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

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Abstract

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 Thermotolerance · Phytohormones · Growth-differentiation balance · Proteomic · Cross-talk

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.

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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 30 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 thylakoid 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 some 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, 2015; Velikova et al. 2014; Brunetti 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 set of cellular networks 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 hypotheses 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 soil 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 soil 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 required for growth, to defense (Lerdau and Coley 2002; Stamp 2003). Coley et al. (1985) expanded the principals of the CNB hypothesis to account for genetically fixed G-D tradeoffs and interspecific differences in adaptation. In formulating the Resource Availability Hypothesis (RAH), they presented the case that the G-D tradeoff reflects selection across a broad range of traits in plants native to environments with different levels of resource availability. According to the RAH, slower growth rates, but higher levels of constitutive defense, occur in plants native to resource-limited environments, compared to plants native to resource-rich environments, and these patterns of trait covariance maximize fitness. In the Growth-Differentiation Balance Hypothesis (GDBH), Herms and Mattson (1992) developed the physiological case for a mutually-exclusive allocation of resources to one or the other function—growth versus differentiation (including defense). The GDBH included the tenets of the CNB hypothesis, but expanded the arguments to include ontogenetic constraints and it framed growth-differentiation tradeoffs in terms of cellular processes. Herms and Mattson (1992) applied their physiological hypotheses to an evolutionary optimality model in an effort to link the processes of cellular tradeoffs with natural selection, and therefore integrate the RAH and GDBH. The Optimal Defense Theory (ODT), which predates the tradeoff hypotheses discussed above, emerged from studies showing that plants deploy their chemical defenses in ways that maximize their effectiveness against herbivores (McKey 1974; Rhoades 1979). These observations led to a general hypothesis that plants have evolved patterns of defense in direct proportion to levels of herbivory and impacts on fitness. The ODT differs from the RAH and GDBH in that the principal determinant in natural selection is the balance between demand and fitness cost, rather than growth and fitness cost.

Tradeoff hypotheses 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 hypotheses. 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, gibberellic acid, 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, which implicitly accompanies many evolutionary optimization models, there is no clear margin for error in the face of environmental fluctuation. In that condition, mistakes in allocation strategy can lead to inefficient resource use and reduced fitness. Transcriptional control provides a means for selection to favor phenotypes that operate conservatively, below the zero-sum constraint, and retain a resource safety 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* × *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 15 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, which is consistent with the data, is that expression of the gene for this protein is suppressed in the presence of isoprene as a means of adjusting 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). Thus, *CAD1* may have a more important role in facilitating growth, rather than defense. 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). 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

