Tree Physiology

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Biology, Controls and Models of Tree Volatile Organic Compound Emissions

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Preface

Ülo Niinemets and Russell K. Monson

Discoveries that plants are in part responsible for the blue haze commonly observed in the atmosphere above many forests (Went 1960; Rasmussen and Went 1965) and for the tropospheric ozone pollution in many forested urban and suburban areas (Rasmussen 1972; Chameides et al. 1988) have compelled researchers to ask what are the plants emitting, how much is being emitted and how do these emissions impact our environment? These very important questions are at the heart of a highly interdisciplinary research field: biogenic volatile organic compounds (BVOC) in the biosphere–atmosphere component of the Earth system.

Fritz Went, a plant physiologist famous for discoveries on plant growth regulators, was also intrigued by the potential for plants to form atmospheric hazes above forests. He hypothesized that the organic compounds emitted from plants, many of which he could detect with his own sense of smell, contributed to this haze. In 1960, he made measurements on the mass of leaf oils in shrubs from the Western United States and estimated global terpene emissions to be 175 Tg C year^{-1} (1 Tg $= 10^{12}$ g) (Went 1960). Since that seminal study, estimates of global BVOC emissions (excluding methane) have been refined through coupled vegetation–atmosphere models and better maps of global vegetation. Currently, the worldwide emissions are estimated to be around 1 Pg C year^{-1} (1 Pg $= 10^{15}$ g) (Guenther et al. 2012). Much of the past research on plant volatile emissions has been, and continues to be, on the simple C5 volatile hydrocarbon, isoprene, which is emitted from leaves in a light- and temperature-dependent manner and is coupled to the metabolic processes of photosynthesis (Sanadze 1956; Rasmussen and Went 1965; Rasmussen 1970). Since its initial discovery, the research on biogenic isoprene has

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focused on understanding its source strength and distribution among different plant taxa, resulting in the construction of first list of isoprene-emitting plants (Rasmussen 1978) and first biogenic emission inventory system (BEIS, Pierce and Waldruff 1991), followed by more biologically oriented emission algorithms and inventories (e.g., Guenther et al. 1991, 1994, 2006, 2012; Arneth et al. 2007; Monson et al. 2012). By now, it is widely acknowledged that multiple plant BVOC emissions play a major role in atmospheric dynamics within the Earth system with highly reactive compounds reacting in the gas phase to affect atmospheric chemistry, including influences on less reactive compounds that affect the atmosphere's global warming potential, e.g., methane (Fuentes et al. 2000). Furthermore, condensation of photo-oxidation products of BVOCs leads to the formation of secondary organic aerosols, which also generate cloud condensation nuclei and have profound implications for the Earth's solar radiation budget and climate (Kulmala et al. 2004; Huff Hartz et al. 2005; Hallquist et al. 2009; Arneth et al. 2010). Trees have been traditionally thought to contribute the most to BVOC emissions due to the presence of multiple tree species with high emission rates (e.g., the oaks and poplars) and large aerial coverage, which gives forests a unique global role in regulating atmospheric chemistry. Apart from strong constitutive emitters, emissions induced by abiotic and biotic stresses have also been identified in many important forest species previously considered "non-emitters" (Holopainen and Gershenzon 2010; Loreto and Schnitzler 2010; Niinemets 2010), implying that there are strong biological controls on the emissions of BVOCs that we still do not understand and that continue to cause large uncertainties in our models and inventories.

From a biological perspective, the key question has been why do plants emit these volatiles? What are the costs and benefits for the plant, and why do some plant species emit these compounds constitutively and in others the emissions must be induced? The role of isoprene emissions was unknown until the mid-1990s when it was discovered that isoprene enhances plant thermotolerance (Sharkey and Singsaas 1995) and increases the resistance of plant metabolism to atmospheric oxidants (Loreto et al. 2001), followed by the development of a hypothesis of general enhancement of cellular oxidative stress tolerance (Vickers et al. 2009a). Lately, breakthroughs in the genetic engineering of plants have allowed us to test these hypotheses with unprecedented specificity in the processes affected (Behnke et al. 2007, 2010; Vickers et al. 2009b; Velikova et al. 2011). Rapid developments in the availability of genomic information have also recently allowed researchers to gain insight into evolutionary patterns and past influences by natural selection on the isoprene emission capacity (Monson et al. 2013; Sharkey et al. 2013). Furthermore, the role of multiple plant BVOCs in abiotic and biotic stress signalling within and among plants, and among plants and insects has drawn strong attention from a broader complement of the ecological research community (Owen and Peñuelas 2005; Dicke et al. 2009; Dicke and Baldwin 2010; Holopainen and Gershenzon 2010).

In the past, three comprehensive treatises have been published on trace gas emission from vegetation (Sharkey et al. 1991; Hewitt 1999; Gasche et al. 2003). Only the book by Sharkey et al. (1991) deals exclusively with biological controls

on BVOC, while the book by Hewitt (1999) focuses on quantification of emissions, and the book by Gasche et al. (2003) only briefly considers biological controls. Since the publication of these books, there has been rapid progress in understanding the controls over isoprene emissions, including the discovery of isoprene synthase (Silver and Fall 1991), its sequence in several organisms (e.g., Miller et al. 2001; Vickers et al. 2010; Sharkey et al. 2013) and establishment of the reaction mechanism (Köksal et al. 2010). Furthermore, there has been a significant increase in our knowledge of multiple terpene synthase structures and regulation (Bohlmann et al. 1998; Fischbach et al. 2001; Degenhardt et al. 2009; Chen et al. 2011; Keeling et al. 2011; Köksal et al. 2011). Due to the development of sensors and detection technology that was in its infancy at the time of the appearance of the book edited by Hewitt (1999), a large volume of literature has emerged on BVOC flux quantifications (Karl et al. 2002, 2007; Müller et al. 2010). Finally, since the publication of the three past books, the field of BVOC emissions has matured greatly in our understanding of BVOC emission models and their connection to underlying metabolic processes (Monson et al. 2012).

This book intends to cover all biological scales of organization from molecular to globe and to make a major leap in summarizing and synthesizing the existing information accumulated since the publication of the past comprehensive summaries of BVOC emissions. The book consists of 17 primary chapters followed by a synthesis. The chapters focus on four major topics: evolutionary diversification and perspectives for genetic engineering of volatile organic compound emissions (chapters 1–4), controls over emissions (chapters 5–7), emissions under stress (chapters 8–11), and emission models (chapters 12–17).

Chapters 1–4 cover tree BVOC emission diversity and evolutionary aspects as driven by abiotic (chapter of Fineschi et al.) and biotic (chapter of Trowbridge and Stoy) stresses, and molecular diversity (chapters of Rajabi Memari et al. and Rosenkranz and Schnitzler), specifically asking how and why the capacity for constitutive volatile production has evolved, what are the key biochemical pathways and factors that determine the blend of emitted volatiles. Here also the diversity in elicited emissions (chapter of Trowbridge and Stoy) and ways of genetic modification to alter emissions (chapter of Rosenkranz and Schnitzler) are analysed.

Chapters 5–7 provide an overview of the cellular and leaf-level mechanisms controlling BVOC emissions. The group of these chapters starts with short-term molecular pathway and leaf-level metabolic controls (chapters of Li and Sharkey, and Monson), then covers longer-term controls by carbon availability and gene expression level acclimation responses to environmental variability (chapter of Monson) and finishes with stomatal and physico-chemical controls driven by variations in compound volatility (chapter of Harley).

Chapters 8–11 review the effects of abiotic and biotic stresses on volatile emissions. Consideration of this topic starts with a chapter analysing the modification of tree abiotic stress tolerance by the capacity for volatile emissions, emphasizing tolerance to heat, drought, salinity and resulting oxidative stress (chapter of Possell and Loreto). Then responses of emissions to flooding (chapter of Kreutzwieser and Rennenberg) and air pollution and elevated [CO_2] (chapter of Calfapietra et al.)

are reviewed. This topic is concluded with a chapter investigating the multitrophic interactions between trees, herbivores and herbivore enemies in future polluted atmospheres that significantly alter the role of volatile compounds as important ecological signals that organize food webs and host parasite relations (chapter of Holopainen et al.).

The chapters 12–17 put the information summarized in previous chapters into the quantitative framework of predictive emission models. This topic starts with a review of leaf-level emission algorithms (chapter of Grote et al.), then covers canopy and landscape (chapters of Niinemets et al. and Guenther) and biome level models (chapter of Ashworth et al.), emphasizing the philosophy of model parameterization and validation. Finally, global feedbacks to climate and atmospheric composition due to tree BVOC emissions are analysed (chapter of Kulmala et al.).

The book concludes with a synthesis section that puts the contents of the book into global biosphere–atmosphere science context and provides a perspective for future developments in the field. In addition to summarizing the state-of-the-art information of tree volatile emissions, the book, in particular, explores the ways that rich archives of molecular, physiological and ecological evidence can be best included in quantitative emission models. The content and focus of the chapters are intended for use by graduate students, researchers and policy professionals interested in the recent developments in the field. As in many interdisciplinary, complex and rich science fields, the challenge in BVOC research is to get to an intellectual place beyond the fragmented, and increasingly more detailed, knowledge provided in the primary literature to one of synthetic understanding, utility for framing further hypotheses and application to devising observation and regulation policies that improve the global human condition. We hope that this book can serve as a springboard for those interested in starting a career in BVOC research, those continuing to pursue research at the frontier of this fascinating field and those interested in applying the research to the better management of our Earth system.

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Chapter 1
Diversification of Volatile Isoprenoid Emissions from Trees: Evolutionary and Ecological Perspectives

Silvia Fineschi, Francesco Loreto, Michael Staudt, and Josep Peñuelas

Abstract Not all tree species are strong constitutive volatile compound emitters, and a variety of hypotheses have been put forward to explain the evolution and the function of the emissions of volatile compounds. This chapter reviews the evolutionary and ecological aspects of volatile compound production in trees, specifically asking how and in which tree species the capacity for constitutive volatile production has evolved. The capacity for volatile emissions is a polyphyletic trait present in several diverse plant groups, but the presence of emission capacity is not directly related to phylogenetic distance among the species and species genera, demonstrating that the trait has evolved multiple times during evolution. We here review present volatile emission inventories highlighting the need for more worldwide, coordinated efforts to obtain realistic data of geographical and taxonomic patterns. We thereafter discuss the past evolution of isoprenoid emissions, and pose the questions of why isoprene emission is particularly widespread in hygrophytes, why it is a characteristic of mostly fast-growing perennial plants and why it is stimulated by low concentrations of CO_2. Finally, we discuss the future, how climate and global change and the corresponding ecological constraints impact the diversification and emission of volatile organic compounds from plants.

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1.1 Introduction: The Spectrum of Volatile Organic Compounds in Plants and the Importance of Constitutive Volatile Isoprenoids

The emission of biogenic volatile organic compounds (BVOCs) by plants was first reported in the 1950s and 1960s (see the Preface; Rasmussen and Went 1965; Sanadze and Kalandaze 1966) and has been studied since by plant biologists and atmospheric chemists. The amount of hydrocarbons emitted into the atmosphere by plants as BVOCs far exceeds the levels from human activity (Sharkey et al. 2008; Peñuelas and Staudt 2010). The large scale of emission, the reactive properties, and the large capacity of many organisms to sense them render several of these products of secondary plant metabolism as particularly important for the chemical and physical properties of the atmosphere. BVOCs also play a critical role in the interaction between the biosphere and atmosphere, in addition to their role in communication amongst organisms (Peñuelas et al. 2009a, b; Dicke and Loreto 2010).

Plants produce several secondary metabolites, which can be classified in three main groups: isoprenoids, phenolics, and nitrogenous compounds. Volatile isoprenoids (isoprene, monoterpenes, and some sesquiterpenes) can be either directly emitted, as is always the case for isoprene, or stored in resin ducts or in glandular trichomes for later release, especially upon mechanical stress. All plant organs can emit isoprenoids; vegetative organs such as leaves and branches (Loreto and Schnitzler 2010), flowers (Knudsen et al. 1993), and roots can all emit volatiles (Steeghs et al. 2004). Volatile isoprenoids are generally easily smelled (e.g., the typical fragrance of flowers, resin, and conifer needles), but isoprene, at its natural concentration, cannot be detected by human olfactory system. Plants release a very large amount of carbon as isoprene, estimated to be more than 500 Tg isoprene $year^{-1}$ (Arneth et al. 2008; Ashworth et al. 2013), more than an order of magnitude of the amount of isoprene emitted by animals (Sharkey and Loreto 1993). The emission of volatile isoprenoids is widespread in terrestrial plants, with no apparent evolutionary or ecological gradient (see below), but high isoprene emitters are generally only trees or perennials. For the purposes of this book on tree physiology, we will therefore present evolutionary and ecological considerations related mostly to isoprene emission.

