016/j.plantsci.2011.04.005

730 Covarrubias, AA, Romero-Perez, PS, Cuevas-Velazquez, CL, Rendon-Luna, DF (2020) The
731 functional diversity of structural disorder in plant proteins. Arch Biochem Biophys 680:108229.
732 https://doi.org/10.1016/j.abb.2019.108229

733 D'Auria JC, Gershenzon J (2005) The secondary metabolism of *Arabidopsis thaliana*: growing
734 like a weed. Curr Opinion Plant Biol 8:308-316. https://doi.org/10.1016/j.pbi.2005.03.012

735 Dani KG, Jamie IM, Prentice IC, Atwell BJ (2014). Evolution of isoprene emission capacity in
736 plants. Trends Plant Sci 19:439–446. https://doi.org/10.1016/j.tplants.2014.01.009

737 Davison PA, Hunter CN, Horton P (2002) Overexpression of beta-carotene hydroxylase
738 enhances stress tolerance in *Arabidopsis*. Nature 418:203-206.
739 https://doi.org/10.1038/nature00861

740 Ding Y, Sun T, Ao K, Peng Y, et al. (2018) Opposite roles of salicylic acid receptors NPR1 and
741 NPR3/NPR4 in transcriptional regulation of plant immunity. Cell 173:1454-1467.
742 https://doi.org/10.1016/j.cell.2018.03.044

743 Dowdle J, Ishikawa T, Gatzek S, Rolinski S, Smirnoff N (2007) Two genes in *Arabidopsis*
744 *thaliana* encoding GDP-l-galactose phosphorylase are required for ascorbate biosynthesis and
745 seedling viability. Plant J 52:673–689. https://doi.org/10.1111/j.1365-313X.2007.03266.x

746 Durrant WE, Dong X (2004) Systemic acquired resistance. Ann Rev Phytopath 42:185-209.
747 https://doi.org/10.1146/annurev.phyto.42.040803.140421

748 Endara MJ, Coley PD (2011) The resource availability hypothesis revisited: a meta-analysis.
749 Functional Ecology 25:389-398. https://doi.org/10.1111/j.1365-2435.2010.01803.x

750 Fernandez-Martinez M, Llusia J, Filella I, et al. (2018) Nutrient-rich plants emit a less intense
751 blend of volatile isoprenoids. New Phytol 220:773-784. https://doi.org/10.1111/nph.14889

752 Feussner I, Wasternack C (2002) The lipoxygenase pathway. Annu Rev Plant Biol 53:275-297.
753 https://doi.org/10.1146/annurev.arplant.53.100301.135248

754 Fini A, Brunetti C, Loreto F, et al. (2017) Isoprene responses and functions in plants challenged
755 by environmental pressures associated to climate change. Front Plant Sci 8:e1281
756 https://doi.org/10.1146/10.3389/fpls.2017.01281

757 Fortunati, A; Barta, C; Brilli, F; Centritto, M (2008) Isoprene emission is not temperature-
758 dependent during and after severe drought-stress: a physiological and biochemical analysis. Plant
759 J 55:687-697. https://doi.org/10.1111/j.1365-313X.2008.03538.x

760 Fraser CM, Chapelle C (2011) The phenylpropanoid pathway in *Arabidopsis*. Arabidopsis Book
761 9:e0152. https://doi.org/10.1199/tab.0152

762 Frey A, Effroy D, Lefebvre V, et al. (2012) Epoxycarotenoid cleavage by NCED5 fine-tunes
763 ABA accumulation and affects seed dormancy and drought tolerance with other NCED family
764 members. Plant J 70:501-512. https://doi.org/10.1111/j.1365-313X.2011.04887.x

765 Gidda SK, Watt SC, Collins-Silva J, et al. (2013) Lipid droplet-associated proteins (LDAPs) are
766 involved in the compartmentalization of lipophilic compounds in plant cells. Plant Signal Behav
767 8:e27141. https://doi.org/10.4161/psb.27141

768 Glanz-Idan N, Tarkowski P, Tureckova V, Wolf S (2020) Root-shoot communication in tomato
769 plants: cytokinin as a signal molecule modulating leaf photosynthetic activity. J Exp Bot 71:247-
770 257. https://doi.org/10.1093/jxb/erz399

771 Guo Q, Major IT, Howe GA (2018a) Resolution of growth–defense conflict: mechanistic
772 insights from jasmonate signaling. Curr Opin Plant Biol 44:72-81.
773 https://doi.org/10.1016/j.pbi.2018.02.009

774 Guo Q, Yoshida, Y, Major, IT, et al. (2018b) JAZ repressors of metabolic defense promote
775 growth and reproductive fitness in Arabidopsis. Proc Natl Acad Sci (USA) 115:10768-10777.
776 https://doi.org/10.1073/pnas.1811828115

777 Hanson DT, Swanson S, Graham LE, Sharkey TD (1999) Evolutionary significance of isoprene
778 emission from mosses. Amer J Bot 86:634-639. https://doi.org/10.2307/2656571

779 Harley PC, Litvak M, Sharkey T, Monson RK (1994) Isoprene emission from velvet bean leaves.
780 The influences of growth light intensity, nitrogen availability, and leaf development. Plant
781 Physiol 105:279-285. https://doi.org/10.1104/pp.105.1.279

782 Harley PC, Monson RK, Lerdau MT (1999) Ecological and evolutionary aspects of isoprene
783 emission from plants. Oecologia 118:109-123. https://doi.org/10.1007/s004420050709

784 Harvey CM, Sharkey TD (2016) Exogenous isoprene modulates gene expression in unstressed
785 Arabidopsis thaliana plants. Plant, Cell Env 39:1251-1263. https://doi.org/10.1111/pce.12660

786 Harvey CM, Li Z, Tjellström H, Blanchard GJ, Sharkey TD (2015). Concentration of isoprene in
787 artificial and thylakoid membranes. J Bioenerg Biomem 47:419–429.
788 https://doi.org/10.1007/s10863-015-9625-9

789 Hatanaka A, Kajiwara T, Sekiya J (1987) Biosynthetic pathway for C6-aldehydes formation
790 from linolenic acid in green leaves. Chem Phys Lipids 44:341–361.
791 https://doi.org/10.1016/0009-3084(87)90057-0

792 Herms DA, Mattson WJ (1992) The dilemma of plants – to grow or defend. Quart Rev Biol
793 67:283-335. https://doi.org/10.1086/417659

794 Houle D (1991) Genetic covariance of fitness correlates – What genetic correlations are made of
795 and why it matters. Evolution 45:630–648. https://doi.org/10.2307/2409916