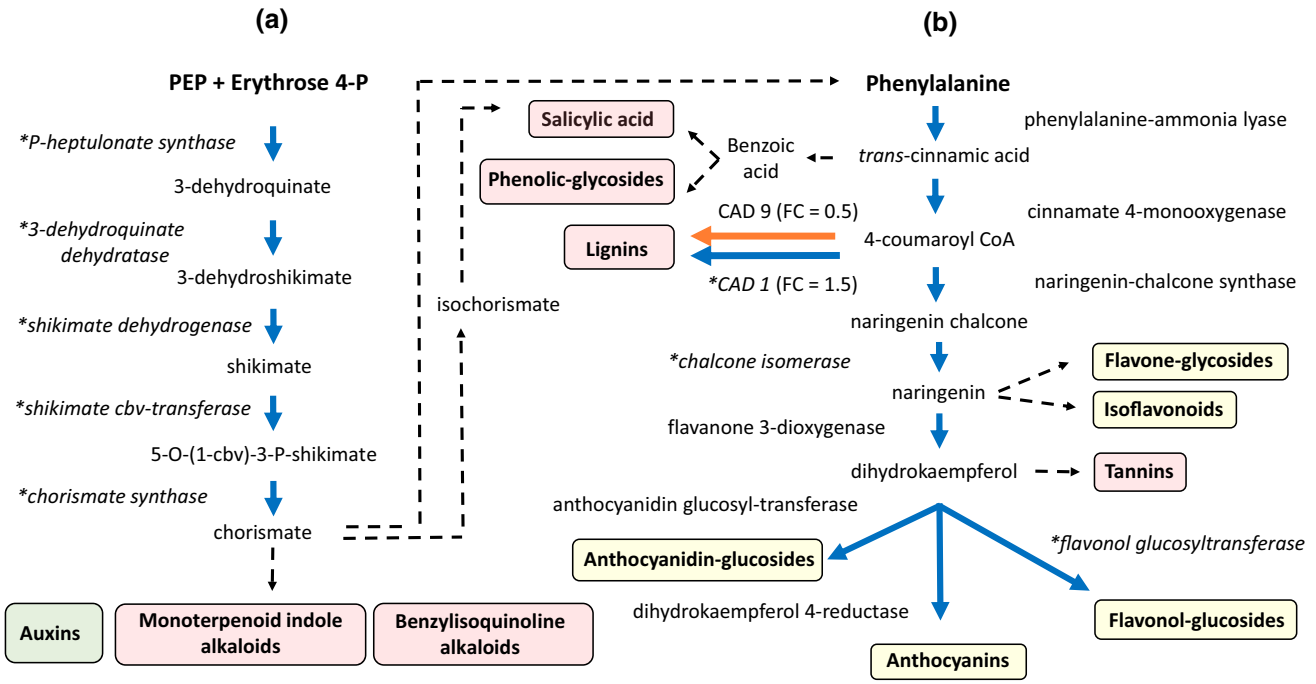

Fig. 1 Protein or transcriptome expression modulation in the **a** shikimate and **b** phenylpropanoid pathways in isoprene-emitting (IE) *Populus x canescens*, compared to non-emitting (NE) transgenic lines. Bold blue or orange arrows indicate increased or decreased expression in the presence of isoprene, 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

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 Fig. 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*