The reason why plants re-emit up to 10 % of their fixed carbon as isoprenoids (and a much larger proportion under conditions of stress that substantially inhibit photosynthesis) remains a debated issue (Sharkey and Yeh 2001; Peñuelas and Llusià 2004; Vickers et al. 2009) despite the many studies of isoprenoid emission. Plants very likely invest such high metabolic costs to protect vital organs from both biotic (insects and pathogens) and abiotic (especially extreme temperatures and atmospheric pollutants) stresses (Possell and Loreto 2013). Emissions, however, appear to be "specialized", because not all isoprenoids function in plant defence. Isoprene was not believed to have a role in defence against biotic stressors until two independent reports indicated its capacity to repel insects (Laothawornkitkul et al. 2008; Loivamäki et al. 2008). On the other hand, the finding that isoprene (and

volatile isoprenoids) protects against abiotic stressors, especially high temperatures (Sharkey and Singsaas 1995; Peñuelas et al. 2005; Velikova et al. 2011) and oxidative stress (Loreto and Velikova 2001; Peñuelas and Llusià 2002; Vickers et al. 2009; Possell and Loreto 2013), has repeatedly been confirmed experimentally. The mechanisms by which isoprene produces its protective effects are also topics of debate. The ideas that isoprene strengthens thylakoid membranes or scavenges reactive oxygen species have been discussed (reviewed by Loreto and Schnitzler 2010). Velikova et al. (2012) have recently suggested that membrane stabilisation may also reduce the production of reactive oxygen and nitrogen species under conditions of stress, thus, reconciling the two putative roles of isoprene. Despite the prominent role of isoprene in plant protection against abiotic stressors, only a relatively few extant plants emit large amounts of isoprene (less than 30 % of the woody species examined to date), the trait having been lost multiple times during the course of evolution (Harley et al. 1999, see below). Another intriguing observation is that plants do not generally emit both isoprene and monoterpenes, but with exceptions, especially in isoprenoid-storing plants. This feature has often been observed and was discussed in detail by Harrison et al. (2013). Even within a single plant species, leaves specialize in emitting either monoterpenes or isoprene at different times during ontogeny, with an apparent trade-off of carbon between the different biosynthetic pathways (Brilli et al. 2009).

Constitutive emissions of monoterpenes may have the same role as that of isoprene. If the mechanism of membrane stabilisation is through lipid solubility and the capacity to delocalize electrons by conjugated double bonds (Loreto and Schnitzler 2010), then several monoterpenes can replace isoprene, as is often seen (e.g., Loreto et al. 2004). Monoterpenes, though, are possibly formed at lower rates, an issue difficult to resolve due to the frequent accumulation of monoterpenes in storage pools and despite the fact that twice as much carbon is required to construct monoterpene skeletons as isoprene skeletons.

Plants that emit monoterpenes and sesquiterpenes, however, accumulate isoprenoids in specialized organs in leaves, stems, or trunks and then massively release them after wounding. The main role of these compounds is thus to act as powerful deterrents to pathogens and herbivores (Dicke and Baldwin 2010) and to contribute to the sealing of wounds (Loreto et al. 2008). Constitutive and induced emissions of volatile isoprenoids may be stimulated by mechanical stresses and metabolic changes in plants attacked by herbivores. The emitted isoprenoids can directly attract or deter herbivores (*direct defence*) or attract parasitoids or predators of herbivores, thus, eliciting an *indirectly induced defence* resulting from the interaction of plants with insects of the third trophic level, i.e., carnivores (Llusià and Peñuelas 2001; Dicke et al. 2003a, b; Matthes et al. 2010; Dicke and Baldwin 2010; Trowbridge and Stoy 2013). After the first report of the interactions amongst the three trophic levels (Price et al. 1980), these relationships became an attractive area for interdisciplinary research involving evolutionary biology, ecology, and plant physiology. Volatile isoprenoids may also activate mutualisms, e.g., with pollinators or ants (Farré-Armengol et al. 2013). In insect-plant mutualisms, the partners involved play different roles. The sedentary partner (the plant) must be easily located

and must offer rewards (mostly food) to the mobile partner (the insect), who offers a service but who also has a choice whether or not to visit a particular individual (Farré-Armengol et al. 2013). This relationship implies that plants have evolved particular traits contributing to mutualism, whereas insects have not. In fact, the behavioral repertoire of mutualistic insects is no different from that of their non-mutualistic relatives. Such an asymmetry in trait evolution is particularly evident in more generalized insect-plant mutualisms (Bronstein et al. 2006).

Phylogenetic studies suggest that insect-plant mutualisms, despite their ecological importance, may have appeared and disappeared several times throughout evolutionary history, as has isoprene emission (see below). Pollination and seed dispersal, where physical factors such as wind, water, or gravity have replaced insect- and bird-facilitated dispersal (Bronstein et al. 2006), clearly illustrate this adaptability. Whether changes in BVOC emissions have also played a role in these shifts is not clear, but is a very interesting question. Notably, in the evolution of plants, interactions with pollinators and seed dispersers appeared after herbivory and environmental stresses had already shaped evolution of plants (Farré-Armengol et al. 2013). More generally, understanding the trade-offs between the costs and benefits of emitting volatiles is challenging and important from the viewpoint of evolutionary ecology.

1.2 The Present: Inventories of Volatile Isoprenoids

1.2.1 State-of-the-Art of Emission Inventories

Numerous screening studies have been performed since the 1970s to identify plant species that emit BVOCs. Beginning in North America but later rapidly expanding to Europe and other continents, most of these studies have focused on isoprene, the most abundant BVOC. The results of these species inventories, together with data on canopy densities and species abundances, were used to calculate BVOC-emission capacities of landscapes that were further integrated into emission models to estimate BVOC fluxes at regional, continental, and global scales (Guenther et al. 2006; Ashworth et al. 2013; Guenther 2013).

In addition to their usefulness in estimating emission inventories, data of the relative abundancies of isoprene-emitting species in regional floras may improve our understanding of the evolutionary origins and ecological significance of isoprene emissions. If isoprene production confers significant protection against stresses at non-negligible metabolic costs, then the patterns of species abundance and species dominance of isoprene emitters may vary worldwide according to site-specific conditions. Terrestrial ecosystems, though, differ greatly in age. While communities of tropical forests may be many millions of years old, those at higher latitudes emerged from ice-age refugia only a few thousand years ago and they may still be far from equilibrium. The adaptive significance of isoprene production in younger, often oligospecific plant communities may differ from that in older, often highly

diversified communities, independent of differences in the occurrence of abiotic stresses associated with each climate. Furthermore, isoprene emissions may have initially evolved in only a limited number of plant taxa whose distributions depend not only on their competitiveness and the potential role of isoprene production therein, but also on the extent of their past geographical isolation that was governed by geological events such as continental drift and changes in sea level.

Monson and co-workers (2013) recently combined a large dataset of isoprene emission with DNA sequence data to reconstruct the taxonomic distribution and evolutionary history of isoprene emitters throughout the plant kingdom. Their compilation found that all major groups of Gymnosperms (Pinophyta) and Angiosperms (Magnoliophyta) contain both isoprene-emitting and non-emitting species, with perhaps a few exceptions. Isoprene emitters seem to be particularly rare in the subclass Asteridae that consists of modern and highly derived plant clades, many of which are herbaceous species. Isoprene emitters are also rare in the subclass Magnoliidae, which is considered a rather ancient clade within the Magnoliophyta comprising numerous trees and shrubs in tropical and subtropical areas around the world. Nevertheless, isoprene-emission capacity appears to be associated with the perennial lifestyle and to be particularly frequent in woody plant species. Isoprene emission is also present in Pinophyta trees, but emissions appear to be generally low and limited to a few genera (e.g., *Abies*).

We examined about 30 emission inventories for the presence of isoprene-emitting species in the floras form various ecosystems and biomes. From each inventory, we calculated the fraction of isoprene-emitting species per vegetation type, defining isoprene emitters as species with reported emission rates clearly exceeding 1 $\mu g\ g^{-1}\ h^{-1}$ (ca. 4 $pmol\ g^{-1}\ s^{-1}$). We did not include species with taxonomically assigned emission properties unless we found reliable corroborating information from other sources. Figure 1.1 displays the mean fraction of emitters per biome. These results imply a relatively homogenous presence of isoprene emitters throughout the world's major biomes, with mean fractions only ranging between about 20–35 %. The range is, however, considerably larger when comparing individual screening studies. For example, the fraction of isoprene-emitting species reported at various locales in China (Fig. 1.2) extends from 10 %, reported by Klinger et al. (2002) amongst 67 species in the humid forests of the Ailao Mountains, to 46 %, observed by Geron et al. (2006a) amongst 95 species screened in the tropical Biological Gardens of Xishuangbanna. Worldwide, the average fraction of isoprene-emitting species across all screening studies considered here is 29 %, which is close to the values we have extracted from the global BVOC databases of NCAR and Lancaster University (approximately 32 and 33 %, respectively, for isoprene-emission potentials $>1\ \mu g\ g^{-1}\ h^{-1}$).

Inferring trends from the geographical variation of the fractions displayed in Figs. 1.1 and 1.2 is appealing. For example, the relative presence of isoprene emitters in the floras of tropical and subtropical America tends to decrease from wet to dry forests, savannas, and desert shrublands. The forests of cold highlands and boreal regions seem to have fewer isoprene emitters than temperate forests (Figs. 1.1 and 1.2), whereas the flora of suburban areas may be particularly rich in isoprene

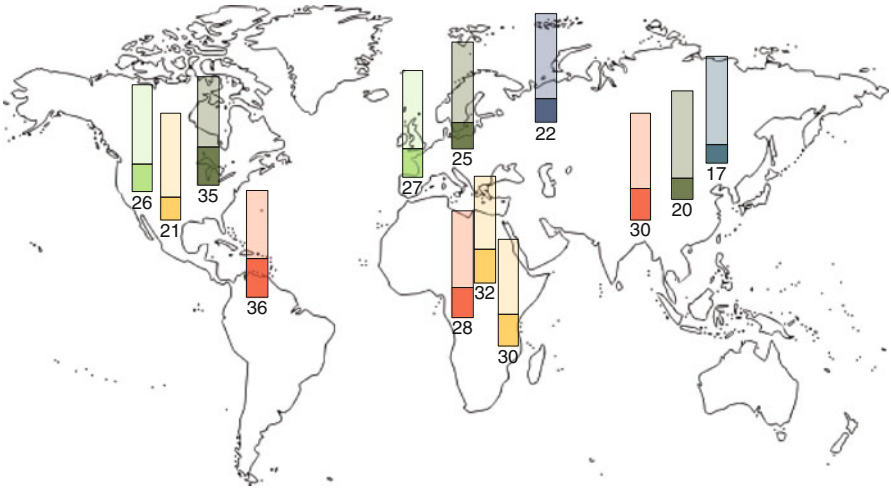

Fig. 1.1 Mean fraction of isoprene-emitting plant species in the floras of various ecosystems around the world (numbers are percentages of isoprene emitters). The colours roughly denote climate classes. *Red*: moist tropical; *yellow*: dry (sub)tropical; *light green*: dry temperate; *dark green*: moist temperate; *blue*: cold (References: Arey et al. 1995; Bracho-Nunez et al. 2012; Chang et al. 2012; Geron et al. 2002, 2006a, b; Guenther et al. 1996a, b, 1999; Harley et al. 2003, 2004; Isebrands et al. 1999; Helmig et al. 1999; Karl et al. 2009; Karlik and Winer 2001; Keenan et al. 2009; Keller and Lerdau 1999; Klinger et al. 1998, 2002; Lerdau and Keller 1997; Lerdau and Throop 1999; Lindfors et al. 2000; Owen et al. 2001; Parra et al. 2004; Rinne et al. 2009; Singh et al. 2008; Tsui et al. 2009; Wang et al. 2003; Xiaoshan et al. 2000; BVOC emission databases of the National Center for Atmospheric Research (http://bvoc.acd.ucar.edu) and the Lancaster University (http://www.es.lancs.ac.uk/cnhgroup/iso-emissions.pdf))

emitters (Beijing, Hangzhou, and Hong Kong in Fig. 1.2). Many ornamental shrubs and trees used for private gardening and urban shading are isoprene emitters that contribute to the atmospheric load of BVOCs in these areas (Niinemets and Peñuelas 2008). Few studies have examined the abundance of isoprene emitters along geographical, climatic/edaphic, and successional gradients in different continents and biomes (Klinger et al. 1994, 1998, 2002; Martin and Guenther 1995). The results of these studies collectively suggest that isoprene emitters, often associated with fast-growing woody species, are more frequent in transitional, mid-successional forests than in late "climax" forests that are dominated by evergreen monoterpene-emitting plants (Harrison et al. 2013).

