Opportunistic emissions of volatile isoprenoids

Susan M. Owen and Josep Peñuelas

Opinión

Isoprenoid emissions and the opportunistic hypothesis

Isoprene, monoterpenes and sesquiterpenes are synthesized and emitted by some plant species, but not all plant species have this ability. These volatile, non-essential isoprenoid compounds share the same biochemical precursors as larger essential isoprenoids such as gibberellic acids and carotenoids. They have many protective and ecological functions for the plant species that produce them, but plant species that do not produce these compounds also grow and reproduce successfully. Here, we develop an ‘opportunist hypothesis’ suggesting that (i) volatile isoprenoid production takes advantage of dimethylallyl diphosphate (DMAPP) and its isomer isopentenyl diphosphate (IPP), which are synthesized primarily to produce essential isoprenoids, and (ii) conditions affecting synthesis of the higher isoprenoids will affect the production and emission of volatile isoprenoids.

By contrast, isoprene, monoterpenes and sesquiterpenes are volatile and semivolatile organic compounds (VOCs) that represent only a small proportion of the diverse group of isoprenoid plant products. They are not emitted by all plant species and so they can have no universally essential role in plant function. However, for the species that emit them, isoprenoid VOCs have certain ecophysiological roles that are, in non-emitting species, fulfilled by other compounds or other mechanisms. Isoprene is not stored but is emitted as soon as it is synthesized, as are monoterpenes from some species (e.g. Quercus ilex). Recent work suggests that isoprene acts on photosynthetic membranes to protect against thermal damage [5]. Other attempts to demonstrate a general thermoprotective role for isoprene have not been successful in all species studied [6]. It is also possible that isoprene can help to prevent oxidative damage of photosynthetic structures in cells [7]; this mechanism is similar to that described for carotenoids [8]. Some studies on non-stored monoterpenes have also indicated a thermor or photoprotective role for these compounds [9]. However, at least some instantly emitted monoterpenes do not confer thermotolerance, but might replace photosynthesis as a way to protect the photosynthetic apparatus at high temperatures under low O2 concentrations [10,11]. It has been suggested that all isoprenoids, including low molecular weight VOCs, represent an evolutionary pool for plant photoprotection [12]. For plants that emit these non-stored isoprenoid VOCs, these putative protective roles are useful, for example, in the relatively recent environment of elevated ozone episodes or for occasions on which leaf temperature temporarily soars. However, non-emitting species survive without these mechanisms.

Most monoterpenes and sesquiterpenes are synthesized and stored in special storage structures within plant tissues before emission. They have diverse ecological functions, such as antiherbivory and antimicrobial defence or pollinator attraction [13]. Some plant species have evolved the facility to produce these compounds as a response to stress (e.g. herbivore attack) [14]. Floral scents comprise a range of compounds, including isoprenoids, that can attract pollinators [15] and some monoterpeno emissions can attract predators of herbivores [16]. Certain biogenic monoterpenes have an allelopathic role, inhibiting the cytochrome pathway of respiration [17] or inhibiting growth and seed germination of neighbouring plants [18]. However, although these stored and induced...
isoprenoid VOCs have useful roles, non-terpene-emitting species also survive the onslaught of herbivores and competition, and can set seed.

It is not known to what extent an isoprenoid VOC-emitting plant will synthesize (and emit) isoprene and monoterpenes relative to the production needs of essential isoprenoid compounds, either for healthy plants at different stages of development or for plants in a stressed condition. Given that isoprenoid VOC emissions from plants are unavoidable as a result of their volatility [19], an ‘opportunist’ hypothesis was recently proposed [20] to suggest that there is not necessarily a specific role for every isoprenoid VOC emitted but that, in some cases, they are emitted purely because they are volatile and that natural selection has retained the benefits of these volatile compounds. The different functions of both essential and nonessential isoprenoids are summarized in Figure 2. In this article, we elaborate on the opportunist hypothesis to suggest that isoprenoid VOCs, although they are useful (and therefore retained) in some plants, are basically

Figure 1. Biosynthesis of isoprenoid compounds in plant foliage.
nonessential compounds. We suggest that isoprenoid VOCs might be making use of isoprenoid precursors (DMAPP and IPP) that could remain after the requirements are met for the synthesis of the essential isoprenoid compounds in some plant species. Over evolutionary time, the volatile isoprenoid-emitting trait might have been adapted in several ways to favour high or inducible volatile isoprenoid production. The opportunist hypothesis suggests that conditions affecting synthesis of the higher isoprenoids might affect production and emission of volatile isoprenoids.