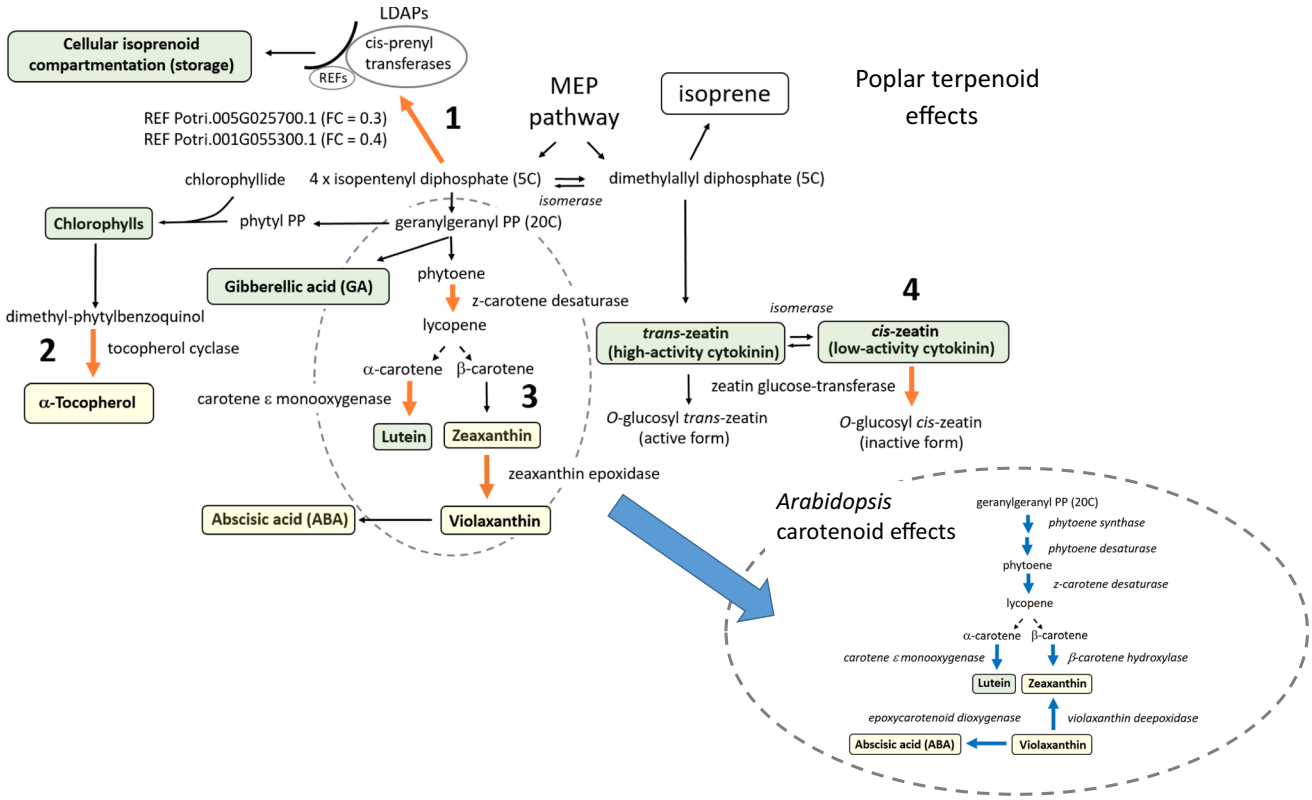

Fig. 2 Proteomic and transcriptomic adjustments in the pathways of terpenoid synthesis in response to the presence or absence of isoprene in *Populus* × *canescens* and *Arabidopsis.* Orange and blue arrows/labels indicate decreased or increased protein contents or transcripts, respectively, in the presence 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) or Harvey and Sharkey (2016)

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 lipid-based 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 Fig. 2). The observation of reduced VTE1 levels is consistent with past studies that showed reduced levels of the associated metabolite, α-tocopherol, in IE poplar leaves (Behnke et al. 2009), and heat-stressed Holm oak (*Quercus ilex*) leaves fumigated with isoprene (Peñuelas et al. 2005). α-tocopherol is an important antioxidant that is active in reducing cellular ROS during physiological stress. In wild-type *Arabidopsis* plants exposed to isoprene, *VTE1* gene transcripts were increased, as well as those for tocopherol methyltransferase (*TMT*) (Harvey and Sharkey 2016), once again, showing the tendency for interspecific differences in terpenoid pathway effects, especially between poplar and *Arabidopsis*.

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

In contrast to the poplar and tobacco systems, transcripts of most carotenoid-related enzymes were significantly increased in wild-type *Arabidopsis* exposed to isoprene (Harvey and Sharkey 2016; Fig. 2). In addition to upregulation in many of the same proteins that were described above, transcripts for the gene encoding β-carotene hydroxylase (*crtZ*) were increased in wild-type *Arabidopsis* exposed to isoprene. The protein, crtZ, converts β-carotene to zeaxanthin and can control the size of the xanthophyll cycle pool, and overexpression of *crtZ* in *Arabidopsis* has been linked to high-light and high-temperature stress tolerance (Davison et al. 2002). Finally, expression of the gene for the enzyme violaxanthin deepoxidase (*VDE*), which converts violaxanthin to zeaxanthin in the flexible, photoprotective part of the carotenoid cycle, was increased in wild-type *Arabidopsis* exposed to isoprene (Harvey and Sharkey 2016; Zuo et al. 2019).

A second component of the antioxidant system in plants, ascorbate, also appears to be differentially expressed in response to isoprene in poplar versus *Arabidopsis*. In poplar, the enzyme ascorbate oxidase (AAO), which leads to a lower ascorbate content in leaves, is present at a 44% higher level in IE lines, compared to NE lines (Monson et al. 2020). This observation is consistent with past observations showing lower ascorbate contents in non-stressed, IE poplar (Behnke et al 2009). In wild-type *Arabidopsis* exposed to isoprene (Harvey and Sharkey 2016; Zuo et al. 2019), two genes for the protein GDP-galactose phosphorylase (*VTC2* and *5*), which has been shown to be the only significant pathway in *Arabidopsis* that produces ascorbate (Dowdle et al. 2007), exhibited increased expression.

In poplar, there were no isoprene-associated shifts in gene expression or protein contents related to the synthesis of abscisic acid (ABA) (Behnke et al. 2010a; Monson et al. 2020). 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 of the experimental *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).

The presence of isoprene increases expression in several *LOX* genes, and thus the potential for GLV production, in all of the experimental systems used in this analysis (Behnke et al. 2010a; Harvey and Sharkey, 2016; Zuo et al. 2019; Monson et al. 2020) (Fig. 3). Beyond this initial step, however, isoprene effects on oxylipin processes among species begin to diverge, especially with respect to JA signaling.