796 Howe GA, Major IT, Koo AJ (2018) Modularity in jasmonate signaling for multistress
797 resilience. Annu Rev Plant Biol 69:387–415. https://doi.org/10.1146/annurev-arplant-042817-
798 040047

799 Jardine KJ, Monson RK, Abrell L, et al. (2012) Within-plant isoprene oxidation confirmed by
800 direct emissions of oxidation products methyl vinyl ketone and methacrolein. Glob Change Biol
801 18:973-984. https://doi.org/10.1111/j.1365-2486.2011.02610.x

802 Jassbi AR, Zare S, Asadollahi M, Schuman MC (2017) Ecological roles and biological activities
803 of specialized metabolites from the genus *Nicotiana*. Chem Rev 117: 12227-12280.
804 https://doi.org/10.1021/acs.chemrev.7b00001

805 Jensen MK, Lindemose S, de Masi F, et al. (2013) ATAF1 transcription factor directly regulates
806 abscisic acid biosynthetic gene NCED3 in Arabidopsis thaliana. FEBS Open Bio 3:321-327.
807 https://doi.org/10.1016/j.fob.2013.07.006

808 Karasov TL, Chae E, Herman JJ, Bergelson J (2017) Mechanisms to mitigate the trade-off
809 between growth and defense. Plant Cell 9:666-680. https://doi.org/10.1105/tpc.16.00931

810 Koo AJ (2018) Metabolism of the plant hormone jasmonate: a sentinel for tissue damage and
811 master regulator of stress response. Phytochem Rev 17:51-80. https://doi.org/10.1007/s11101-
812 017-9510-8

813 Koo YM, Heo AY, Choi HW (2020) Salicylic acid as a safe plant protector and growth
814 regulator. Plant Path J 36:1–10. https://doi.org/10.5423/PPJ.RW.12.2019.0295

Lantz AT, Allman,J, Weraduwage SM, Sharkey TD (2019) Isoprene: New insights into the control of emission and mediation of stress tolerance by gene expression. Plant, Cell Env 42:2808-2826. https://doi.org/10.1111/pce.13629

Lau N-S, Makita Y, Kawashima M, et al. (2016) The rubber tree genome shows expansion of gene family associated with rubber biosynthesis. Sci Rep 6:28594. https://doi.org/10.1038/srep28594

Lerdau M, Coley PD (2002) Benefits of the carbon-nutrient balance hypothesis. Oikos 98:534-536. https://doi.org/10.1034/j.1600-0706.2002.980318.x

Li Q, Song J, Peng S, Wang JP, et al. (2014) Plant biotechnology for lignocellulosic biofuel production. Plant Biotech J 12:1174-1192. https://doi.org/10.1111/pbi.12273

Lindermayr C, Saalbach G, Durner J (2005) Proteomic identification of S-nitrosylated proteins in *Arabidopsis*. Plant Physiol 137:921–930. https://doi.org/10.1104/pp.104.058719

Litvak ME, Loreto F, Harley PC, et al. (1996) The response of isoprene emission rate and photosynthesis rate to growth photon flux density and nitrogen supply in aspen and white oak trees. Plant, Cell & Env 19:549-559. https://doi.org/10.1111/j.1365-3040.1996.tb00388.x

Liu B, Zhang L, Rusalepp L, et al. (2020) Heat-priming improved heat tolerance of photosynthesis, enhanced terpenoid and benzenoid emission and phenolics accumulation in *Achillea millefolium*. Plant, Cell Env (in press). https://doi.org/10.1111/pce.13830

Logan BA, Anchordoquy TJ, Monson RK, Pan RS (1999) The effect of isoprene on the properties of spinach thylakoids and phosphatidyl-choline liposomes. Plant Biol 1:602–606. https://doi.org/10.1111/j.1438-8677.1999.tb00269.x

836 Logan BA, Monson RK (1999) Thermotolerance of leaf discs from four isoprene-emitting
837 species is not enhanced by exposure to exogenous isoprene. Plant Physiol 120:821–825.
838 https://doi.org/10.1104/pp.120.3.821

839 Loivamäki M, Gilmer F, Fischbach RJ, et al. (2007) *Arabidopsis*, a model to study biological
840 functions of isoprene? Plant Physiol 144:1066-1078. https://doi.org/10.1104/pp.107.098509

841 Loivamäki M, Mumm R, Dicke M, Schnitzler JP (2008) Isoprene interferes with the attraction of
842 bodyguards by herbaceous plants. Proc Natl Acad Sci (USA) 105:17430-17435.
843 https://doi.org/10.1073/pnas.0804488105

844 Loreto F, Fineschi S (2015) Reconciling functions and evolution of isoprene emission in higher
845 plants. New Phytol 206:578-582. https://doi.org/10.1111/nph.13242

846 Loreto F, Schnitzler J-P (2010) Abiotic stresses and induced BVOCs. Trends Plant Sci 15:154-
847 166. https://doi.org/10.1016/j.tplants.2009.12.006

848 Loreto F, Sharkey TD (1990) A gas-exchange study of photosynthesis and isoprene emission in
849 *Quercus rubra* L. Planta 182:523-531. https://doi.org/10.1007/BF02341027

850 Loreto F, Sharkey TD (1993) Isoprene emission by plants is affected by transmissible wound
851 signals. Plant, Cell Env 16:563-570. https://doi.org/10.1111/j.1365-3040.1993.tb00904.x

852 Loreto F, Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus
853 against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular
854 membranes. Plant Physiol 127:1781–1787. https://doi.org/10.1104/pp.010497

855 Marino G, Brunetti C, Tattini M, et al. (2017) Dissecting the role of isoprene and stress-related
856 hormones (ABA and ethylene) in *Populus nigra* exposed to unequal root zone water stress. Tree
857 Physiol 37:1637-1647. https://doi.org/10.1093/treephys/tpx083

858 Matsui K (2006) Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. Curr
859 Opin Plant Biol 9:274–280. https://doi.org/10.1016/j.pbi.2006.03.002

860 McKey D (1974) Adaptive patterns in alkaloid physiology. Amer Nat 108:305–320.
861 https://doi.org/10.1086/282909

862 Miloradovic van Doorn M, Merl-Pham J, Ghirardo A, Fink S, et al. (2020) Root isoprene
863 formation alters lateral root development. Plant, Cell Env (in press).
864 https://doi.org/10.1111/pce.13814

865 Monson RK (2002) Volatile organic compound emissions from natural ecosystems: a primary
866 control over tropospheric chemistry. Israel J Chem 42:29-42. https://doi.org/10.1560/0JJC-
867 XQAA-JX0G-FXJG