**Resource allocation**

Isoprenoid VOC emissions can represent a large loss (up to 20%) of carbon fixed by photosynthesis [21]; several authors have tried to explain emissions in terms of ecological hypotheses of carbon allocation to either growth or to carbon-based secondary compounds with defensive or structural roles [22]. In some cases, the ecological models can contribute to predicting isoprenoid VOC emissions, such as light-dependent synthesis of terpenes filling storage pools during leaf expansion in conifers [23] and light-dependent terpene emission from Mediterranean Quercus spp. [24]. In other cases, emissions are not well predicted by carbon allocation models – for example, there is no proven increase in isoprenoid VOC emission from leaves growing in elevated CO2 concentrations.

Because carbon allocation hypotheses are not expected to explain changing requirements in a plant for essential isoprenoid production, it is not surprising that these hypotheses cannot consistently explain the production of isoprenoid VOCs. However, it is possible that regulation of DMAPP and IPP supply by the biosynthetic demands of essential isoprenoids might contribute to the control of isoprenoid VOC biosynthesis. There is no quantitative literature that reports the production rate of DMAPP or its relative allocation to volatile and essential isoprenoids throughout the life cycle of a plant. This would be a fruitful and interesting multidisciplinary line of research. It is possible that DMAPP production is tightly regulated to avoid wasting resources, but it is also possible that synthesis of essential isoprenoids is regulated by DMAPP supply, thereby reducing the need for synthesizing the many enzymes of isoprenoid synthesis. If the precursors for essential isoprenoid compounds are synthesized in excess to requirements then all plants are only a single enzyme away from synthesizing isoprene [25].

**Biochemistry of isoprenoid synthesis**

Isoprenoids are synthesized in all green plants through the condensation of the five-carbon intermediates IPP and DMAPP (Figure 1) (see Ref. [13] for a review of the evolutionary and functional history of the two pathways for IPP and DMAPP synthesis). Some exchange and/or cooperation is thought to exist between these two pathways [26,27]. The two pathways probably operate under different physiological conditions within the cell and depend on the developmental state of plastids [27]. In an investigation of enzyme activity in Quercus robur leaf extracts, IPP isomerase activity was always greater than isoprene synthase activity, which in turn was greater than isoprene emission rate [28]. This is not surprising in the context of our opportunist hypothesis because we would expect production of DMAPP for essential isoprenoids over and above that for nonessential compounds. The availability of cellular DMAPP is an important regulatory
factor of leaf isoprene emission [29]. This suggests that, if essential isoprenoid compound synthesis was needed for urgent repair or protection (for example), DMAPP pools might be diminished for a while, which would reduce isoprene emission rates.

**Essential isoprenoid biosynthesis**

Controls of essential isoprenoid biosynthesis include genetic control by the DNA encoding the biosynthesis pathway enzymes, control by enzyme activity and control by the availability of the enzyme substrate. Several studies have investigated the effect of altering gene and enzyme activity on the production of a target-essential isoprenoid, as well as on the concentrations of other essential isoprenoid compounds [30]. For example, tomato plants were transformed with a copy of the cytosolic synthase cDNA that is expressed in fruit [31]. Phytoene synthase converts geranylgeranyl diphosphate to phytoene and thereby diverts this intermediate away from the gibberellin and phytohormone biosynthetic pathways (Figure 1). Transformed plants were dwarves, with a reduction by a factor of 30 in levels of gibberellins, and showed ectopic production of carotenoids [31]. This shows that rerouting of isoprenoid biosynthesis intermediates is possible, so it is reasonable to expect that, in some plant species, non-essential isoprenoid VOC synthesis is the result of using excess of IPP and DMAPP.

In a similar experiment, transgenic *Arabidopsis* plants were produced that over- or underproduced deoxyxylulose-5-phosphate synthase (DXS) [32]. This is the enzyme that catalyses the first step of one pathway producing IPP and hence DMAPP. Transgenic plants that overproduced DXS accumulated greater concentrations of various isoprenoids (e.g. chlorophylls, tocopherols, carotenoids, abscisic acid and gibberellins) than did wild-type plants, whereas underproduction of DXS resulted in lower concentrations of these compounds. It would be interesting to repeat this experiment with a species that emits high levels of volatile isoprenoids, or with *Arabidopsis* transformed with an isoprene synthase-encoding gene. This would determine whether or not volatile isoprenoid synthesis is sufficiently important for a plant to sustain in conditions where high rates of essential isoprenoid synthesis draw heavily on pools of IPP and DMAPP.