The active form of JA occurs when it forms a macromolecular complex with the amino acid, isoleucine (Ile). A principal receptor of the JA-Ile conjugate is the Coronatine Insensitive 1 (COI1) F-box protein, which is part of an E3 ubiquitin ligase complex. When JA-Ile reaches a threshold level, COI1 associates with JA-Ile to form a multi-protein transcriptional modifier (Ruan et al. 2019). The JA-Ile-COI1 complex targets a large family of proteins known as Jasmonate ZIM (JAZ), which generally function as negative transcriptional regulators (Fig. 3). In the absence of JA-Ile-COI1, JAZ proteins bind to TFs with a high degree of specificity, blocking their ability to participate in the formation of transcription initiation complexes. The COI1-JAZ interaction triggers ubiquitin-dependent degradation of the JAZ proteins, freeing the TFs and activating transcription.

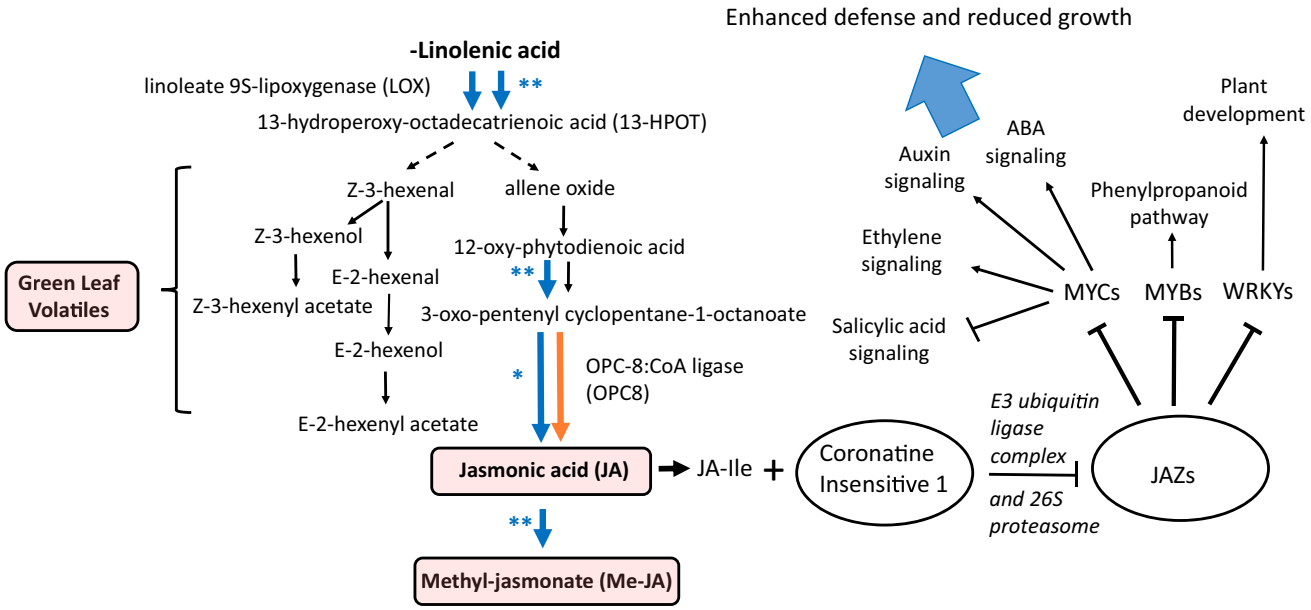

Fig. 3 Proteomic or transcriptomic adjustments in the oxylipin pathways in response to the presence or absence of isoprene in *Populus x canescens*, *Nicotiana tabacum* (tobacco) and *Aribodopsis.* Blue and orange bold arrows indicate increased or decreased expression, respectively, in the presence of 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)

From the past studies in *Arabidopsis*, there are antagonistic interactions involving proteins in the JAZ and DELLA families, which are associated with the JA and gibberellic acid (GA) pathways, respectively. JA synthesis leads to an enhancement in DELLA transcription and degradation of JAZ proteins, which leads to decreased growth and increased defense, respectively—in essence, providing a molecular context for the growth-defense tradeoff and, in this case, favoring defense (Wild et al. 2012; Campos et al. 2016). GA synthesis leads to degradation of DELLA proteins and an enhancement of GA-activator signaling, which enhances growth and, at the same time, increases the number of free JAZ proteins, which suppress JA-associated defense signaling (Campos et al. 2016). Once again, these molecular interactions enable the growth-defense tradeoff; but, in this case, favoring growth. Antagonism between JA and GA signaling in determining the growth-defense tradeoff is also influenced by extrinsic environmental cues involving spectral shifts in incident light (Ballarè 2014), and it can be uncoupled by phytochrome *b* gene mutations (Campos et al. 2016).