868 Monson RK, Fall R (1989) Isoprene emission from aspen leaves. The influence of environment
869 and relation to photosynthesis and photorespiration. Plant Physiol 90:267-274.
870 https://doi.org/10.1104/pp.90.1.267

871 Monson RK, Holland E (2001) Biospheric trace gas fluxes and their control over tropospheric
872 chemistry. Ann Rev Ecol System 32:547-576.
873 https://doi.org/10.1146/annurev.ecolsys.32.081501.114136

874 Monson RK, Jones RT, Rosenstiel TN, Schnitzler JP (2013). Why only some plants emit
875 isoprene. Plant, Cell & Environ 36:503–516. https://doi.org/10.1111/pce.12015

876 Monson RK, Winkler B, Rosenstiel TN, et al. (2020) High productivity in hybrid-poplar
877 plantations without isoprene emission to the atmosphere. Proc Natl Acad Sci (USA) 117:1596-
878 1605. https://doi.org/10.1073/pnas.1912327117

879 Mooney HA (1972) The carbon balance of plants. Ann Rev Ecol Syst 3:315-346.
880 https://doi.org/10.1146/annurev.es.03.110172.001531

881 Nathan C (2003) Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell
882 signaling. J Clin Invest 111:769-778. https://doi.org/10.1172/JCI200318174

883 Neill SJ, Desikan R, Clarke A, et al. (2002). Hydrogen peroxide and nitric oxide as signalling
884 molecules in plants. J Exp Bot 53:1237–1247. https://doi.org/10.1093/jexbot/53.372.1237

885 Parveen S, Harun-Ur-Rashid M, Inafuku M, et al. (2019a) Molecular regulatory mechanism of
886 isoprene emission under short-term drought stress in the tropical tree *Ficus septica*. Tree Physiol
887 39: 440-453. https://doi.org/10.1093/treephys/tpy123

888 Parveen S, Iqbal MA, Mutanda I, et al. (2019b). Plant hormone effects on isoprene emission
889 from tropical tree in *Ficus septica*. Plant, Cell Env 42:1715–1728.
890 https://doi/org/10.1111/pce.13513

891 Peñuelas J, Llusià J, Asensio D, Munné-Bosch S (2005) Linking isoprene with plant
892 thermotolerance, antioxidants and monoterpene emissions. Plant, Cell Env 28:278–286.
893 https://doi.org/10.1111/j.1365-3040.2004.01250.x

894 Pike RC, Young PJ (2009). How plants can influence tropospheric chemistry: The role of
895 isoprene emissions from the biosphere. Weather 64:332–336. https://doi.org/10.1002/wea.416

Pollastri S, Tsonev T, Loreto F (2014). Isoprene improves photochemical efficiency and enhances heat dissipation in plants at physiological temperatures. J Exp Bot 65:1565–1570. https://doi.org/10.1093/jxb/eru033

Raes J, Rohde A, Christensen JH, et al. (2003) Genome-wide characterization of the lignification toolbox in Arabidopsis. Plant Physiol. 133:1051-1071. https://doi.org/10.1104/pp.103.026484

Rasmussen RA, Jones CA (1973) Emission of isoprene from leaf disks of *Hamamelis*. Phytochem 12:15–19. https://doi.org/10.1016/S0031-9422(00)84618-X

Reich PB (2014) The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. J Ecol 102:275-301. https://doi.org/10.1111/1365-2745.12211

Ren N, Timko MP (2001) AFLP analysis of genetic polymorphism and evolutionary relationships among cultivated and wild *Nicotiana* species. Genome 44:559-571. https://doi.org/10.1139/gen-44-4-559

Rhoades DF (1979) Evolution of plant chemical defense against herbivores. In: Rosenthal GA, Janzen DH (eds) Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York, pp. 1-55

Roitsch T, Ehneß R (2000) Regulation of source/sink relations by cytokinins. Plant Growth Reg 32:359–367. https://doi.org/10.1023/A:1010781500705

Ruan J, Zhou Y, Zhou M, et al. (2019) Jasmonic acid signaling pathway in plants. Int J Mol Sci 20:2479. https://doi.org/10.3390/ijms20102479

915 Ryan AC, Hewitt CN, Possell M, et al. (2014) Isoprene emission protects photosynthesis but
916 reduces plant productivity during drought in transgenic tobacco (*Nicotiana tabacum*) plants. New
917 Phytol 201:205–216. https://doi.org/10.1111/nph.12477

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

920 Sasaki K, Saito T, Lamsa M, et al. (2007). Plants utilize isoprene emission as a thermotolerance
921 mechanism. Plant Cell Physiol 48:1254–1262. https://doi.org/10.1093/pcp/pcm104

922 Schuman MC, Baldwin IT (2016) The layers of plant responses to insect herbivores. Ann Rev
923 Entmol 61:373-394. https://doi.org/10.1146/annurev-ento-010715-023851

924 Seo JS, Koo YJ, Jung C, et al. (2013) Identification of a novel jasmonate-responsive element in
925 the AtJMT promoter and its binding protein for AtJMT repression. PLoS ONE 8: e55482.
926 https://doi.org/10.1371/journal.pone.0055482

927 Sharkey TD (2013) Is it useful to ask why plants emit isoprene? Plant, Cell & Environ 36:517–
928 520. https://doi.org/10.1111/pce.12038

929 Sharkey TD, Chen X, Yeh S (2001). Isoprene increases thermotolerance of fosmidomycin-fed
930 leaves. Plant Physiol 125:2001–2006. https://doi.org/10.1104/pp.125.4.2001

931 Sharkey TD, Monson RK (2017) Isoprene research-60 years later, the biology is still enigmatic.
932 Plant, Cell & Env 40:1671-1678. https://doi.org/10.1111/pce.12930

933 Sharkey TD, Preiser AL, Weraduwage SM, Gog L (2020) Source of ^{12}C in Calvin Benson cycle
934 intermediates and isoprene emitted from plant leaves fed with $^{13}CO_2$. Biochem J (in press).
935 https://doi.org/10.1042/bcj20200480

936 Sharkey TD, Singsaas EL (1995) Why plants emit isoprene. Nature 374:769.
937 https://doi.org/10.1038/374769a0

938 Sharkey TD, Wiberley AE, Donohue AR (2008) Isoprene emission from plants: Why and how.
939 Ann Bot 101:5-18. https://doi.org/10.1093/aob/mcm240

940 Sharkey TD, Yeh SS (2001) Isoprene emission from plants. Ann Rev Plant Physiol Plant Mol
941 Biol 52: 407-436. https://doi.org/10.1146/annurev.arplant.52.1.407