**Biosynthesis of nonessential isoprenoid VOCs**

To our knowledge, there are no studies investigating the biosynthesis of monoterpene VOCs in relation to DMAPP, so the discussion here is restricted to the biosynthesis of isoprene. Recently, researchers have explored the relationship between DMAPP availability and production, and isoprene emission rates. Control of isoprene by isoprene synthase activity rather than by DMAPP supply has been demonstrated, but only under conditions of saturating DMAPP [33,34]. Indeed, DMAPP is not always present in excess for isoprene production [35]. Regulation of isoprene emissions by availability of DMAPP has also been suggested [36]. These findings support the opportunist hypothesis of isoprenoid VOC production: that the demands on the DMAPP pool in leaf tissue are multifarious and that, at any time, the plant might need to synthesize a wide range of isoprenoid compounds for growth, reproduction, repair, constitutive protection and protective response to stress. This might contribute to the control of isoprenoid VOC synthesis and emissions.

A large variation in DMAPP concentrations was found between plant species, with typical midday concentrations of 0.13–3.00 mM for chloroplastic DMAPP in leaves of *Populus deltoides* [35]. Absolute concentrations of DMAPP varied hugely regardless of whether the species were isoprene emitters but, generally, isoprene emitters contained the highest concentrations of DMAPP. Emitters generally showed an increase in DMAPP levels between the pre-dawn and midday measurements. However, species that did not emit isoprene also had light-dependent DMAPP synthesis, which could support the production of higher isoprenoids during periods of high photosynthetic activity, including the replacement and repair of chlorophylls, carotenoids and pigment–protein complexes that become damaged at high light intensities [35]. Because light-dependent DMAPP synthesis is not restricted to isoprene-emitting plants, it follows that light-dependent DMAPP biosynthesis did not evolve only for light-dependent isoprene emissions. Instead, isoprene-emitting plants capitalize on a biochemical infrastructure that primarily services the synthesis of essential isoprenoids. This offers further support for the opportunist hypothesis. However, further studies of turnover rates of DMAPP, isoprene (and other isoprenoid VOCs) and essential isoprenoids are required for quantitative proof of the concept. Similarly, young isoprene-emitting leaves of *P. deltoides* contained significantly less DMAPP than did fully mature leaves [35], possibly because of the depletion of DMAPP pools as a result of increased chlorophyll and carotenoid biosynthesis during leaf development. This supports the observations of delay of isoprene emissions in young plants [37] and is also consistent with the opportunistic hypothesis.

We suggest that the rate of IPP and DMAPP synthesis is normally controlled partly on demand by metabolic ‘pulling’ from downstream essential isoprenoid synthesis pathways and partly by metabolic ‘pushing’ by photosynthate accumulation. Metabolic pushing could be the result of the observed relationship between passive phloem loading and the ability to emit isoprene [38]. Any elevation in concentrations of IPP (and DMAPP) resulting from these metabolic pushes and pulls might opportunistically provide the raw materials for isoprenoid VOC biosynthesis in isoprene-emitting plant species.

A continuous supply of IPP and DMAPP is needed to produce essential isoprenoids in the chloroplasts; it has been suggested that isoprene synthase functions as a safety device to prevent unnecessary accumulation of sequestered phosphate in the form of DMAPP [39]. We postulate that the role of volatile isoprenoids in using excess IPP and DMAPP precursors might be a contribution to other mechanisms for regulating concentrations of DMAPP and IPP that are likely to exist in all plant species. For example, cytosolic competition for phosphoenolpyruvate regulates chloroplast DMAPP supply [39]. Furthermore, a negative feedback of DMAPP (or other
Monoterpenes are produced by plants and are a common group of volatile organic compounds (VOCs). Different environmental and ecological stresses, particularly herbivory, affect emission rates of isoprene and monoterpenes from different plant species. For example, monoterpene emission rates are increased by mechanical and water stress resulting from injury or herbivory. Studies of the stress effects of atmospheric pollutants such as elevated tropospheric ozone concentrations on isoprenoid VOC emissions are conflicting, but isoprenoid VOC emissions from native Mediterranean species and from Pinus sylvestris increased overall at higher tropospheric ozone concentrations. Extremes of radiation and temperature can also affect isoprenoid VOC emissions, as can nutrient stress linked to an excess or deficiency of minerals in the soil, soil salinity and soil pH. Limited water availability can restrict terpenoid biosynthesis. Moderate water stress has no effect on isoprenoid VOC emissions from young plants but more severe drought reduces emissions.