1.2.2 What Are We Missing in Emission Inventories?

The current species inventories, however, can only be taken as rough estimates of the true prevalence of isoprene emitters in terrestrial ecosystems. The fraction of isoprene emitters determined by screening studies at one location can differ by as

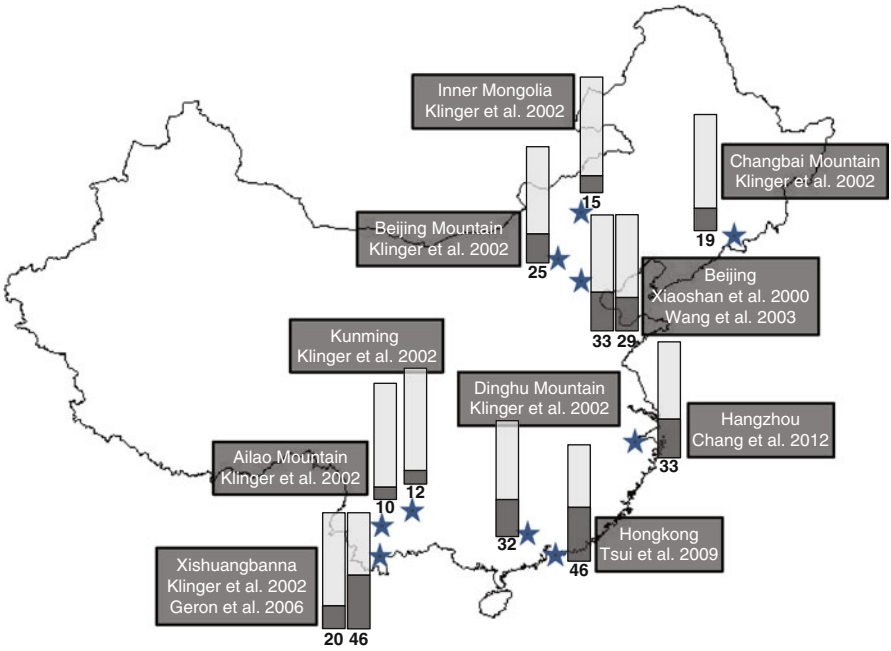

Fig. 1.2 Fractions of isoprene-emitting plant species observed in screening studies conducted in various regions of China (*numbers* are percentages). *Asterisks* indicate the approximate locations of the studies

much as a factor of two (for example, see the inventories of the Xishuangbanna region in southern China in Fig. 1.2). Inventories are unreliable for several reasons. First, fewer than 2,000 woody species have been screened for isoprene emission, and in many inventories, only few species have been measured in particularly species-rich floras that are composed of thousands of vascular plant species. Second, screening studies have usually focused on large woody plant species, neglecting small herbaceous species, probably because the latter are difficult to measure with current enclosure systems and are considered to contribute less biomass to the vegetation cover. Thus, if isoprene emission is indeed mostly confined to woody perennial growth habits, then the real fraction of isoprene-emitting species present in the world's floras is likely much lower than those inferred from current emission inventories (Figs. 1.1 and 1.2) in which annual and biennial plant species are largely underrepresented. Third, inventories are usually conducted at a single time of year (generally in summer), but the biosynthesis of constitutive volatile isoprenoids is under strong ontogenetic, seasonal, and even circadian control (for overviews, see Loreto and Schnitzler 2010; Niinemets et al. 2010). The situation is particularly complicated for monoterpenes, amongst which are some compounds such as the β-ocimenes that are known to be induced by stress and/or to occur only during a limited time of the year (Staudt et al. 1997, 2000, 2003; Grote et al.

2013). Fourth, inventories are usually conducted on only a few individuals and ignore the possibility of intra- and inter-population variability in the emission of isoprenoids. For example, the emission of volatile isoprenoids by the Mediterranean oak species *Quercus ilex* and *Q. suber* can change qualitatively, and can also be absent amongst individuals and populations (Loreto et al. 2009; Staudt et al. 2001, 2004, 2008). Fifth, screening studies differ greatly in their applied methods (i.e., enclosure systems and analytical facilities), although some efforts have been made to develop standardized methods and measuring protocols (for an overview, see Niinemets et al. 2011). Optimal identification and quantification of different classes of BVOCs require the use of several different sampling and analytical techniques. Consequently, the same plants, which have been screened for the emission of isoprene, have often not been screened for the emission of other volatile isoprenoids. Finally, many plant species previously classified as isoprenoid non-emitters now emerge as emitters when monitored with more sensitive methods of detection (e.g., Peñuelas et al. 2009b).

Our knowledge of the present distribution of BVOC-producing plants in terrestrial ecosystems has rapidly progressed in recent decades, but more worldwide coordinated efforts are needed to obtain realistic data on geographical and taxonomic patterns of volatile isoprenoid production by plants. These systematically gained data are a prerequisite for understanding and reliable assessment of the past and future evolution of volatile isoprenoid production.

1.3 The Past: Evolution of Volatile Isoprenoids

1.3.1 Volatile Isoprenoids: "Secondary" or "Primary" Metabolites?

The evolution of volatile isoprenoids is currently being intensively investigated. Using the traditional definitions of "secondary metabolites", volatile isoprenoids should not be essential for plants. The production of volatile isoprenoids has been proposed to take advantage of dimethylallyl diphosphate (DMADP) and its isomer, isopentenyl diphosphate (IDP), both of which are synthesized primarily to produce essential isoprenoids. Conditions affecting synthesis of the higher isoprenoids will thus affect the production and emission of volatile isoprenoids (Owen and Peñuelas 2005). According to Peñuelas and Llusià (2004), every BVOC emitted does not necessarily have a specific role, given that their emission is unavoidable due to their volatility. In many cases, however, natural selection has taken advantage of this volatility and conferred upon them important roles in defence or communication (Peñuelas and Llusià 2004).

Thus, volatile isoprenoids, while traditionally classified as secondary metabolites, are in fact key molecules that require large fractions of fixed carbon (Sharkey and Yeh 2001) and serve multiple very important physiological and ecological

functions, especially those related to communication and protection against biotic and abiotic stressors (Loreto and Schnitzler 2010; Possell and Loreto 2013). Evolution forced by selection can work on available genotypic diversity and modify the roles of isoprenoids to serve a broad diversity of adaptive roles (Peñuelas and Llusià 2004), e.g., due to high levels of ozone (Lerdau 2007; Calfapietra et al. 2013) or low levels of CO_2 (Way et al. 2011). The human factor may also be an important driver of evolution by selection, at least after plant domestication. Non-volatile terpenes, which are also considered as "secondary metabolites", are often not "secondary" for humans. For example, morphine and codeine, two alkaloids produced by *Papaver somniferum*, link "ecology, evolution, and human affairs" very well, as noted by Theis and Lerdau (2003). In other cases, intraspecific differences in the emission of volatile isoprenoids may have no evolutionary significance *per se*, but are associated with suitable traits for cultivation. A similar hypothesis has been invoked by Loreto et al. (2009) to explain why Portuguese cork oaks that have been selected for the quality of cork also emit a blend of constitutive monoterpenes characterized by a much higher emission of limonene compared to the blend emitted elsewhere in the range of this plant species.

The "raison d'être of secondary plant substances" has been investigated for more than 50 years (Fraenkel 1959) and is still being discussed (Berenbaum and Zangerl 2008), but we now believe that plants synthesize their secondary compounds, including volatile isoprenoids, to fulfill specific needs. Just as floral scents and pigments were selected to attract pollinators, or toxic non-volatile secondary compounds to repel herbivores and pathogens, evolution may have selected the biosynthesis of volatiles emitted from leaves. Thus, research into the ecological and physiological roles of isoprenoids have led us to question the firm meaning of the term "secondary plant compound". In fact, secondary and primary roles are often indistinguishable with regard to enhancing plant fitness.

1.3.2 Loss and Gain of Isoprene Emission Capacity: When and Why?

Past inventories suggest that the emission of isoprene and monoterpenes is scattered across plant divisions (Harley et al. 1999; Kesselmeier and Staudt 1999; Bagnoli et al. 2012). In fact, isoprene emission occurs in Bryophyta (mosses), Pteridophyta (ferns), Pinophyta (conifers), and Magnoliophyta (angiosperms), both monocots and dicots, independent of phylogeny. This evidence has suggested, as commented above, that the capacity for isoprene emission may have been gained and lost several times during the evolutionary history of plants (Sharkey et al. 2008; Monson et al. 2013).

If isoprenoid emission is under evolutionary control, then an evolutionary perspective can help us to understand why only some plants emit isoprene or monoterpenes. According to Harley et al. (1999), the enzyme responsible for isoprene

biosynthesis, isoprene synthase (IspS), may have evolved several times independently, but Hanson et al. (1999) proposed that the trait of isoprene emission evolved only once and was lost many times, accounting for its heterogeneous distribution among taxa. More recently, Lerdau and Gray (2003) suggested an independent origin of isoprene emission in Pinophyta and Magnoliophyta, with multiple losses of the trait accounting for the distribution of isoprene emission within Magnoliophyta. In fact, recurrent losses and neo-formations of isoprenoid synthase genes have been indicated by several independent studies. For example, Welter et al. (2012) observed that the production of isoprenoids by *Q. afares*, an oak species resulting from an ancient hybridization between an isoprene-emitting and a monoterpene-emitting oak, has strongly diverged from its parental species, including the complete suppression of isoprene production. The most recent examination of phylogenetic relationships (Monson et al. 2013) has: (i) confirmed the "multiple gain – multiple loss" model of isoprene evolution in both Pteridophyta (ferns) and Magnoliophyta, (ii) suggested that isoprene synthase genes arose frequently from mutations of terpene synthase genes (incidentally, this origin is reminiscent of the finding that mono- and sesquiterpene synthases in Pinophyta and Magnoliophyta have evolved from an ancestral diterpene synthase (Chen et al. 2011)), and (iii) indicated that isoprene production has been widely lost, and retained only under a few conditions. Sharkey et al. (2013), basing their analysis on the chronology of rosid evolution, suggest that isoprene-emitters appeared during the Cretaceous, and that the trait was subsequently lost multiple times until present. Monson et al. (2013) hypothesize that the loss of isoprene may have had different causes depending on whether isoprene emission was an adaptive or neutral trait. The idea that the trait is adaptive is more appealing, because the trait would be lost whenever the cost of isoprene biosynthesis exceeded its adaptive value. The frequency of loss during evolution suggests only a narrow range of conditions in which isoprene has adaptive value.

If isoprene has adaptive significance, what are the conditions under which the trait is retained?

(a) Isoprene emission is particularly widespread in hygrophytes. Hanson et al. (1999) and Vickers et al. (2009) therefore suggested that isoprene evolution could be beneficial when plants are under more recurrent and stronger oxidative stress in terrestrial than in aquatic environments. About 80 % of European hygrophytes emit isoprene, a figure significantly higher than in xerophytes (Loreto et al. unpublished data). However, it may be possible that hygrophytes diversified less than other plant functional types in which the trait was lost more often. Even resurrection plants that can survive in extremely dry environments have recently been found to emit isoprene (Beckett et al. 2012).

(b) Isoprene emission is a characteristic of perennial plants. This restriction may suggest a relationship with the phloem-loading type, being active phloem loading widespread in trees, and associated with lower concentration of leaf non-structural carbohydrates, which are then made available for growth (Turgeon 2010). However, it was also suggested that high sugar content in the mesophyll is needed to provide substrate for isoprene (Logan et al. 2000;

Kerstiens and Possell 2001). A relationship between isoprene emission and sugar accumulation has not yet been demonstrated.

(c) Isoprene emission is a characteristic of fast-growing perennial plants. Again, this restriction postulates a direct link between the rapid metabolism of carbon and the need to produce isoprene. In both cases, the benefit of producing isoprene is unclear, unless plants need to release extra carbon and energy when the machinery of carbon metabolism is maximally active, an idea reminiscent of the "overflow valve" hypothesis (Logan et al. 2000) and consistent with the idea that unstressed plants produce more isoprene because the carbon is not needed for structural or defensive compounds (the "opportunistic hypothesis", Owen and Peñuelas 2005). Not all strong isoprene emitters, though, are fast-growing (e.g., some deciduous oaks are slow-growing), and even amongst fast-growing plants, no clear relationship has been found between primary metabolism (photosynthesis) and isoprene emission (Guidolotti et al. 2011). Nevertheless, there is evidence of prevalence of isoprene emission among early-successional trees that typically grow in high light environments and have greater growth rates than late-successional species (Niinemets and Valladares 2006; Valladares and Niinemets 2008; Harrison et al. 2013).