942 Shetty NP, Jorgensen HJL, Jensen JD, et al. (2008) Roles of reactive oxygen species in
943 interactions between plants and pathogens. Eur J Plant Path 121: 267-280.
944 https://doi.org/10.1007/s10658-008-9302-5

945 Siwko ME, Marrink SJ, de Vries AH, et al. (2007) Does isoprene protect plant membranes from
946 thermal shock? A molecular dynamics study. Biochim Biophys Acta 1768: 198–206.
947 https://doi.org/10.1016/j. bbamem.2006.09.023

948 Spadaro D, Yun BW, Spoel SH, et al. (2010) The redox switch: dynamic regulation of protein
949 function by cysteine modifications. Physiol Plant 138:360-371. https://doi.org/10.1111/j.1399-
950 3054.2009.01307.x

951 Stamp N (2003) Out of the quagmire of plant defense hypotheses. Quart Rev Biol 78:23-55.
952 https://doi.org/10.1086/367580

953 Tattini M, Loreto F, Fini A, Guidi L, et al. (2015) Isoprenoids and phenylpropanoids are part of
954 the antioxidant defense orchestrated daily by drought-stressed *Platanus* x *acerifolia* plants during
955 Mediterranean summers. New Phytol 207:613-626. https://doi.org/10.1111/nph.13380

956 Tattini M, Velikova V, Vickers C, et al. (2014). Isoprene production in transgenic tobacco alters
957 isoprenoid, non-structural carbohydrate and phenylpropanoid metabolism, and protects
958 photosynthesis from drought stress. Plant, Cell & Environ 37:1950–1964.
959 https://doi.org/10.1111/pce.12350

960 Taylor TC, Smith MN, Slot M, Feeley KJ (2019) The capacity to emit isoprene differentiates the
961 photosynthetic temperature responses of tropical plant species. Plant, Cell & Environ 42:2448-
962 2457. https://doi.org/10.1111/pce.13564

963 Tingey DT, Manning M, Grothaus LC, Burns WF (1979) The influence of light and temperature
964 on isoprene emission rates from live oak. Physiol Plant 47:112–118.
965 https://doi.org/10.1111/j.1399-3054.1979.tb03200.x

966 Tretner C, Huth U, Hause, B (2008) Mechanostimulation of *Medicago truncatula* leads to
967 enhanced levels of jasmonic acid. J Exp Bot 59:2847-2856. https://doi.org/10.1093/jxb/ern145

968 Ullah C, Tsai C-J, Unsicker SB, et al. (2019) Salicylic acid activates poplar defense against the
969 biotrophic rust fungus *Melampsora larici-populina* via increased biosynthesis of catechin and
970 proanthocyanidins. New Phytol 221:960–975. https://doi.org/10.1111/nph.15396

971 van der Schoot C, Paul LK, Paul SB, Rinne PL (2011) Plant lipid bodies and cell-cell signaling:
972 a new role for an old organelle? Plant Signal Behav 6:1732-1738.
973 https://doi.org/10.4161/psb.6.11.17639

974 van Noordwijk AJ, de Jong G (1986) Acquisition and allocation of resources—their influence on
975 variation in life history tactics. Amer Nat 128:137–142. https://doi.org/10.1086/284547

976 Vanzo E, Merl J, Lindermayr C, et al. (2014) S-nitroso-proteome in poplar leaves in response to
977 acute ozone. PLoS One 9:e106886. https://doi.org/10.1371/journal.pone.0106886

978 Vanzo E, Merl-Pham J, Velikova V, et al. (2016). Modulation of protein s-nitrosylation by
979 isoprene emission in poplar. Plant Physiol 170:1945–1961. https://doi.org/10.1104/pp.15.01842

980 Velikova V, Bruneti C, Tattini M, Doneva D, et al. (2016) Physiological significance of
981 isoprenoids and phenylpropanoids in drought response of Arundinoideae species with contrasting
982 habitats and metabolism. Plant, Cell Env 39:2185-2197. https://doi.org/10.1111/pce.12785

983 Velikova V, Edreva A, Loreto F (2004). Endogenous isoprene protects *Phragmites australis*
984 leaves against singlet oxygen. Physiol Plant 122:219–225. https://doi.org/10.1111/j.0031-
985 9317.2004.00392.x

986 Velikova V, Ghirardo A, Vanzo E, et al. (2014). Genetic manipulation of isoprene emissions in
987 poplar plants remodels the chloroplast proteome. J Proteome Res 13:2005–2018.
988 https://doi.org/10.1021/pr401124z

989 Velikova V, Loreto F (2005). On the relationship between isoprene emission and
990 thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the
991 recovery from a heat stress. Plant, Cell & Environ 28:318–327. https://doi.org/10.1111/j.1365-
992 3040.2004.01314.x

993 Velikova V, Fares S, Loreto F (2008) Isoprene and nitric oxide reduce damages in leaves
994 exposed to oxidative stress Plant, Cell & Environ 31: 1882-1884. https://doi.org/10.1111/j.1365-
995 3040.2008.01893.x

996 Velikova V, Tsonev, Tattini, M, Arena C, et al. (2018) Physiological and structural adjustments
997 of two ecotypes of *Platanus orientalis* L. from different habitats in response to drought and re-
998 watering. Conserv Physiol 6:coy073. https://doi.org/10.1093/conphys/coy073

999 Velikova V, Varkonyi Z, Szabo M, et al. (2011) Increased thermostability of thylakoid
1000 membranes in isoprene-emitting leaves probed with three biophysical techniques. Plant Physiol
1001 157:905–916. https://doi.org/10.1104/pp.111. 182519

1002 Vickers CE, Gershenzon J, Lerdau MT, Loreto F (2009a) A unified mechanism of action for
1003 volatile isoprenoids in plant abiotic stress. Nat Chem Biol 5:283–291.
1004 https://doi.org/10.1038/nchembio.158

1005 Vickers CE, Possell,M, CojocariuCI, et al. (2009b) Isoprene synthesis protects transgenic
1006 tobacco plants from oxidative stress. Plant, Cell & Environ 32:520-531.
1007 https://doi.org/10.1111/j.1365-3040.2009.01946.x

1008 Walther TC, Farese RV Jr (2012) Lipid droplets and cellular lipid metabolism. Ann Rev
1009 Biochem 81:687-714. https://doi.org/10.1146/annurev-biochem-061009-102430

1010 Wild M, Daviere JM, Cheminant S, et al. (2012) The *Arabidopsis* DELLA RGA-LIKE3 is a
1011 direct target of MYC2 and modulates jasmonate signaling responses. Plant Cell 8:3307-3319.
1012 https://doi.org/10.1105/tpc.112.101428