The expression of three genes important for JA synthesis were increased by isoprene in *Arabidopsis* and tobacco. For example, in addition to LOX, 12-oxo-phytodienoic acid reductase (*OPR*) was enhanced in transgenic IE *Arabidopsis* and IE tobacco, while 3-oxo-2-(2′-[*Z*]-pentenyl) cyclopentane-1-octanoate CoA ligase (*OPC*-8:CoA ligase) increased in IE tobacco. In addition, expression of the gene for MYB59 transcription factor was reduced in both the fumigated and transgenic *Arabidopsis* systems in response to isoprene (Harvey and Sharkey 2016; Zuo et al. 2019). MYB59 suppresses expression of certain genes important for oxylipin biosynthesis (e.g., the gene for allene oxide synthase, *CYP74A,* and *OPR3*), as well as the gene for the MYC2 TF that regulates JA signaling (Boter et al. 2004). Expression of the gene for jasmonate *O*-methyltransferase (*JMT1*) was increased in wild-type *Arabidopsis* exposed to isoprene. JMT catalyzes the reaction leading to methyl jasmonate (Me-JA), a volatile hydrocarbon that can function as a long distance chemical signal with significant effects on JA related gene expression (Benevenuto et al. 2019). Both Me-JA and isoprene have been shown to be produced in response to wounding (Loreto and Sharkey 1993; Lantz et al. 2019; Benevenuto et al. 2019); a response that seemed out of place with the past literature emphasizing a role for isoprene in abiotic stress tolerance, but that now makes more sense within the context of a multi-pathway signaling network. In addition to an isoprene-induced increase in *JMT1* expression in 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 make 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 associated with REF proteins, while in *Arabidopsis* expression for all three were increased. These differences are likely to reflect the different selection regimes experienced by the species. Interestingly, *Arabidopsis* and tobacco were capable of responding to the introduction of isoprene, despite no past history of selection for phenotypes expressing the trait. This indicates that isoprene is a trait that, once evolved, can rapidly become incorporated into naïve phenotypes. Many of the regulatory sequence motifs in the *ISPS* promoter of IE poplar are shared with the promoter of *1,8-cineole synthase* (*AtTPS-Cin*) from NE *Arabidopsis*, the gene for a terpene synthase that is closely-related to *ISPS* (see Fig. S3 in Miloradovic van Doorn et al. 2020). These include regulatory elements that respond to ABA, ME-JA, SA and binding with the MYB transcription factor family. Thus, at least one terpene synthase, unrelated to isoprene biosynthesis, exists in *Arabidopsis* with pre-existing metabolic connections to several cellular signaling pathways. These factors might facilitate rapid (generationally-speaking) directional selection during the evolution of isoprene signaling within naïve lineages, and provide high selective value to the trait early during its appearance.

The adaptive value of isoprene is likely also linked to its association with photosynthesis. From the earliest days of isoprene research, it has been recognized that variances in emission rate and photosynthesis are correlated across many determining variables (Sanadze et al. 1972; Rasmussen and Jones 1973; Tingey et al. 1979). Subsequent research showed numerous biochemical dependencies of isoprene synthesis on photosynthate and photoreductant (Sharkey and Yeh 2001; Sharkey and Monson 2017; Sharkey et al. 2020). These dependencies provided explanations for: (1) the positive correlations of isoprene emission and photosynthetic capacity (Monson and Fall 1989; Loreto and Sharkey 1990), (2) the role of increased resources, such as nitrogen, in supporting higher isoprene emission rates (Harley et al. 1994; Litvak et al. 1996; Fernandez-Martinez et al. 2018), and (3) the observations that within a phylogenetic clade, isoprene emissions are found in species with higher photosynthetic capacities, higher growth rates, and niche affinities that favor sunny habitats (Harley et al. 1999; Dani et al. 2014; Loreto and Fineschi 2015). The many metabolic and ecological associations between isoprene and photosynthesis likely elevate the selection differential for isoprene. (Selection differential is the difference between the average value to fitness of a quantitative trait in an entire population and the average value in those individuals selected to reproduce and form the next generation). With the new insight into isoprene's role in regulating gene expression, we can expand the scope of isoprene's trait value to include plant defense. Metabolically, isoprene sits in a position wedged between the processes that determine the supply of photosynthetic substrates and the demand of secondary metabolite

products—the two economic determinants underlying most contemporary growth-defense theories.