(d) Isoprene emission is stimulated by low concentrations of CO_2. This characteristic has been interpreted as evidence that isoprene may have had an adaptive advantage during those epochs in geological history when atmospheric CO_2 concentration was low (Way et al. 2011). Epochs characterized by low levels of CO_2, however, were also cold, whereas isoprene emission is stimulated by high temperatures, and its function is associated with foliar thermotolerance (Sharkey and Yeh 2001). The evolution of the isoprene trait in epochs with low concentrations of CO_2 would be difficult to explain within this scenario. However, it may be argued that even in cold epochs, parts of the globe experienced a temperate climate. On more physiological grounds, isoprene might be needed when photosynthesis is constrained by low CO_2, and associated with environmental stresses (Possell and Loreto 2013). This would explain why isoprene evolved under low CO_2 but does not explain why isoprene is today a widespread trait in fast-growing trees (see (c) above).

1.4 The Future: Impacts of Climate Change and Ecological Constraints on the Diversification and Emission of Volatile Organic Compounds

The pace and extent of climate change will affect isoprenoid emissions. Temperature and CO_2 are key climate change factors controlling isoprene emissions. Light, water availability, and pollution, are also likely going to interfere with isoprene production and emission capacity by plants (Calfapietra et al. 2013; Holopainen et al. 2013). Because of the well-known dependence of synthesis and volatilization

of volatile isoprenoids on temperature (Niinemets et al. 2004), any further increase in temperature (as foreseen by IPCC 2007) will cause an increase in isoprenoid emission by plants, thus, inducing (alluding to monoterpene odor) 'a more fragrant world' (Peñuelas and Staudt 2010). Indeed, rising temperatures since the late nineteenth century may have already induced higher emissions worldwide.

Higher temperatures are mainly due to the accumulation of greenhouse gases, primarily CO_2. Rising levels of CO_2 have a negative effect on isoprene, documented by many reports since Sanadze (1964) and reviewed elsewhere (Loreto and Schnitzler 2010; but see Sun et al. 2012; Calfapietra et al. 2013; Monson 2013). The impacts of simultaneous increases in temperature and CO_2 levels on isoprene emission may thus virtually cancel out, with a residual stimulatory effect due to higher CO_2-driven biomass production and leaf area index (Arneth et al. 2008). A similar conclusion was reached by Heald et al. (2009) using a different algorithm, and therefore, this scenario is rather probable. Rising levels of CO_2 appear not to have a similar inhibitory effect on monoterpenes, so the future impact of rising CO_2 levels on these compounds cannot be predicted using the same parameterization as for isoprene (Arneth et al. 2008). Although clearly there are more data needed on [CO_2] effects on monoterpene emissions. More importantly, volatile isoprenoid emissions can be transiently enhanced by high temperatures that are repeatedly occurring worldwide (Rennenberg et al. 2006). In the absence of a concurrent increase in CO_2 levels, this effect appears to be much more dramatic and deserves thorough analysis, especially if the frequency of episodic extremely high temperatures will indeed increase in the future.

In areas where rising temperatures and enhanced evapotranspiration will reduce water availability, isoprenoid emission may also be affected by drought. Stress from drought appears to have a complex impact on isoprenoids, but isoprene biosynthesis is generally resistant to drought and increases the metabolic cost of isoprenoids when carbon acquisition by photosynthesis is inhibited (Loreto and Schnitzler 2010; Possell and Loreto 2013). Interestingly, the dependence of isoprenoid emission on temperature changes in leaves severely stressed by, or recovering from drought, suggesting that a further feedback operates on the main driver of isoprene emission; this can be a possible additional factor reducing isoprenoid emission in a warmer world (Bertin and Staudt 1996; Fortunati et al. 2008).

Changes in incident light, for example through an increase in the atmospheric load of aerosols that increase the fraction of diffuse light (Mercado et al. 2009), may have large impacts on the future release of isoprenoids from vegetation (Kulmala et al. 2013). Foliar isoprenoid emissions normally increase with increasing incident radiation. Combined with extreme temperatures or drought, though, strong radiation amplifies oxidative stress inside leaves, which can efficiently reduce the total amount, alter the composition, and modify the thermal responses of isoprenoid emissions (Staudt and Lhoutellier 2011). Such unaccounted interactive effects of environmental drivers on isoprenoid emissions may explain some of the observed variability in the response of emissions to single factors. For example, monoterpene emissions from Mediterranean oaks appear to be much more resistant to drought when studied in controlled mono-factorial laboratory and greenhouse conditions

(Bertin and Staudt 1996; Staudt et al. 2008) than under field conditions (Staudt et al. 2002; Lavoir et al. 2009). More studies of the interactions caused by stress are certainly needed to reliably predict the future evolution of isoprenoid emissions.

The impact of pollutants on isoprenoid emission is controversial. Air pollution appears to stimulate or maintain isoprenoid biosynthesis and emission, although not always (Peñuelas et al. 1999; Loreto et al. 2004; Calfapietra et al. 2009, 2013; Holopainen et al. 2013). Calfapietra et al. (2009) hypothesized a hormetic response, with pollution stimulating isoprenoid emission until the inhibition of photosynthesis no longer allows for a sufficient production of isoprenoid substrates. Again, as in the case of drought, pollutants will increase the costs of carbon and energy of isoprenoid formation. Why is this? We suggest that this results from the circumstance that isoprenoids interact with reactive oxidative species, thereby reducing membrane damage and stabilising photosynthesis (Loreto and Schnitzler 2010). Enhanced isoprenoid emission may thus reflect the activation of a defensive system by plants against stress.

If volatile isoprenoids protect plants against high temperatures and oxidative stresses, then high emitters should be fitter in a warmer, drier and more polluted world. In fact, because most natural ecosystems are composed of both emitters and non-emitters of volatile isoprenoids, emitters may ultimately be selectively favored by evolution (Lerdau 2007). On the other hand, because most isoprene emitters are woody species, man- and climate-driven conversion of forested areas to cropland, savanna, arid shrubland, and grassland may dramatically change the pattern of emission and reduce the emission of isoprene (Wiedinmyer et al. 2006). Moreover, because many isoprene emitters are deciduous, and warming will favor evergreen plants, isoprene emitters may be disfavored, e.g., in boreal areas that will experience milder temperatures. Another important alteration in the agro-ecosystems may come from changes in land use involving intensive cultivation of fast-growth plantations. Most of these fast-growing plants including poplars (*Populus* spp.), willows (*Salix* spp.), eucalypts (*Eucalyptus* spp.), oil palm (*Elaeis guineensis*), and reed (*Phragmites australis*), are all strong isoprene emitters, with eucalypts also emitting monoterpenes from storage organs. An expansion of plantations of these species worldwide is thus expected to vastly increase the biogenic emission of isoprene. For example, very large areas of rainforest in China, Malaysia, and neighboring countries have been converted to plantations of rubber trees (*Hevea brasiliensis*) or oil palms, which release five to ten times more monoterpenes or isoprene than the natural vegetation (Wang et al. 2007; Hewitt et al. 2009). In contrast, biofuel-producing C_4 plants do not emit isoprenoids in large amounts (Graus et al. 2011), though low emissions of isoprene and monoterpenes have been reported in switchgrass (*Panicum virgatum*) (Eller et al. 2012) and corn (*Zea mays*) may emit several isoprenoids, predominantly sesquiterpenes, when and after enduring pest attacks (Ton et al. 2007). The massive cultivation of C_4 biofuel plants in the future may thus not affect or may decrease regional fluxes of isoprene. These crops, though, release significant amounts of oxygenated BVOCs of low molecular weight, such as methanol, accounting for several grams per liter of biofuel

produced. These BVOCs are less reactive than volatile isoprenoids and are therefore less efficient as drivers of atmospheric chemistry.

As is evident from all of the above, the way the emissions of volatile isoprenoids respond to future conditions is still unclear. The overall emission of isoprene may increase if large forested areas are converted to tree plantations at the current pace, and if a "greening of the world" would occur because of photosynthesis stimulation by rising CO_2 levels (unlikely given the accompanying warming and drying; Peñuelas et al. 2011). In any case, the combined effect of climatic and anthropogenic factors, in particular, the interaction between rising temperature, CO_2, drought, and pollution, may offset the predicted (Lerdau 2007) evolutionary shift in favor of isoprene emitters in natural ecosystems. Rather, monoterpene-emitting plants, which are often evergreens and resistant to drought, with substantial limitations to CO_2 entry in the leaves, may take the highest advantage in terms of photosynthesis and growth from rising CO_2 levels (Niinemets et al. 2011). Because monoterpene emission is stimulated by rising temperatures and levels of pollutants, and is not inhibited by rising levels of CO_2, monoterpene emitters may have the largest evolutionary advantage, making a warmer world indeed more "fragrant".

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Chapter 2
BVOC-Mediated Plant-Herbivore Interactions

Amy M. Trowbridge and Paul C. Stoy

Abstract Plants release unique blends of biogenic volatile organic compounds (BVOCs) into the atmosphere, part of a silent language used to communicate with other organisms in their community. Within this high traffic chemical environment, plants and insects, among other organisms, are receiving, processing, modifying, and responding to information conveyed through varying suites of molecules. Because plants and insects are part of an integrative complex of food web relationships, one common topic of conversation is defence. Plants maintain a baseline level of BVOC emissions as a bottom-up constitutive defence, emitting compounds that act as repellents or deterrents to feeding and/or egg deposition by herbivores. Due to the autonomy of their attackers, plants can also employ an indirect top-down defence strategy, releasing induced volatiles in response to feeding that attract the natural enemies of their herbivore attackers, such as predators and parasitoids. Both bottom-up and top-down BVOC-mediated strategies have important consequences for herbivore preference, performance, and survival with even broader ecological and evolutionary consequences for tritrophic interactions. In this chapter we discuss how constitutive BVOCs mediate aspects of plant defence within a hierarchical spatiotemporal framework. Next we bring to light some of the most recent research on oviposition- and herbivore-induced BVOC synthesis and subsequent effects on the recruitment of natural enemies. We follow up by discussing the ecological effects of induced BVOCs in the context of multiple herbivores, expression from various plant organs, time-lags associated with BVOC induction, and heterogeneity within the infochemical environment. The critical feature of insect learning is described and we highlight some of the major evolutionary implications of BVOC-mediated plant defence syndromes that rely on the unique timing of events at the biochemical, atmospheric, organismal, and community scales.

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2.1 Introduction

Organisms are in continuous communication with each other to facilitate survival, defence, cooperation, and social connections. The study of the ability of organisms to interpret and communicate information is known as biosemiotics (von Uexküll 1926) and extends far beyond the signals that human are most adept at perceiving. We are constantly in the midst of countless mutualistic, antagonistic, and uninformative dialogs that are carried out in a chemical language that developed long before humans walked the Earth. The terrestrial fossil record provides evidence of volatile chemical signalling at the beginnings of the evolutionary arms race between species via structures such as plant essential oil glands (Fahn 2002; Krings et al. 2002) and olfactory appendages (Strausfeld and Hildebrand 1999; Labandeira 2002). The ability of plants to *communicate* with plants and other organisms via volatile organic compounds (BVOCs) is fascinating, and influences important evolutionary and ecological processes that we are only beginning to understand.

Definitions

Allelopathy: Biogenic phenomenon where secondary chemicals produced by one organism affect the development, growth, survival, and reproduction of other organisms.
Constitutive defence: A plant defence strategy that is always expressed in the plant.
Direct plant defence: Plant traits that negatively influence the physiology or behavior of herbivores.
Indirect plant defence: Plant traits that enhance the efficacy of the natural enemies of herbivores, such as herbivore-induced BVOCs.
Induced response: Change in a plant following stress or damage.
Induced resistance: An induced response that reduces herbivore survival, reproduction, or preference for the plant of interest, but may not necessarily benefit the plant.
Induced defence: A response that decreases the negative *fitness* consequences of attacks on plants, but may not necessarily affect herbivores.
Infochemical: A chemical that conveys information between two organisms or individuals resulting in a physiological or behavioral response in the receiver that is adaptive to one or both parties.
Tritrophic interactions: Relationship between a plant, an herbivore, and the natural enemies of the herbivore, mediated by plant chemistry and/or BVOCs.