1013 Wu Y, Zhang D, Chu JY, Boyle P, et al. (2012) The Arabidopsis NPR1 protein is a receptor for
1014 the plant defense hormone salicylic acid. Cell Rep 1:639-647.
1015 https://doi.org/10.1016/j.celrep.2012.05.008

1016 Yan XJ, Liu J, Kim H, (2019) CAD1 and CCR2 protein complex formation in monolignol
1017 biosynthesis in *Populus trichocarpa*. New Phytol 222: 244-260.
1018 https://doi.org/10.1111/nph.15505

1019 Zhao Q, Tobimatsu Y, Zhou R, et al. (2013) Loss of function of cinnamyl alcohol dehydrogenase
1020 1 leads to unconventional lignin and a temperature-sensitive growth defect in *Medicago*
1021 *truncatula*. Proc Natl Acad Sci (USA)110:13660-13665.
1022 https://doi.org/10.1073/pnas.1312234110

1023 Zhou ML, Sun ZM, Ding MQ, et al. (2017) FtSAD2 and FtJAZ1 regulate activity of the
1024 FtMYB11 transcription repressor of the phenylpropanoid pathway in *Fagopyrum tataricum*.
1025 New Phytol 216:814-828. https://doi.org/10.1111/nph.14692

1026 Zuo Z, Weraduwage SM, Lantz AT, et al. (2019). Isoprene acts as a signaling molecule in gene
1027 networks important for stress responses and plant growth. Plant Physiol 180:124–152.
1028 https://doi.org/10.1104/pp.18.01391

1029 Züst T, Agrawal AA (2017) Trade-offs between plant growth and defense: an emerging
1030 mechanistic synthesis. Ann Rev Plant Biol 68:513-534. https://doi.org/10.1146/annurev-arplant-
1031 042916-040856

Figure Legends

Figure 1. Protein or transcriptome expression modulation in the shikimate (A) and phenylpropanoid (B) pathways in IE *Populus x canescens*, compared to NE transgenic lines. Bold blue or orange arrows indicate increased or decreased expression in the presence of isoprene, respectively. Protein labels in italics with asterisks indicate transcriptome data from Behnke et al. (2010a); otherwise data are from the proteome study of Monson et al. (2020). Fold-change (FC) multipliers are shown in parentheses of some steps and refer to isoprene-emitting/non-emitting (IE/NE) plants. Not all pathway steps are shown; only those with significant ($P < 0.05$; $n = 4$ replicate trees) changes in transcription or protein amount (bold arrows; $0.5 \leq FC \geq 1.5$) or steps that produce metabolites that are not directly regulated, but are of importance to defense, growth or stress tolerance as discussed in the main text (broken arrows). Metabolite boxes highlighted in green, red or yellow refer to those most influencing growth, defense or stress tolerance, respectively.

Figure 2. Proteomic and transcriptomic adjustments in the pathways of terpenoid synthesis in response to presence or absence of isoprene in *Populus x canescens* and *Arabidopsis.* Orange and blue arrows/labels indicate decreased or increased protein contents or transcripts, respectively, in the presence of isoprene. Fold-change (FC) multipliers are shown in parentheses for some steps and refer to isoprene-emitting/non-emitting (IE/NE) plants. Not all pathway steps are shown; only those with significant ($P<0.05$) changes in protein amount (bold arrows) or steps that produce metabolites of importance to defense, growth or stress tolerance (dashed arrows) as discussed in the main text. Metabolite boxes highlighted in green,or yellow refer to those most influencing growth or stress tolerance, respectively. **Inset:** Bold arrows represent changes in *Arabidopsis* determined as observations that showed a consistent directional change in isoprene-

fumigated wild-type *Arabidopsis*). All values are derived from Monson et al. (2020) and Harvey and Sharkey (2016).

Figure 3. Proteomic or transcriptomic adjustments in the oxylipin pathways in response to presence or absence of isoprene in *Populus x canescens*, *Nicotiana tabacum* (tobacco) and *Aribodopsis.* Blue and orange bold arrows indicate increased or decreased expression, respectively, in the presence of isoprene. Arrows with one or two asterisks represent changes in tobacco and/or *Arabidopsis* determined as observations that showed a consistent directional change in one or two, respectively, of the three experimental systems that were examined (wild-type, isoprene-fumigated *Arabidopsis* plants and transgenic IE *Arabidopsis* and tobacco). Not all pathway steps are shown; only those with significant ($P<0.05$) changes in transcription or protein amount (bold arrows) or steps that produce metabolites of importance to defense, growth or stress tolerance as discussed in the main text. Data from Monson et al. (2020), Harvey and Sharkey (2016) and Zuo et al. (2019).

Figure 4. Graph showing relationships among growth, defense and isoprene emissions in the presence of abiotic stress. From the perspective of plant carbon budgets, the growth-defense tradeoff can be described in relation to a limit set by the plant's maximum photosynthetic capacity. In the absence of isoprene emissions, environmental stresses will reduce the photosynthetic capacity to a hypothetical limit defined by the dashed line. The evolution of isoprene emissions in taxonomic lineages will improve the potential for allocation to defense with reduced cost to growth, compared to the case for non-emitters. Based on concepts originally presented in Stamp (2003) and Züst and Argawal (2017).

Figure 5. Responses to stresses or isoprene involve perception (yellow chevrons) or signal transduction pathways (yellow arrows). Pathways stimulated (blue) or inhibited (orange) by stress are also shown, as is isoprene modulation of these pathways. Direct effects are shown as dark grey arrows. Four possible roles for isoprene in stress responses are depicted. Pathway 1 depicts effects of isoprene on an isoprene-specific signal cascade that can affect growth and / or defense. Pathway 2 suggests isoprene can modify signal cascades, for example JA or GA signaling pathways. Pathways may be upregulated (blue) or downregulated (orange) by stress and isoprene could modulate the changes in pathway regulation caused by stress. By modulating signal transduction pathways, isoprene could prevent the buildup of ROS and NO rather than quenching as a mechanism for keeping their concentrations low. Pathway 3 (right side) is direct quenching of H_2O_2 and other ROS. This pathway was highly favored in earlier studies, but is now considered unlikely to account for the effects of isoprene on plants. Pathway 4 shows isoprene signaling causing changes in metabolites that can quench ROS and or NO. Disruption of transduction pathways initiated by H_2O_2 or NO (e.g., the hypersensitive response) are suppressed by isoprene effects and could indirectly affect programmed cell death (PCD; see Vickers et al. 2009a; Vanzo et al. 2016).