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

This view of isoprene emissions is compatible with previous observations and theories 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 hypothesis 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 hypothesis 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 1991). From our hypothesis, 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.

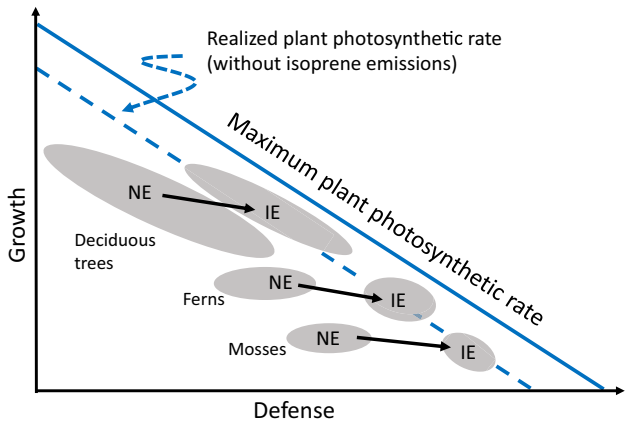

Fig. 4 Graph showing relationships among growth, defense and isoprene emissions in the presence of abiotic stress. From the perspective of plant carbon budgets, the growth-defense tradeoff can be described in relation to a limit set by the plant's maximum photosynthetic capacity. Without isoprene emissions, environmental stress will reduce the photosynthetic capacity to a hypothetical limit defined by the dashed line. 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. Redrawn from growth-defense concepts originally presented in Stamp (2003) and Züst and Agrawal (2017)

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

Conventional signal-receptor interactions are based on macromolecular shape complementarity (Covarrubias et al. 2020). In the case of the plant immune signal, salicylic acid (SA), SA-protein binding interactions, involving a set of cooperating transcriptional activators (NPR1) and repressors (NPR3 and NPR4), occurs in a conventional manner and controls SA signal reception (Wu et al. 2012; Ding et al. 2018). However, redox-driven interactions are also common, especially in signaling networks based on reactive oxygen (ROS) and nitrogen species (RNS) (Nathan 2003; Shetty et al. 2008; Vickers et al. 2009a). For ROS, signaling occurs through the oxidation of key amino acids, such as cysteine, on the surface of transcription-modifier proteins, leading to direct control over gene expression (Neill et al. 2002). In the

case of RNS, protein activities are modified by post-translational modification (PTM) involving nitric oxide (NO), a process known as *S*-nitrosylation (Spadaro et al. 2010). *S*-nitrosylation most commonly reduces enzyme activities in plants, leading to reduced rates of metabolite production (Lindermayr et al. 2005). *S*-nitrosylation is known to regulate inducible responses to abiotic stress (Corpas et al. 2011; Vanzo et al. 2014; Begara-Morales et al. 2018), and past studies in poplar have shown that isoprene (through an unknown mechanism) modulates the H_2O_2-dependent translational cascade and NO-dependent post-translational cascade (Vanzo et al 2016). Several past theories concerning the adaptive functions of isoprene have posited that isoprene's potential role as an antioxidant molecule provides a mechanism for direct chemical reactions that reduce ROS or RNS to a level that causes pathway modulation (Velikova and Loreto 2005; Velikova et al. 2008; Vickers et al. 2009a; Behnke et al. 2010a). Such past theories of isoprene directly affecting cellular redox signaling through its role as an antioxidant are too narrow to account for the broad set of influences and diverse set of pathways indicated in the recent multi-omic analyses.