Plants emit more than 30,000 different BVOCs including terpenoids, green leaf volatiles, phenylpropanoids, benzenoids, and methyl esters of plant hormones (i.e., methyl jasmonate and methyl salicylate). These BVOCs mediate plant-plant, plant-insect, and multitrophic interactions and play critical roles in plant defence (Yuan et al. 2009; Fineschi et al. 2013). Effective defence is critical for plant survival and reproduction as plants are subject to constant attack from pathogens, fungi, insects, mammalian herbivores, and other biotic agents. To combat herbivory by

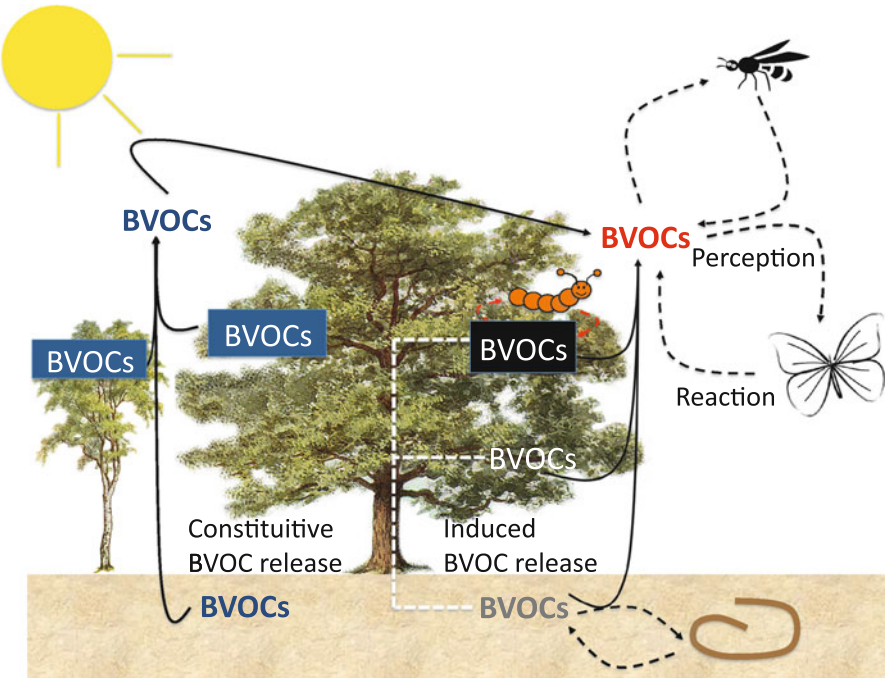

Fig. 2.1 Scheme highlighting the role of biogenic volatile organic compounds (BVOCs) in mediating interactions between trees and herbivores. Plants constitutively synthesize and release BVOCs from leaves, stems, and roots, as demonstrated on the *left hand side* of the figure. Constitutive BVOC emissions from different organs within a tree and trees within a forest (*blue*) are transported to the atmosphere (*black lines*) where they are mixed by turbulence. Different mixtures and concentrations of BVOCs are present in different plant parts, as indicated by coloration. Constitutive BVOC emissions are also mixed with BVOCs whose synthesis and/or emission was induced by herbivores, as demonstrated on the *right-hand side* of the figure, resulting in the total BVOC signal in *red*. Herbivores perceive and respond to BVOC concentrations within the plant (*red dashed lines*), and their feeding activities may induce plants to produce and release a different combination of BVOCs locally, or in other parts of the plant as demonstrated by the *dashed white lines*. Other organisms perceive and respond to BVOCs in their environment (or *Umwelt* following von Uexküll 1926), as represented by *dashed black lines*. The multitrophic interactions that are mediated by BVOCs, including herbivore activity and parasitoid oviposition behavior, are central components of forest population, community and ecosystem ecology

heterotrophic organisms that span multiple taxa and developmental stages, plants employ a diverse arsenal of chemical defence mechanisms that include BVOCs. Thus, BVOCs are an integral part of a comprehensive bottom-up and top-down plant defence strategy because they enable plants to resist herbivory within and beyond the leaf, stem, and root (Fig. 2.1).

This chapter focuses on the role BVOCs play in interactions between plants and insects. We place particular emphasis on spatial and temporal aspects of both constitutive and insect-induced BVOC dynamics to highlight the role played by BVOCs in tritrophic ecological interactions. We also discuss above- and belowground factors

that control BVOC emissions, and the influence of the resulting emissions on tritrophic interactions associated with both roots and leaves. Our focus here is on biotic interactions in natural unpolluted environments, and we refer the reader to the chapter of Holopainen et al. (2013) in this book for a discussion of the modification of biotic interactions in polluted atmospheres and under global change. We further refer the reader to Peñuelas and Llusià (2003), Loreto and Schnitzler (2010), and Possell and Loreto (2013) and Calfapietra et al. (2013) in this book about the role of abiotic stresses, including climate change, in altering BVOCs, and subsequent consequences for ecological signalling. We conclude with a brief overview of the evolutionary implications of BVOCs in the context of insect learning in complex environments.

2.2 Constitutive BVOCs

Constitutive BVOCs serve as constant barriers to herbivore attack by deterring colonization through their antixenotic function and inhibiting growth, reproduction, development, and/or survival through their antibiotic function (Paiva 2000; Walling 2000). We refer to these characteristics as 'bottom-up' defences as they directly impact herbivore performance. In a natural setting, both the rate at which volatiles are released and their spatial and temporal persistence in the environment determine their relevance in ecological signalling. However, complex feedbacks exist between the phylogenetic constraints on biosynthesis, environmental controls on emissions, and compound-specific physico-chemical responses to the environment. As a result, the spatial and temporal heterogeneity of BVOC emission rates is often pronounced, with important implications for multispecies interactions. We discuss consequences of BVOC emissions at the leaf level and then describe controls on and consequences of BOC emissions at the organ, whole plant, and community scale.

2.2.1 Role of Constitutive BVOCs in Defence and Host Selection

Trees maintain a baseline level of volatile metabolites that are released from the leaf upon production, or some time after production from storage sites (e.g., resin canals) (Paré and Tumlinson 1999). Within the last decade, an emphasis has been placed on understanding the role of constitutive vegetative BVOCs in deterring herbivory. Unfortunately we still lack a mechanistic understanding of how BVOCs released from plants *directly* repel herbivores and/or inhibit feeding. One possibility is that constitutive emissions are directly toxic to impending attackers, potentially affecting physiological and neurological processes by influencing gene expression and/or interfering with the macronutrient digestion. However, studies have yet to

demonstrate how relatively low-emission rates, typically on the scale of nano- or micrograms per hour per gram of leaf tissue, elicit toxic physiological effects (Unsicker et al. 2009).

Herbivores must discriminate between true ecological signals that might increase their fitness, and background noise resulting from a highly variable infochemical environment. Most phytophagous insects are specialist feeders that utilise a limited number of species as resources, and chemical cues are critical in selecting suitable hosts. It is not in the best interest of a plant to attract herbivores; however, specialists are capable of exploiting the characteristic volatiles emitted by their hosts to gain information on quality, susceptibility, and natural enemy status. Reddy and Guerrero (2004) give two examples of how insects exploit BVOCs with different pheromone-mediated implications for plant resistance. Some insects can use constitutive BVOCs for their own defence. For instance, green leaf volatiles (GLVs) released from non-host tree species serve as repellents in host selection and inhibit the pheromone response of several bark beetles. Other insects can use constitutive BVOCs to their advantage in finding mates. When male *Ips* and *Dendroctonus* spp. are exposed to the volatile myrcene, they modify the compound, which results in the production of oxygenated pheromones in their hindgut. However, predators and parasitoids attacking adults and eggs have learned to eavesdrop on these pheromone signals to locate prey more effectively (Stowe et al. 1995). As a consequence, constitutive BVOC signals that are attractive to specialists can be intercepted and used by the herbivore's natural enemies as a type of *indirect* defence. Thus, constitutive and induced defence strategies should be assessed in an integrative fashion to fully appreciate the ecological role played by BVOCs.

2.2.2 Spatiotemporal Patterns

2.2.2.1 Leaf-Level Responses

BVOC emissions exhibit significant variations across space and time at the scales of leaves, organs, whole plants, and ecosystems. At the leaf level, rates of constitutive BVOC production and emission are sensitive to a number of abiotic factors, including light and temperature as discussed by Niinemets et al. (2004) and other chapters in this volume. These factors can interact with ontogenetic changes in emissions and result in variable emission rates from leaves close in proximity. For instance, constitutive monoterpene (C_{10}) emissions can be detected from young hybrid poplar leaves (*Populus deltoides* x *Populus nigra*). However, mature leaves on the same tree were found to emit only isoprene (C_5) and at a rate of about five times greater than monoterpenes released from young leaves (Brilli et al. 2009), suggesting that the amount of carbon invested towards isoprenoid biosynthesis changes with leaf ontogeny. Differences in BVOC quality and quantity between leaves have

important consequences for herbivore host selection, and these differences can result from the presence of specialized structures and/or the vascular connections within the tree. Even subtle spatial variation can be critical for specialists that have learned to associate proximate volatile signals with host quality. For example, BVOC emissions tend to be significantly higher in sunlit leaves when compared to shaded leaves on the same plant and/or branch, due largely to an increase in light and temperature and higher rates of physiological activity (Harley et al. 1996). While this increase in emissions may have consequences for herbivore preference, it is also possible that BVOCs serve multiple ecological functions simultaneously (e.g., protecting leaves from photodamage at high temperature and light conditions) (Peñuelas and Llusià 2002) with evolutionary implications via multiple selection pressures. Furthermore, some plant BVOCs with low vapour pressures tend to condense on leaf surface as a function of leaf temperature and position in the canopy and may serve as defence agents against herbivores and pathogens (Holopainen and Gershenzon 2010), but it is unknown if this mode of BVOC function represents an effective defence.

2.2.2.2 BVOC Variation Between Vegetation and Flowers

The suite of volatiles released by healthy trees can also vary spatially within a plant among organs including leaves, stems, flowers, and roots (Fineschi and Loreto 2012; Mumm and Hilker 2006; Takabayashi et al. 1994). The survival of a plant is determined by investment in reproduction (e.g., flowers, nectar) and growth (e.g., vegetative biomass). Thus, allocation of resources to defence may come at the expense of investment in reproductive tissues and affect mutualistic relationships, pollination events, and fitness (Mothershead and Marquis 2000). For instance, floral and vegetative BVOC emissions are differentially attractive and repellent to species that specifically feed on those organs, yet BVOCs from both plant parts are likely to be mixed in the atmosphere before their perception by an insect. Thus, chemical information from other plant organs may confound perception and host identification, particularly in response to damage (see *Induced Responses*). Because both herbivores and pollinators are selective agents on floral chemistry and emissions, it is critical to understand the degree to which defensive vegetative BVOC production alters nectar and pollen quality and thereby affects fitness. Furthermore, compound type and emission rate can vary substantially between organs as a function of the predictability of attack on various plant parts (see optimal defence theory, Rhoades (1979); Zangerl and Rutledge (1996)) and ecophysiological constraints (e.g., tissue-specific biosynthesis or allocation, Niinemets et al. 2004). While many BVOCs appear to be exclusively produced in either flowers or leaves, compounds can also be passively transported into the nectar and/or pollen and subsequently released into the atmosphere. Future studies should focus on plant allocation and tissue type to fully understand the dynamics of plant-pollinator-herbivore-interactions (Kessler and Halitschke 2009).

2.2.2.3 Belowground BVOC-Mediated Interactions

While most studies focus on BVOC-mediated interactions between aboveground plant parts and herbivores, plants must also defend themselves against soil-dwelling herbivores including rodents, insects, and nematodes, all of which play key roles in terrestrial ecosystem community structure. Due to the limited mobility of soil organisms and the low transport rates of root compounds, interactions in the soil occur over significantly smaller spatial scales than aboveground interactions (van der Putten et al. 2001). Despite the difference in characteristic spatial scales of influence, the role of BVOCs might be more important for belowground communities where visual cues are lacking and with soil-dwelling herbivores often exhibiting poor eyesight (Rasmann et al. 2005; van Dam and Heil 2011). A number of compound classes emitted from aboveground organs have been shown to mediate plant-herbivore interactions belowground, but in different compositions (Wenke et al. 2010). Nonetheless, BVOCs can directly or indirectly influence belowground communities, support symbioses, combat competitive plant species, and defend against pathogenic fungi, bacteria, and herbivores (Nardi et al. 2000).

Herbivore host choice for oviposition is critical for the next successful generation of soil dwelling herbivores. Adults of the large pine weevil (*Hylobius abietis*) are attracted to suitable hosts in a dose-dependent manner, depending on BVOC concentrations released from conifer roots (Nordlander et al. 1986). BVOC composition is also important; larvae of the forest cockchafer (*Melolontha hippocastani*) exhibited a noted preference for monoterpenes released from carrots over the fatty acid derivatives emitted from oak roots (Weissteiner and Schütz 2006). Despite being separated in space, roots, shoots, leaves, and flowers are connected and so are their resources, metabolic activities, and defences. Belowground organisms can induce defence responses aboveground and vice versa, the cost: benefit ratio of these induced responses and their consequences are discussed in more detail in the Sect. 2.3: *Induced BVOCs*.