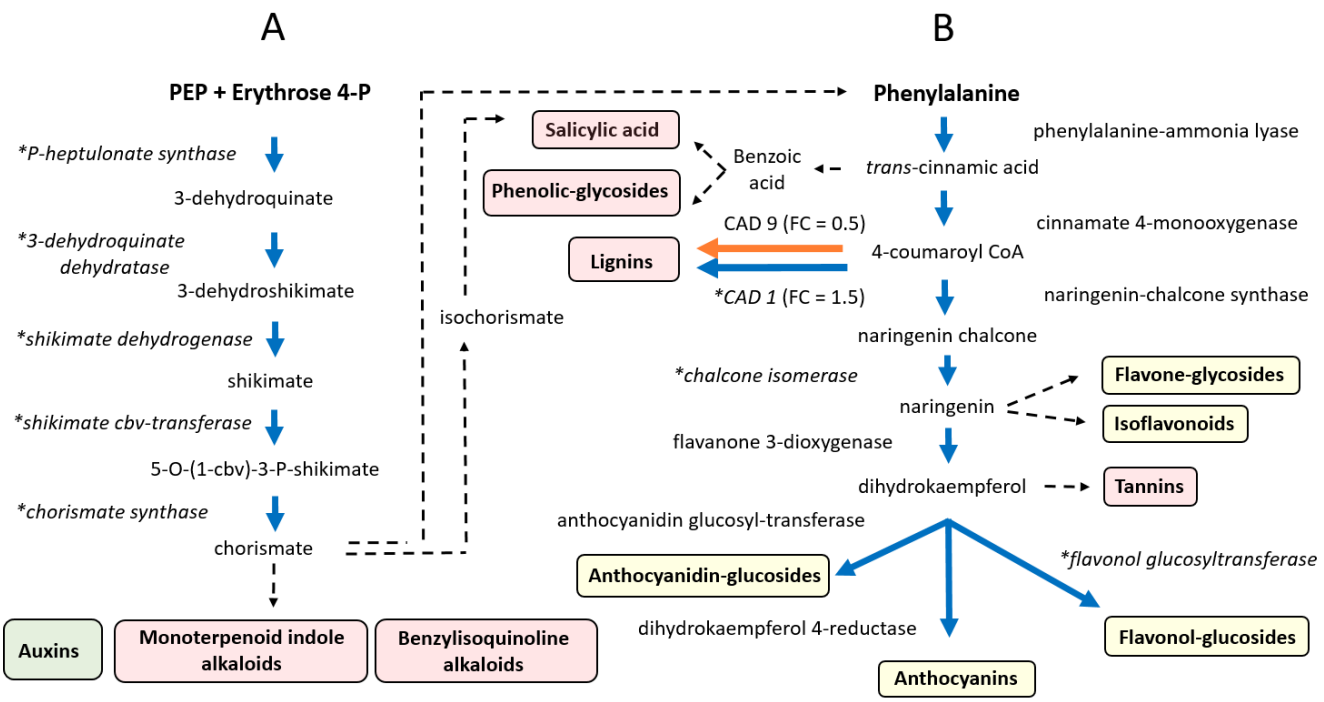

A
B
PEP + Erythrose 4-P
*P-heptulonate synthase
3-dehydroquinate
*3-dehydroquinate dehydratase
3-dehydroshikimate
*shikimate dehydrogenase
shikimate
*shikimate cbv-transferase
5-O-(1-cbv)-3-P-shikimate
*chorismate synthase
chorismate
isochorismate
Salicylic acid
Phenolic-glycosides
Lignins
Benzoic acid
Phenylalanine
phenylalanine-ammonia lyase
trans-cinnamic acid
CAD 9 (FC = 0.5)
cinnamate 4-monooxygenase
4-coumaroyl CoA
*CAD 1 (FC = 1.5)
naringenin-chalcone synthase
naringenin chalcone
*chalcone isomerase
naringenin
Flavone-glycosides
Isoflavonoids
flavanone 3-dioxygenase
dihydrokaempferol
Tannins
anthocyanidin glucosyl-transferase
*flavonol glucosyltransferase
Anthocyanidin-glucosides
dihydrokaempferol 4-reductase
Flavonol-glucosides
Anthocyanins
Auxins
Monoterpenoid indole alkaloids
Benzylisoquinoline alkaloids