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

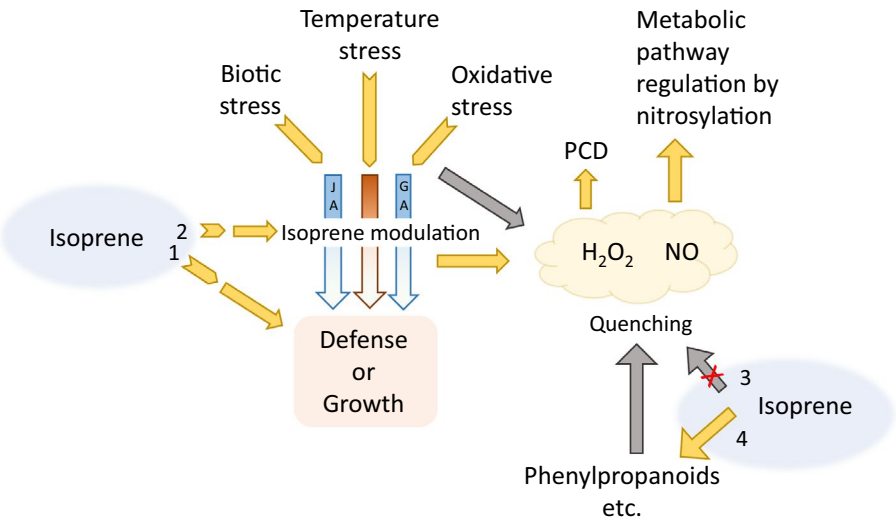

Fig. 5 Responses to stress or isoprene are shown as involving perception (yellow chevrons) or signal transduction pathways (yellow arrows). Pathways stimulated (blue) or inhibited (orange) by stress are also shown, as is isoprene modulation of these pathways. Direct effects are shown as dark grey arrows. 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 depicts isoprene as a modifier of signal cascades; for example, the jasmonic acid (JA) or gibberellic acid (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. Pathway 3 (right side) is direct quenching of H_2O_2 and other reactive oxygen species (ROS). This pathway was highly favored in earlier studies, but is now considered unlikely to account for the effects of isoprene on plants (shown as red "*x*"). Pathway 4 shows isoprene signaling causing changes in metabolites that can quench ROS and or NO, disrupting the hypersensitive response and nitrosylation, and potentially indirectly affecting programmed cell death (PCD; see Vickers et al. 2009a; Vanzo et al. 2016)

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

At this time, it is not clear as to how the dependency of isoprene emissions on diurnal and seasonal environmental change fits into its role as a signaling metabolite. In some ways, isoprene is similar to an inducible defense trait—its expression is promoted by high temperature, high light, mechanical wounding and high nitrogen availability (Harley et al. 1999; Sharkey et al. 2008; Monson et al. 2012). Observations have also revealed higher emission rates during drought recovery periods (Fortunati et al. 2008; Tattini et al. 2015; Vanzo et al. 2015; Velikova et al. 2016, 2018), and in the isoprene-emitting species *Arundo donax* the increased emission rates were accompanied by increased production of selected phenylpropanoid compounds (Velikova et al. 2016; Ahrar et al. 2017). Generally, the seasonal environments that promote isoprene emission also provide reliable cues of herbivory risk—warm, high light, midsummer weather with high soil fertility, and/or following the stressful conditions of a drought. The work by Tattini et al. (2015) showed that metabolic adjustments that promote tolerance of abiotic stress extremes can also occur on the scale of hours, and are timed for midday stress extremes; including isoprene emission. Thus, like the cues for inducible defenses, high isoprene emission rates could condition plants to anticipate future episodes of combined abiotic and biotic stress on quite short time scales, providing an effective form of phenotypic plasticity.

Conclusions

The explanation of the effects of isoprene on plants proposed here 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. The presence of isoprene affects a number of transcription factors important in signaling processes involved in shikimate, phenylpropanoid, terpenoid and oxylipin synthesis, and in the production of numerous compounds involved in plant growth, defense, and abiotic stress tolerance. This suggests that isoprene can affect the outcome of several regulatory cascades. We presume that in some conditions the altered regulatory landscape must be deleterious, to account for the frequent losses of the isoprene emission trait. The research challenges that lie ahead include developing an improved context for how the multiple effects of isoprene are adjusted in the face of interactive influences involving growth, herbivory and threats from extreme climate stress, to optimize fitness. Improved knowledge of how environmental variation influences the processes that control trait tradeoffs, including those influenced by isoprene, will not only lead to more accurate ecological theories concerning plant adaptation, but will also facilitate better strategies for the development of sustainable agriculture. With the rapidly expanding opportunities for genetic modification using CRISPR-Cas9 technology, the possibility exists to develop finely-targeted strategies to mediate the G-D tradeoff to simultaneously improve yield through the combined effects of higher rates of biomass increase and lower rates of biomass loss. Isoprene might have an important role in the design of such strategies.

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Author contribution 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.

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

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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