2.2.2.4 BVOC Variability Between Plants

At the whole-plant level, BVOC emission rates are dependent on a number of environmental and biotic factors, including developmental stage as well as plant species, genotype, and age (see Dicke 1999; Paré and Tumlinson 1999). Constitutive plant defences are expressed differentially with ontogeny, and while BVOC emissions have been shown to decrease in mature cultivated herbs (Cole 1980), information about their ontogenetic patterns in mature trees and seedlings remains largely unknown (Boege et al. 2011). Studies focused on ontogenetic changes in tree BVOC emissions, foliar chemistry, and predator/parasitoid foraging dynamics may offer mechanistic insight into tritrophic patterns observed in the field. In addition to age, specific chemotypes and plant varieties have differential growth and resistance properties (Staudt et al. 2001). For example, mango (*Mangifera indica*) tree cultivars that are the most susceptible to mango gall flies (*Procontarinia* spp.)

emit significantly higher levels of α-pinene and β-pinene throughout the growing season; these volatiles are highly attractive to this pest (Augustyn et al. 2010). At the plant population level, BVOCs emitted by particular genotypes of seedlings of *Pinus pinaster* in plantations have been found to be susceptible to a generalist phloem-feeding pine weevil (*Hylobius abietis*). Blanch et al. (2012) showed that under high nutrient availability, susceptible trees exhibited higher terpene emission rates, including α-pinene, an attractant for *H. abietis* (Moreira et al. 2008), which could explain the pattern of weevil damage observed in the field (Zas et al. 2008). Thus, BVOC emission rates must be investigated in the context of plant genetics, development, *and* the environment to better-understand herbivore-natural enemy development, behavior, and natural ecological patterns.

2.2.2.5 Community-Level Variability

Observed spatial variations in foliar BVOC emissions range over several orders of magnitude at the community level due to changes in species composition and foliar density (Guenther 1997). Spatial patterns in both the magnitude and composition of BVOCs that herbivores and their natural enemies perceive are critical for their behavior, and therefore, for their reproduction and survival. However, the identification of specific cues signalling host availability and quality may be influenced by the background of chemicals present in the habitat from other species, requiring adaptive and integrative abilities to extract useful search information from the milieu of chemicals present in the environment (Hilker and McNeil 2008). Drastic community shifts, via invasive species or changes in climate, can influence plant-herbivore interactions by altering the proportion of species that produce varying types and quantities of BVOCs. Depending on the ability of the insect to learn (see Sect. 2.4, *Insect Perception and Learning*) and the time required to make new host-BVOC associations, changing habitats can modify the probability of a particular plant-herbivore interaction occurring, the intensity of the interaction, and coevolutionary processes (Agrawal and Fishbein 2006).

2.2.2.6 Variation in Time: Diurnal Cycles of BVOC Release

The effects of time on BVOC emissions extend beyond ontogeny. BVOC emissions are a product of day length (light), corresponding changes in temperature and water status, as well as seasonality. Many BVOCs released constitutively from trees exhibit diurnal cycles, increasing rapidly in the morning with temperature and solar radiation, peaking in the middle of the day, and decreasing during the afternoon and evening (Pio et al. 2005; Grabmer et al. 2006). High emissions make plants more “apparent” to insects, and may determine the employment of other defensive strategies used by plants to protect against herbivory (Feeny 1976; Rhoades and Cates 1976). However, “apparency” due to high daytime emissions and their role in increasing plant vulnerability to herbivores is dependent upon the peak foraging

time of the insect attacker as well as the searching behaviors of their natural enemies. Furthermore, because vegetative and reproductive tissues exhibit diurnal variability in emissions, plasticity in volatile production from both types of plant organs can be critical, as the specificity with which insects choose to visit flowers on fruiting trees may be the result of the quantitative relationship between the attractant and repellent components in the blend contributed from leaves (Euler and Baldwin 1996).

2.2.2.7 Variation in Time: Seasonal Trends

Seasonal trends in BVOC emissions have been observed in a number of forest types, including mixed hardwood (Karl 2003), boreal coniferous (Hakola et al. 2003), and Mediterranean (Owen et al. 2001; Pio et al. 2005). Similar to emission rates observed over a shorter time scale, the seasonal release of BVOCs is a function of changes in light, temperature, and compound-specific physico-chemical controls (see Niinemets et al. 2004 and Grote et al. 2013 for detailed discussion of controls on seasonal changes in emissions). For example, within a mixed hardwood forest, Karl (2003) found, among other patterns, a spring peak for methanol and attributed it to rapid leaf expansion. While the large amount of methanol released from vegetation has long been assumed to be a metabolic waste product, studies have shown that herbivory can also elicit its release, suggesting the potential role of methanol in mediating plant-insect relationships (Peñuelas et al. 2005). Furthermore, application of methanol to plants in quantities mimicking herbivore-elicited release affects bottom-up controls by decreasing plant foliar defences (e.g., trypsin proteinase inhibitors) and enhancing the performance of the herbivore (von Dahl et al. 2006). Thus, seasonally-driven BVOC-specific spikes may not only impact plant-insect signalling, but also force within-plant feedbacks that negatively impact defence capabilities resulting in higher herbivore pressure at key developmental times of the year.

2.3 Induced BVOCs

Plants are the primary food source for millions of insect species, each using unique strategies to obtain nutrients from both above- and belowground tissues. In contrast to constitutive 'hard-wired' plant traits that confer resistance to insect pests regardless of insect infestation risk, herbivore-challenged plants can exhibit phenotypic plasticity and can mount active defence responses that are induced by insect behavior. Trees are thought to have evolved induced defences to save on allocation costs when pressure from herbivores is low (Heil 2002). When expressed following herbivory, induced responses can serve as *direct* defences, affecting the herbivore through immediate toxicity (i.e., a 'bottom-up' defence) or as an *indirect* ('top-down') defence, affecting the herbivore via recruiting its

natural enemies (Dicke and Vet 1999). Both induced direct and indirect defences can alter herbivore behavior and development. BVOCs released immediately after herbivory consist of preformed volatiles, some resulting from the bursting of storage structures, and depend on the mode of damage such as wounding, egg deposition, and herbivore feeding (Walling 2000). Other BVOCs released with feeding are synthesized *de novo* and exhibit delayed emissions on time scales of minutes, hours, days, and potentially seasons. These emissions can also be expressed both locally and systemically (Paré and Tumlinson 1999). Plant BVOCs are not only mediators of aboveground plant-insect interactions, but also affect herbivore dynamics in the soil. In fact, damage by below- and/or aboveground herbivores has been found to affect pollinators and higher trophic levels, particularly the natural enemies of herbivores in both root and shoot food webs (see van Dam and Heil (2011) and references therein). Here we briefly expand upon these topics, with particular focus on herbivory and oviposition-induced BVOCs and the consequences of the spatiotemporal dynamics of emissions on above- and belowground defence.

2.3.1 Herbivore and Oviposition-Induced BVOCs: Induction Depends on Mode of Damage, Elicitors, and Signal Transduction

The synthesis of novel BVOCs in response to herbivory is not part of a general syndrome in response to stresses (i.e., drought, ozone, temperature, etc.), but is a *specific* response to herbivory with a well-documented defensive role in trophic interactions (Staudt and Lhoutellier 2007). Some of the earliest studies of induced host volatiles were performed on trees, (e.g., *Populus* spp. (Baldwin and Schultz 1983)). With the ability to release hundreds of BVOCs, how do trees release such specific chemical signals in response to herbivore attack? The quality and quantity of herbivore-induced BVOCs are dependent on a variety of factors, including the plant species, plant age, the tissue type being attacked, as well as the herbivore species, feeding mode, and its developmental stage (De Moraes et al. 1998). The mode, frequency, and severity of physical damage by herbivores and herbivore-specific chemical elicitors initiate highly regulated modifications in the plant's transcriptional and metabolic processes by activating signalling pathways (Kessler and Halitschke 2007). While these pathways are well known in herbaceous species, there exists a gap in our knowledge regarding signals and pathways that induce resistance in many tree systems. In light of a few recent studies, many assume similar signal cross-talk and activation in trees as observed in herbaceous plants (Eyles et al. 2010).

A number of elicitors initiating signal cascades involved in BVOC synthesis have been isolated and characterized from insect saliva, regurgitants, and oviposition fluids, and include enzymes, fatty acid-amino acid conjugates, and bruchins (Paré et al. 2005). Once in contact with plant cells, these elicitors activate signal

transduction pathways (e.g., the octadecanoid (C_{18}-fatty acids) pathway) that ultimately lead to gene expression and the synthesis of particular defence-related BVOCs (for more detail on signal cascades see Kessler and Baldwin (2002)). Numerous studies in trees have shown that in the absence of wounding, pathways can also be induced through the application of herbivore oral secretions, elicitors themselves, or phytohormones such as jasmonic acid (JA), ethylene, and salicylic acid (SA) (Dicke et al. 1999; Eyles et al. 2010; Van Poecke et al. 2001). The early steps in the herbivore elicitation process remain to be elucidated as well as the mechanisms responsible for plant recognition of these herbivore-specific compounds. Nonetheless, the result of these signal cascades is an herbivore-induced BVOC blend comprised of tens to hundreds of compounds. While a number of these compounds are species-specific and actively produced in response to herbivory, many BVOCs also "leak out" or are released simply due to mechanical damage. These compounds, known as green leaf volatiles (GLVs), consist of saturated and unsaturated C_6 alcohols, aldehydes, and esters produced by the oxidative breakdown of membrane lipids (Paré and Tumlinson 1999). Within this complex blend of GLVs and novel compounds synthesized *de novo*, however, only a subset of compounds play a biological role in mediating higher trophic level interactions with herbivores and natural enemies (see Dicke (2009) and references therein).

Individual signal cascades, as described above, have the ability to serve a variety of functions. The involvement of several signal cascades in response to specific forms of herbivory may help explain the specificity of BVOC profiles (Kessler and Baldwin 2002). As such, plants must be able to not only identify the source of damage, but also prioritize and tailor the signalling pathway that will mount the most effective defence strategy (Reymond and Farmer 1998). A rich body of literature exists regarding induced plant responses to attack by *chewing* insects and the subsequent interactions between the two organisms (e.g., Karban and Baldwin 1997). There are also many studies that demonstrate the specific and differential chemical response of plants to chewing insect species (e.g., De Moraes et al. 1998). However, plants are constituents of complex communities, and as such, are rarely attacked by a single herbivore. Multiple biotic stressors can significantly alter herbivore-induced BVOC emissions as concurrent feeding may induce cross-resistance (Kessler and Halitschke 2007) or competing plant defence pathways, both of which have important implications for defence and evolution (Rodriguez-Saona et al. 2005). While far less is known about the induction of BVOCs by herbivores of feeding guilds that cause less tissue damage (i.e., miners, galls, and piercing-sucking insects), a study by Delphia et al. (2007) demonstrated that simultaneous herbivory by insects with different feeding habits significantly alters BVOC emission and defence strategies. However, ways in which plants simultaneously integrate responses to multiple herbivores and the ecological and evolutionary consequences for plant-insect interactions after attack, remains largely unknown.

Herbivores not only induce changes in plant leaf BVOCs through feeding, but also through egg deposition. Hilker and Meiners (2002) describe the mechanism involved in oviposition-induced BVOCs in Scots pine (*Pinus sylvestris*) and field

elm (*Ulmus minor*). In both systems, the adult female wounds the surface of the leaf and needle just before oviposition. The eggs are then laid into the wounded tissue along with oviduct secretions that surround the eggs securing them to the plant. Only when the secretions, containing an elicitor, make it past the cuticular barrier do leaves release parasitoid attractant BVOCs (Hilker and Meiners 2006). To date, only a few oviposition-associated elicitors have been identified, including bruchins (Doss 2005) and benzyl cyanide (Fatouros et al. 2008). The application of jasmonic acid can also elicit the release of BVOCs that attract the egg parasitoids associated with each species, suggesting the involvement of the octadecanoid pathway in driving oviposition-induced responses (Hilker and Meiners 2002; Meiners and Hilker 2000). Similar to herbivory, insect egg deposition induces plant responses that are specific to both the plant and herbivore species attacking it, yet whether this specificity is due to species-specific elicitors or a dosage-dependent response remains unknown for most systems (Hilker and Meiners 2010). While the induced BVOCs produced via feeding and oviposition differ in composition, both BVOC blends may be perceived by the herbivore and parasitoid with either negative or positive consequences. For example, herbivore-induced BVOCs have been shown to deter female herbivores from oviposition in an attempt to avoid competition (Kessler and Baldwin 2001; De Moraes et al. 2001). Future work aimed at understanding the interaction of herbivore- and oviposition-induced signalling pathways, the BVOCs emitted, and consequences for herbivores and their natural enemies will offer insights into the evolutionary importance of these compounds.