Figure 1

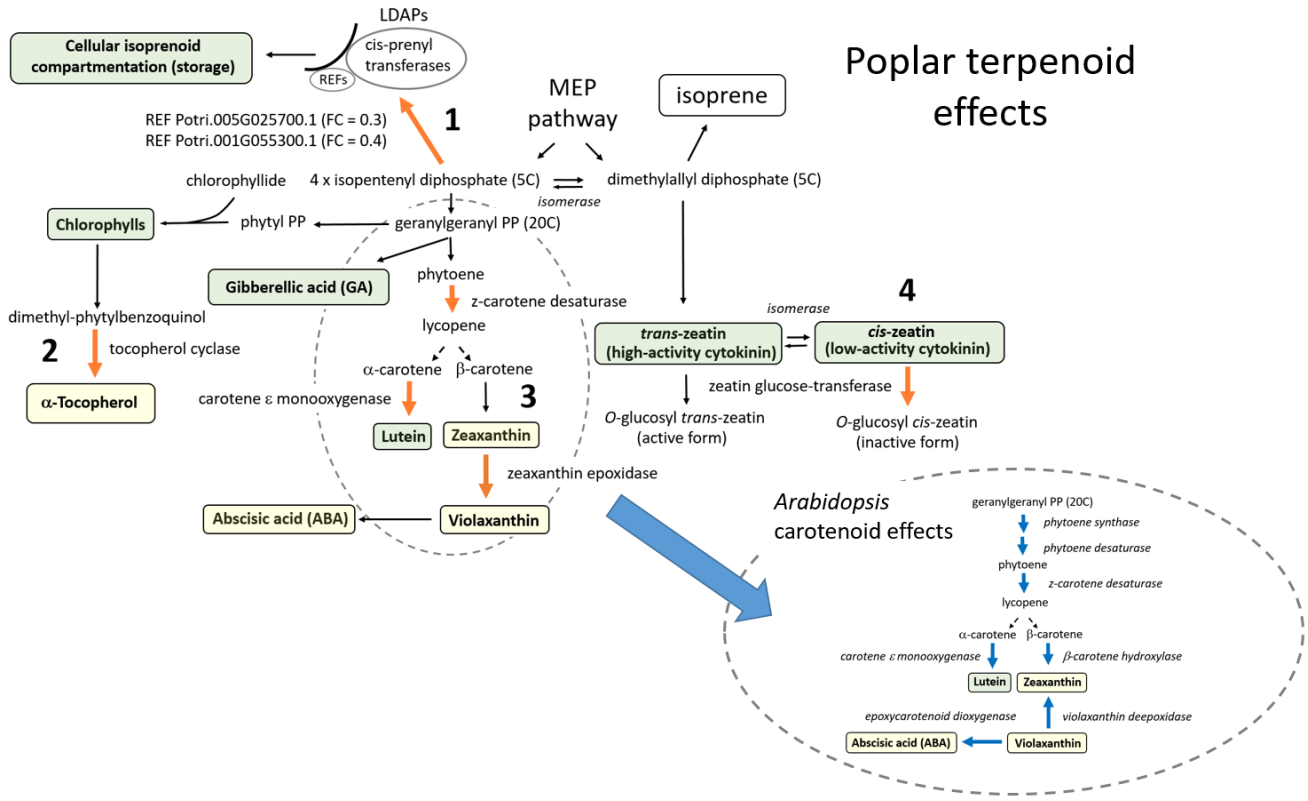

Poplar terpenoid effects
LDAPs
cis-prenyl transferases
REFs
Cellular isoprenoid compartmentation (storage)
REF Potri.005G025700.1 (FC = 0.3)
REF Potri.001G055300.1 (FC = 0.4)
1
MEP pathway
isoprene
chlorophyllide
4 x isopentenyl diphosphate (5C)
isomerase
dimethylallyl diphosphate (5C)
Chlorophylls
phytyl PP
geranylgeranyl PP (20C)
Gibberellic acid (GA)
phytoene
z-carotene desaturase
dimethyl-phytylbenzoquinol
2
tocopherol cyclase
α-Tocopherol
lycopene
α-carotene
β-carotene
carotene ε monooxygenase
3
Lutein
Zeaxanthin
zeaxanthin epoxidase
Violaxanthin
Abscisic acid (ABA)
isomerase
4
trans-zeatin (high-activity cytokinin)
cis-zeatin (low-activity cytokinin)
zeatin glucose-transferase
O-glucosyl trans-zeatin (active form)
O-glucosyl cis-zeatin (inactive form)
Arabidopsis carotenoid effects
geranylgeranyl PP (20C)
phytoene synthase
phytoene desaturase
phytoene
z-carotene desaturase
lycopene
α-carotene
β-carotene
carotene ε monooxygenase
β-carotene hydroxylase
Lutein
Zeaxanthin
epoxycarotenoid dioxygenase
violaxanthin deepoxidase
Abscisic acid (ABA)
Violaxanthin

Figure 2

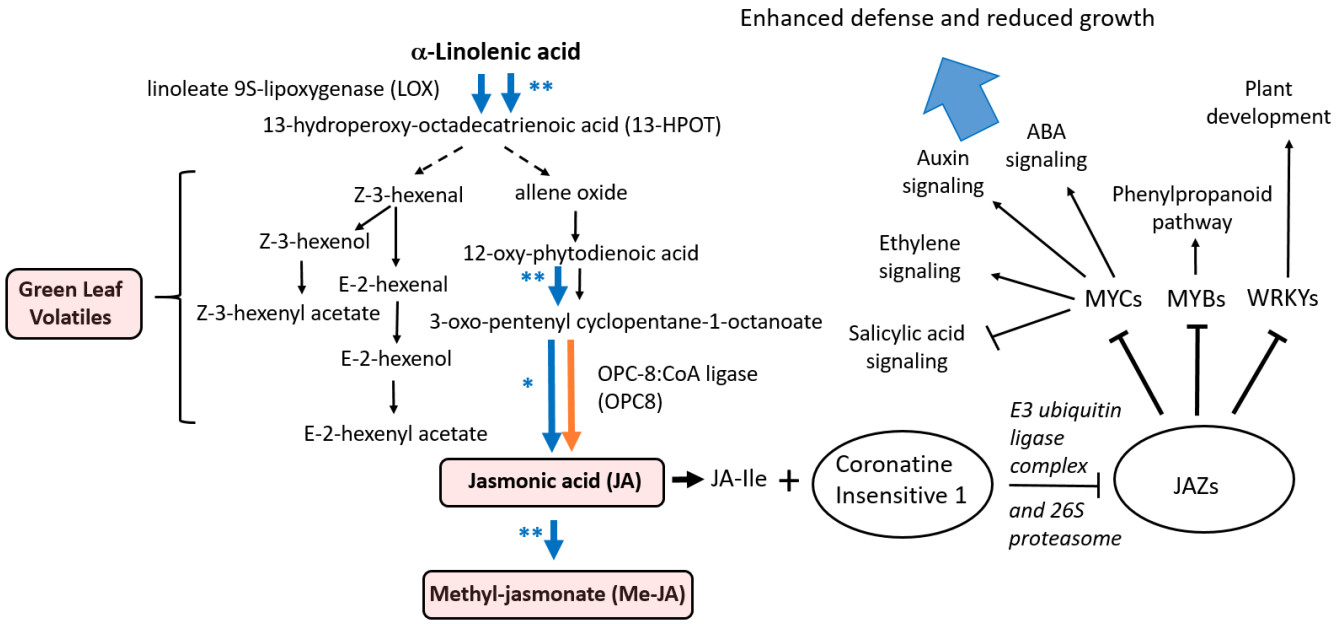
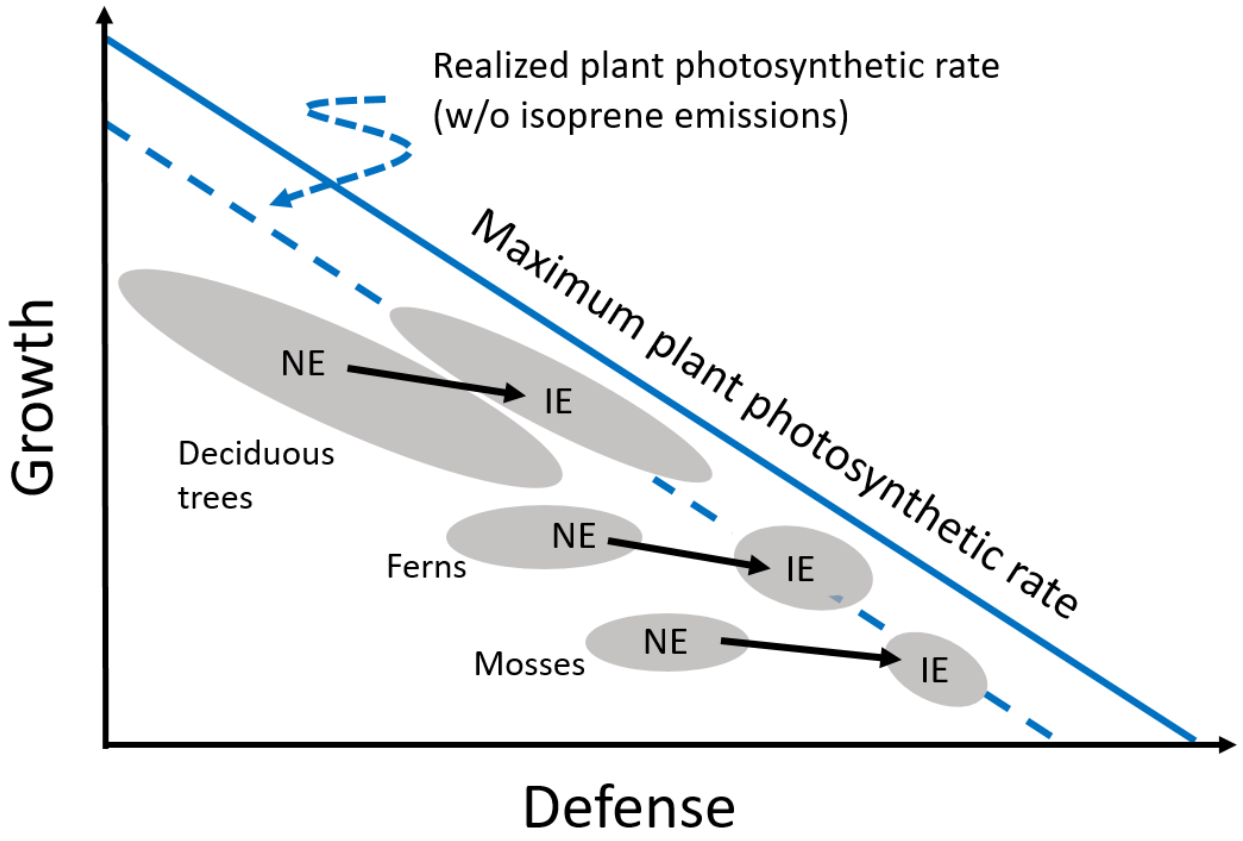