These observations might be partly explained by changes in DMAPP allocation in the isoprenoid biochemical pathway to respond chemically to the needs of the stressed plant. For example, carotenoids (e.g. lycopene, rhodoxanthin and others) were found to accumulate in green tissues experiencing stress conditions. These essential isoprenoids provide efficient protection against oxidative stress. This example, and indeed any demand for essential isoprenoids (e.g. carotenoids, abscisic acid or gibberellic acid) under stress conditions, might change the magnitude of isoprenoid VOC emissions according to the opportunistic hypothesis. To our knowledge, this has not yet been investigated and it warrants further study. The rate of turnover of DMAPP and isoprenoids are more meaningful measures of competition between different isoprenoids for DMAPP resources, but turnover rates for different isoprenoids under different conditions have not been measured. Thus, the relative concentrations of isoprenoid VOCs [expressed as μg (g leaf tissue)^−1] might not be comparable to the increase or decrease in essential isoprenoid concentrations in stressed conditions unless turnover rates are also taken into account.

**Whole plant investigations**

Biosynthesis occurs at the subcellular level but whole-plant events also exert some control over biosynthetic activities. For example, a decrease in isoprene emissions was observed from leaves of young P. deltoides plants that had been partially defoliated. Isoprene emission rate was closely related to the number of leaves remaining on the shoot in defoliated plants. This was explained in terms of increased export of carbon from source leaves as a stress response, leaving less carbon for nonessential isoprenoid synthesis. Whereas isoprene emission is controlled at the leaf level in undamaged plants, emissions from leaves on damaged plants are controlled by whole branch allocation of carbon resources. These findings support the opportunistic hypothesis, which predicts lower isoprenoid VOC emissions from partially defoliated plants in simple terms of IPP and DMAPP pools being required for necessary isoprenoid stress response compounds.

Extending the idea of whole plant allocation of resources, some plants (such as oil palm and some banana species) that form large carbohydrate stores in their fruits also emit isoprenoid VOCs. However, it is not known whether they emit lower levels of isoprenoid VOCs when
fruits are forming. Considerable isoprenoid hormone and pigment formation is associated with carbohydrate storage, flower, seed and fruit production. However, there is no comparative study of concentrations of essential isoprenoids and isoprenoid VOC emissions from fruiting species for the complete duration of their life cycle. Northern latitude and Mediterranean forest species with high emissions of isoprenoid VOCs show a decline in emissions as the season progresses [49,50]. Does this reflect a diversion of DMAPP and IPP from nonessential isoprenoid VOCs to essential isoprenoid pools and compounds associated with propagating the next generation? Observed seasonal variation in isoprenoid VOC emissions and unexplained variability in emission rate measurements from a single species might be caused by the more important demands of hormone and pigment biosynthesis, and rate of carbon storage.

**Conclusions**

For isoprenoid-emitting species, isoprene functions at short temporal scales, at the small spatial scale of the plant cell or even cell parts. Some non-stored monoterpene emissions appear to play similar roles to isoprene and there are many reports of the rapidly inducible production of nonessential isoprenoids as a response to stress [14]. Monoterpene emissions from stored pools also play important roles at longer time scales and at larger spatial scales of tissue, organ, plant and habitat. Although these roles are important for the isoprenoid-emitting species, they are not ubiquitous. We suggest that emissions of isoprenoid VOCs should be considered in the context of the larger isoprenoid synthesis scheme, in terms of DMAPP and IPP precursor allocation and provide support from literature (Box 1). The implications might explain some of the variability in isoprenoid VOC emission potentials on both short and longer time scales (Figure 3). Although turnover of essential isoprenoids is often slow, there are also episodes of quick *de novo* synthesis, for example, to repair tissue damage, to deal with biotic or abiotic stress, or to contribute to pigment accumulation. We suggest that DMAPP pools in plant tissue are synthesized for the essential isoprenoids and that variations in the demands of a plant for essential isoprenoids might contribute to the observed variability in isoprenoid VOC emission potential. In the absence of data from experiments designed specifically to explore this hypothesis, there is considerable potential for further research.

**Acknowledgements**

We thank the anonymous reviewers for constructive and helpful comments. Our research was partly supported by ISONET (Marie Curie network contract MC-RTN-CT-2003 504720) from the European Union, by grants REN2003-04871 and CGL2004-01402/BOS from the Spanish Government, and by a 2004 grant from the Fundación BBVA.

**References**


---

**Figure 3.** Principles of the opportunist hypothesis for synthesis and emission of isoprenoid volatile organic compounds. Environmental factors controlling isoprenoid and biomass synthesis are indicated by broken arrows. Biomass and whole plant control of dimethylallyl diphosphate (DMAPP) is indicated by the green arrow. The synthesis of essential isoprenoids affects DMAPP concentrations and turnover rate (purple arrow). Abbreviations: PAR, photosynthetically active radiation.
50 Hakola, H. et al. (2003) Seasonal variation of VOC concentrations above a boreal coniferous forest. Atmos. Environ. 37, 1623–1634