What is a parasitoid anyway?!?

Parasitoids spend only part of their lifecycle associated with a host. They feed exclusively in or on the body of another arthropod, eventually killing it. Only a single host is required for the parasitoid to complete its lifecycle.

Predators kill their prey, usually more than one species, but do not need a host to complete any part of their lifecycle.

Parasites spend their entire life associated intimately with its host, usually at the host's expense, but without causing death.

2.3.2 Induced Volatiles Serve as Direct Plant Defences

Immediately following release, herbivore- and/or oviposition-induced BVOCs carry a vast array of information through the environment with the potential to directly influence the behavior of different members of the ecological community. Some herbivore-induced volatiles have been shown to function as a plant defence by deterring herbivore feeding and oviposition (Kessler and Baldwin 2001; Laothawornkitkul et al. 2008; De Moraes et al. 2001). For instance, when foraging, starved adult willow leaf bugs (*Plagiodera versicolora*) orient towards odors elicited from

willow leaves infested by conspecifics as opposed to intact leaves, perhaps due to increased quality or lower concentrations of secondary compounds (Yoneya et al. 2009). Deception is another way in which plants use herbivore-induced BVOCs to their advantage, such as in the case of the sesquiterpene, (*E*)-β-farnesene, which is also an aphid alarm pheromone that signals aphids to stop feeding and disperse (Bernasconi et al. 1998). Even oviposition-induced BVOCs can affect the egg laying choice of other female herbivores. To avoid inter- and intraspecific competition and a site attractive to egg parasitoids, laboratory choice tests showed adult *Xanthogaleruca luteola* to prefer BVOCs from field elm (*Ulmus minor*) leaves without eggs over those with eggs and/or feeding damage (Hilker and Meiners 2002). In addition to directly resisting the attacking herbivore, induced BVOCs can also influence herbivores on neighboring plants by priming non-infested plants to chemically respond faster to future insect attacks. One study showed that rates of herbivory were lower in black alder (*Alnus glutinosa*) trees growing close to damaged conspecifics (Dolch and Tscharntke 2000). This is similar to observations made by Rhoades (1983), who reported that undamaged Sitka willow trees (*Salix sitchensis*) in close proximity to herbivore-infested conspecifics mounted a more aggressive chemical defence in response to fall webworm larvae (*Hyphantria cunea*) than distant controls. If induced BVOCs can directly influence the chemical defences within neighboring trees, it is not surprising that they can also elicit defence responses in undamaged parts of the same tree. For instance, gypsy moth (*Lymantria dispar*) feeding on branches previously exposed to herbivore-induced BVOCs from nearby damaged branches was reduced by 70% compared with controls (Frost et al. 2007). In addition, extrafloral nectaries have been shown to increase in output when undamaged leaves are exposed to herbivore-induced BVOCs emitted from damaged leaves on the same plant, resulting in increased visits from predators (Heil and Silva Bueno 2007). Despite evidence from experimental observations, we lack an understanding of *how* the signals that induce priming are received by plants, which compounds are biologically active within an herbivore-induced mixture, and the signalling cascades responsible for indirect BVOC-mediated plant defence.

2.3.3 Induced Volatiles Serve as Indirect Plant Defences

The attraction of the natural enemies of herbivores by damage-induced volatiles is a well-established phenomenon in many plant species, and probably the first defence strategy that comes to mind when discussing induced BVOCs. For over 40 years (Green and Ryan 1972), a vast array of herbivore-induced plant BVOCs has been shown to effectively recruit insects of the third trophic level that prey upon or parasitize larval herbivores, as well as eggs. By doing so, BVOCs reduce the preference and/or performance of herbivores, serving as an indirect defence and an important mediator of tritrophic interactions (Karban and Baldwin 1997). Nonetheless, because most palatable herbivores are cryptically coloured and well hidden on the undersides of leaves, the probability of parasitoids effectively finding their hosts using visual cues and random searches is relatively low. However, the

suite of compounds released following herbivore-damage is quite sophisticated and unique, differing in total abundance and composition following attack by different herbivores (e.g., De Moraes et al. 1998). The species-specific plumes present within the local environment contain critical host-location information for parasitoids, which have developed the ability to learn chemical cues associated with the presence and quality of their specific host (see Sect. 2.4, *Insect Perception and Learning*). For instance, some parasitoids are capable of differentiating between parasitized and unparasitized larval hosts in flight due to the different odor blends induced by each caterpillar (Fatouros et al. 2005). While herbivore-induced volatile blends can be quite complex, a number of individual BVOCs involved in attraction of parasitoids have been identified (e.g., Halitschke et al. 2008; Ibrahim et al. 2005). However, it is highly unlikely that a parasitoid will be exposed to only one BVOC in nature, and the context within a BVOC blend is perceived may be important. In fact, the eulophid egg parasitoid (*Chrysonotomyia ruforum*), while attracted to the herbivore-induced release of (*E*)-β-farnesene, requires the presence of background non-induced pine odor to locate its sawfly host (*Diprion pini*) (Mumm and Hilker 2005). While individual herbivore-induced BVOCs may be involved in parasitoid host location, it is often critical that they are perceived in the context of other BVOCs so as to distinguish variation in quality and quantity.

Although most available information on BVOC-mediated tritrophic interactions comes from studies in agricultural or herbaceous species, a number of studies has also demonstrated the attraction of the natural enemies to herbivore-induced BVOCs elicited by pests attacking trees, particularly in fruit trees [e.g., apple (*Malus domestica*), mango (*Mangifera indica*), and grapefruit (*Citrus paradisi*)], conifers [e.g., Scots pine (*Pinus sylvestris*) and loblolly pine (*Pinus taeda*)], as well as some deciduous species [e.g., elm (*Ulmus minor*) and black poplar (*Populus nigra*)] (see Dicke (1999) and Mumm and Dicke (2010)). For instance, fruit fly parasitoids (*Diachasmimorpha longicaudata*: Braconidae) significantly preferred BVOCs from infested mangoes (with higher BVOC concentrations) and their extracts (particularly 2-phenylethyl acetate) over healthy and mechanically damaged fruits, suggesting that parasitoids use induced BVOCs to locate hosts in this system (Carrasco et al. 2005). In conifers, a dosage-dependent synergistic effect among pine terpenoids and bark beetle pheromones can attract predators and parasitoids to their hosts. For instance, the attraction of the predatory beetle *Thanasimus dubius* was positively correlated with the concentration of α-pinene when mixed with the pheromones of its scolytid prey (Mumm and Hilker 2006). Parasitized herbivores have also been shown to induce different BVOC blends compared to unparasitized herbivores, affecting parasitoid choice (Fatouros et al. 2005). However, mutualistic interactions with herbivores (e.g., aphids and ants) can also alter induced BVOCs with potential consequences for parasitoid host location (Paris et al. 2011). There is also evidence that oviposition-induced plant BVOCs successfully recruit egg parasitoids, such as in the case of *Pinus sylvestris* and egg deposition by *Diprion pini* (Meiners and Hilker 2000). In the deciduous species *Ulmus minor*, terpenoid hydrocarbons induced by oviposition of the elm leaf beetle (*Xanthogaleruca luteola*) are exploited by the egg parasitoid *Oomyzus gallerucae*. Despite the work

in these systems, more studies are needed to fill gaps in our knowledge pertaining to the importance of induced indirect defences employed by trees, particularly in determining community structure and outbreak dynamics.

2.3.4 Spatiotemporal Aspects of Induced Indirect Defence

Similar to constitutive BVOCs, herbivore-induced BVOCs not only vary over space and time, but their associated costs to plant fitness vary as well. Most factors influencing constitutive emissions have similar effects on herbivore-induced volatile defences as discussed in previous sections here and in other chapters in this volume. Thus, rather than reiterating these points, we place the spatial and temporal variability of herbivore-induced BVOCs in an ecological framework by discussing ecological interactions influenced by the location of induction in a plant and the timing and patterns associated with the response.

2.3.4.1 Local vs. Systemic Induced Responses

Induced BVOCs that serve a resistant or defensive role for plants can be expressed locally at the wounding site or systemically via mobile signals and phloem transport (Turlings and Tumlinson 1992; Heil and Ton 2008). The ability to respond systemically to herbivory enables a plant to have a larger BVOC response, potentially serving as long range cues capable of recruiting natural enemies that forage over spatial scales of metres to kilometres (Puente et al. 2008). Furthermore, these systemic signals can give parasitoids an initial estimate of patch quality (e.g., number of hosts in a habitat) to aid in determining whether or not to pursue hosts in a given area. However, once parasitoids orient themselves within the general vicinity of their host, the specific blend associated with the herbivore at the damage site itself becomes more important, and constitutes a reliable indicator of host location (Cortesero et al. 1997). BVOC communication between branches or leaves of the same individual could enable faster responses, particularly when signalling via phloem and xylem is thwarted by limited vascular connections or distance, for example in larger trees (Dicke 2009). For instance, Frost et al. (2008) demonstrated that both mechanically-injured and gypsy moth-damaged leaves of hybrid poplar (*Populus deltoides x P. nigra*) primed defence responses in undamaged leaves of the same plant. This "second route" for signal transduction within plants can provide a relatively large benefit to the emitting plant in lieu of synthesis costs. Herbivore-infested plants also mediate plant-plant interactions in unattacked neighboring plants, thus increasing their attractiveness to natural enemies and decreasing their susceptibility to herbivory (Baldwin et al. 2006). While within-plant BVOC signalling has gained much interest, interspecific volatile signalling between plants has remained a topic of debate (Agrawal 2000; Baldwin and Schultz 1983; Bruin and Dicke 2001; Dudareva et al. 2006; Farmer and Ryan 1990).

2.3.4.2 Spatially Separated but Connected: Above- and Belowground Induced Responses

Herbivores can attack spatially-disparate plant organs, such as roots and leaves simultaneously, leading to variation in herbivore-induced BVOCs and consequences for above- and belowground defences. Due to vascular connections, aboveground herbivory can change the quantity and composition of root BVOCs and *vice versa*. While the majority of studies focus on BVOC-mediated tritrophic interactions above-ground, these relationships occur belowground as well and have important effects on aboveground communities (Van der Putten et al. 2001). To our knowledge, no studies to date have described the integration of above and belowground BVOC-mediated tritrophic interactions in trees. While such interactions are likely to exist, they will likely occur at different temporal time scales due to longer generation times and contributing phenological factors. Recent studies focused on herbaceous plants have shown that the vegetative portions of plants experiencing belowground herbivory emit lower total BVOC emissions and produce different BVOC profiles compared to plants solely attacked by an aboveground herbivore (Rasmann and Turlings 2007). Thus, the presence of soil herbivory in this case appears to lower the potential for defence, particularly when the belowground herbivore causes increased damage as a function of development and size (Soler et al. 2007). Another study demonstrated the effects of belowground herbivory on aboveground indirect defence by showing that black mustard (*Brassica nigra*) plants experiencing root herbivory emit high levels of sulfur-containing BVOCs, highly toxic for insects, and low levels of (*E*)-β-farnesene, an attractant for parasitoids (Soler et al. 2007). In addition to soil herbivores, mutualistic mycorrhizal associations can also effect aboveground signalling, as in the case with parasitoids of aphids attracted to mycorrhizal plants in the absence of their aphid hosts (Guerrieri et al. 2004). Because of signal cross-talk, the timing of attack by above- and belowground herbivores can be crucial when examining the extent to which communities are affected. Clearly, more studies investigating BVOC responses to simultaneous attacks by above- and belowground herbivores in forest species are needed.

2.3.4.3 Induced BVOCs Exhibit Diurnal Patterns

The release of herbivore-induced BVOCs occurs both locally and systemically in space and also varies over time. For example, hybrid poplar leaves (*Populus trichocarpa* × *P. deltoides*) attacked by forest tent caterpillars (*Malacosoma disstria*) released similar characteristic blends of volatiles, including mono-, sesqui-, and homoterpene compounds, that peaked during the light period (Arimura et al. 2004). This diurnal pattern could be critical depending on parasitoid and predator foraging patterns and the biologically-active compounds present. In *Nicotiana tabacum*, several herbivore-induced BVOCs are exclusively released at night and repel female moths (*Heliothis virescens*) searching for oviposition sites (De Moraes et al. 2001). Because most parasitoids search during the day, these

nighttime emissions may not be relevant as top-down defences, but the impact of daytime indirect forcings and nighttime bottom-up effects may have significant multiplicative consequences for herbivore densities. Currently, we lack studies describing exclusive and additive diurnal ecological relationships mediated by herbivore-induced emissions in tree systems.