Enhanced defense and reduced growth
α-Linolenic acid
linoleate 9S-lipoxygenase (LOX)
**
13-hydroperoxy-octadecatrienoic acid (13-HPOT)
Z-3-hexenal
allene oxide
Z-3-hexenol
12-oxy-phytodienoic acid
Green Leaf Volatiles
E-2-hexenal
**
Z-3-hexenyl acetate
3-oxo-pentenyl cyclopentane-1-octanoate
E-2-hexenol
*
OPC-8:CoA ligase (OPC8)
E-2-hexenyl acetate
Jasmonic acid (JA)
JA-Ile
+
**
Methyl-jasmonate (Me-JA)
Auxin signaling
ABA signaling
Plant development
Phenylpropanoid pathway
Ethylene signaling
MYCs
MYBs
WRKYs
Salicylic acid signaling
E3 ubiquitin ligase complex and 26S proteasome
Coronatine Insensitive 1
JAZs

Figure 3



1114

1115

1116

1117

1118 **Figure 4**

1119

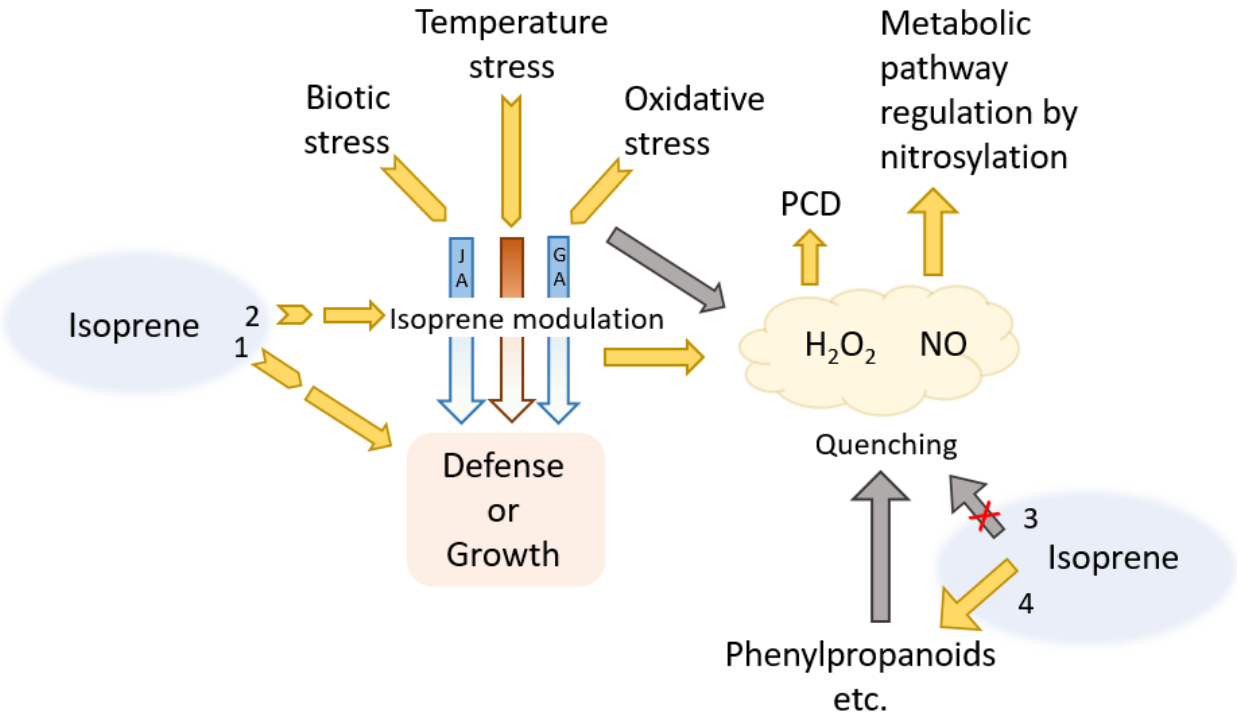

Temperature stress
Biotic stress
Oxidative stress
Metabolic pathway regulation by nitrosylation
PCD
JA
GA
Isoprene
2
1
Isoprene modulation
H_2O_2
NO
Defense or Growth
Quenching
3
Isoprene
4
Phenylpropanoids etc.

Figure 5