2.3.4.4 Induced BVOCs: Immediate vs. Delayed Responses

A limitation of inducible volatile defences is the time-lag between damage, induction, signalling, and the actual plant response (Dicke 2009). Upon feeding or oviposition, the plant can emit BVOCs within seconds to minutes. Most of these emissions, are not under control of the plant, but rather released as a consequence of exposure to the atmosphere (Maffei et al. 2007). Compounds synthesized in response to metabolic changes and involved in indirect plant defence are usually expressed within hours or days following damage (Kunert et al. 2002). Thus, parasitoids and predators must perceive and respond to these compounds within a critical window of time before the benefits of effective host-location are missed. Abiotic conditions and emissions from other organisms in the environment can also influence herbivore-induced cues with important implications for top-down controls over time, the scope of which is beyond this chapter. Rapid herbivore-induced BVOCs active in plant resistance may stabilise insect densities; however, delayed induced resistance, via foliar chemistry, potentially contributes to population cycles (e.g., Roden and Mattson 2008). Some studies demonstrate that needles of previously defoliated trees exhibit higher suitability for subsequent defoliator generations (Lyytikäinen 1992; Clancy et al. 2004), while others have demonstrated induced resistance after defoliation (Hódar et al. 2004; Šmits and Larsson 1999). The susceptibility or resistance of previously defoliated trees depends on a number of variables (i.e., tree age, intensity of defoliation, herbivore species, etc.), yet the influence of BVOCs emitted during and after defoliation on higher trophic level interactions has not been studied.

2.4 Insect Perception, Learning, and Evolutionary Considerations

Insects must perceive and process enormous amounts of sensory information, including chemical information, to locate their hosts within dynamic heterogeneous environments (Vet 2001). BVOC infochemicals are sensed by olfactory sensory neurons, primarily within antennae but also located within chemosensory sensilla on other parts of the insect's body, to aid in perceiving chemical signals in the atmosphere. The ability to perceive BVOCs plays a key role in host location for both herbivores and their natural enemies (Meiners et al. 2003), and many insects are capable of identifying compounds present in the atmosphere at levels much lower than some of our most sensitive analytical instruments.

The ability of an insect to perceive and respond to stimuli is not fixed, and can change upon association with favorable or unfavorable stimuli. For example, female parasitic wasps have a well-developed learning capacity to associate herbivore-induced plant BVOCs with the presence of suitable hosts (de Boer and Dicke 2006). The ability of insects to exploit information from BVOCs can be both innate (Gandolfi et al. 2003a; Steidle and van Loon 2003; Wang et al. 2003) and learned (Dicke 1999; Wäckers and Lewis 1999). We briefly highlight studies that focus on parasitoid learned behavior to emphasize the important role of behavioral ecology in BVOC-mediated multitrophic interactions.

Regardless of the strict categorical descriptions, learning a given task in nature likely involves a combination of stimulus-stimulus and stimulus-response associations, allowing a parasitoid to take advantage of a variety of cues, including unrewarding experiences, which might aid in future decisions and actions (Vet et al. 2003). Learning behavior tends to be more prevalent in generalist parasitoids (Geervliet et al. 1998) than in specialists (Mumm et al. 2005), suggesting that specialist parasitoid species are more 'hardwired' when it comes to responding to plant BVOCs expressed in response to their specific host. While this genetic component may be beneficial in relatively stable environments, changes in climate and/or community composition could confound host-associated signals with important consequences for parasitoid adaptations and herbivore dynamics. Furthermore, not all generalist species have the capability to learn (Tamò et al. 2006), which complicates our ability to generalize parasitoid behavior in response to dynamic chemical cues.

The ability of parasitoids to remember learned behavior also varies with time and stimulus. Recollection of unrewarded activities often fade within a few hours to days (Peri et al. 2006), but learning responses to odors of advantageous activities tend to be more persistent (Takasu and Lewis 2003), and can even occur at preimaginal stages before the adult stage (Gandolfi et al. 2003b). The coordination of plant BVOC emissions with the window of parasitoid 'memory' is thus critical for eliciting parasitoid response. Importantly, learning behavior has been found to positively impact fitness (Dukas and Jun 2000) and contributes to coexistence between parasitoids and their insect hosts (Hastings and Godfray 1999), which emphasizes that learning plays an important role in not only chemical ecology, but also in insect evolution and plant-insect coevolution.

BVOCs impact evolutionary pressures on herbivores and parasitoids through their role in determining fitness. Biogenic BVOCs are involved in a range of ecological functions (Fig. 2.1), and as a consequence, their role in plant evolution is dynamic (Yuan et al. 2009). Adaptive explanations have been offered to address the diversity of BVOCs found among and within plant families (Lerdau and Gray 2003; Wink 2003). It has also been argued that natural selection also exploits the volatility of the compounds themselves and thereby the context in which they are perceived by herbivores and their natural enemies (Peñuelas and Llusià 2004). The precise ecological functions and evolutionary consequences of every BVOC are not yet known (Niinemets et al. 2004), so their full contribution to plant-insect evolution

has yet to be characterized. However, the importance of BVOCs to plant, herbivore, and parasitoid fitness highlights their role in the evolution of each taxonomic group, and their role in ecological signalling suggests that they play a substantial role in coevolution among taxonomic groups.

2.5 Conclusions

BVOCs influence plant-insect interactions across multiple levels of ecological organization and play active roles in bottom-up and top-down defences against herbivory. Important ecological consequences stem from feedbacks that exist between constitutive and herbivore-induced BVOC emissions, herbivore and parasitoid behavior, and the environment. Researchers are only beginning to uncover the role BVOCs play in mediating tritrophic interactions and influencing coevolutionary processes that exist between plants and insects. An improved understanding of the impacts of global change on plant and insect ecology and evolution will help us understand the full consequences of BVOC-mediated plant-insect interactions in forested ecosystems, including the role of insects in recently observed forest die-off (Raffa et al. 2008; Rhoades 1983). Models of surface-atmosphere exchange have long had the capability to include BVOC dynamics (Guenther et al. 1995), and their mechanistic representation of BVOC emissions is continuously being improved (Monson et al. 2012). We suggest that including plant-insect interactions into models of BVOC emissions will improve our understanding of the impacts of these interactions on ecosystems, and to the entire Earth system.

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Chapter 3
The Biochemistry and Molecular Biology of Volatile Messengers in Trees

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Abstract All tree species possess genes encoding terminal enzymes responsible for volatile isoprenoid synthesis. However, only in some species, these genes are expressed constitutively in leaves, while terpenoid emissions can be triggered by abiotic and biotic stress factor in essentially all species. This chapter analyses the biochemical diversity of volatile isoprenoid synthases and investigates the genomic modifications responsible for constitutive volatile production in trees. Plant terpenoids are up to three-domain proteins with either one active center in monofunctional synthases, or two active centers in bifunctional synthases. There is evidence of monophyletic origin of modern plant terpenoid synthases from a three-domain synthase in an ancient progenitor followed by extensive gene duplication and domain loss. The terpenoid synthase sequence similarity can be low among distant plant groups, but terpenoid tertiary structure is remarkably similar in different synthases, and this structural similarity is even conserved across domains of life. However, only minor changes in active center structure can lead to major changes in product profiles, indicating that presence of rich terpenoid genetic diversity constitutes an important means for rapid evolutionary adaptations to novel biotic interactions, and to new abiotic stresses in plant habitats.

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3.1 Introduction

Humans have acknowledged the existence of terpenoids (isoprenoids) in plants from ancient times without any information about their chemistry and role in plants. The term terpene comes from the word turpentine, which is the resin that oozes out from the bark of pine trees after wounding. This sticky resin is rich in a variety of terpenoid compounds with widely differing chemical structure and physico-chemical characteristics (Crozier et al. 2006). All terpenoids consist of isoprene (C5) building blocks and are conditionally divided between primary metabolites such as carotenoids and hormones and secondary metabolites such as volatile terpenoids isoprene and monoterpenes (C10), and semivolatile terpenoids sesquiterpenes (C15) and diterpenes (C20) fulfilling a variety of functions, some of which are not yet fully understood (Modolo et al. 2009; Fineschi et al. 2013). Altogether, these volatiles and semivolatiles constitute the most import class of biogenic volatile organic compounds (BVOCs) (Kesselmeier and Staudt 1999; Dudareva et al. 2004; Holopainen 2004; Guenther et al. 2006; Laothawornkitkul et al. 2009).

Volatile terpenoids are released by different plant tissues including leaves, buds, flowers, fruits and roots (Dudareva and Pichersky 2008), but vegetative plant parts, in particular, leaves are believed to contribute the most to plant emissions due to high leaf mass fraction and generally the highest emission rates per foliage mass (Kesselmeier and Staudt 1999). Vegetative plant parts can release diverse mixtures of terpenoids, including isoprene, monoterpenes, sesquiterpenes and some diterpenes (Keeling and Bohlmann 2006a). These volatiles play different roles in plants including enhancement of abiotic stress tolerance by serving as antioxidants (Loreto et al. 2001b; Vickers et al. 2009a; Possell and Loreto 2013) and possibly also by modifying membrane fluidity (Sharkey and Singsaas 1995; Singsaas et al. 1997; Behnke et al. 2007), and serving as direct defences against omnivorous insects, e.g., oleoresin is a common direct defence in conifers against pathogens and herbivores (Bohlmann and Croteau 1999; Raffa et al. 2005; Heijari et al. 2008; Chen et al. 2011). In addition, these volatiles serve as indirect defences participating in within-plant and among-plant communication and stress priming and in communication with herbivore enemies (Arimura et al. 2000; Dicke and Bruin 2001; Dudareva et al. 2006; Pieterse and Dicke 2007; Choudhary et al. 2008; Dicke et al. 2009).

Due to the importance of volatile terpenoids in plant life, there is a continuing interest in plant terpenoid synthesis pathways and terpene synthase (Tps) genes as targets to increase plant stress tolerance by enhanced terpenoid content and emission of volatiles attracting herbivore parasites and predators (Pichersky and Gershenzon 2002; Degenhardt et al. 2003; Dudareva and Pichersky 2008). Understanding structural, functional and evolutionary features of Tps family in trees can help to discover defence systems in these plants and use this information for breeding and tree protection in forestry. Furthermore, some of these volatiles or their derivatives

have cosmetic or medicinal properties, which have made them as important targets for fragrance and pharmaceutical science and industry, for selection of promising genotypes and genetic engineering to overexpress the pathway in the given or a new host organism (Seigler 1998; Braun et al. 2001; Schepmann et al. 2001; Huang et al. 2004; Aharoni et al. 2005; Bohlmann and Keeling 2008; Saranitzky et al. 2009; Trusheva et al. 2010). Also, in last decades, the pathways of volatiles have been widely used and engineered to produce different types of biofuels (Peralta-Yahya et al. 2012).

From air chemistry and climate perspective, volatile isoprenoids significantly contribute to photochemical reactions in the atmosphere, participating in the formation of ozone, secondary organic aerosols and cloud condensation nuclei, thereby altering air quality, and solar radiation penetration (Huff Hartz et al. 2005; Guenther et al. 2006; Lee et al. 2006; Engelhart et al. 2008; Ashworth et al. 2013; Kulmala et al. 2013). For example, the hemiterpene (C5) isoprene (2-methyl-1,3-butadiene) is worldwide the most important volatile isoprenoid with global emissions about 440–660 Tg C $year^{-1}$ (Guenther et al. 2006; Ashworth et al. 2013). Isoprene is emitted constitutively by leaves of several deciduous angiosperm tree species like *Salix* spp., *Populus* spp., and *Quercus* spp., but also from several North-American evergreen *Quercus* spp. (Kesselmeier and Staudt 1999). Furthermore, some gymnosperms and a number of herb, moss and fern species release isoprene as well (Sharkey and Yeh 2001; Sharkey et al. 2008). Carbon loss due to isoprene emission in constitutive emitters is typically between 1 and 2 % of photosynthetic carbon fixation, but this percentage may increase to more than 50 % under stress conditions (Sharkey and Yeh 2001).

During the past decades, major progress has been made in identification and functional characterization of volatile terpenoid biosynthesis genes, enzymes and in metabolic engineering, and this has greatly contributed to improved understanding of terpenoid biosynthesis (Crozier et al. 2006; Keeling and Bohlmann 2006b; Bohlmann and Keeling 2008; Degenhardt et al. 2009; Nagegowda 2010; Chen et al. 2011). Latest developments in molecular techniques, such as new technologies for identification of large genome parts up to full genomes and rapid assessment of plant transcriptome are strongly contributing to identifying and isolating new terpenoid genes, studying the synthase reaction mechanisms and understanding their function in plants. In this chapter, we briefly describe the key pathways for immediate substrates for plant terpenoid biosyntheses. Then we analyse isoprene, monosesqui- and diterpene biosynthesis, characteristics of involved terpenoid synthases, their regulation and corresponding gene families with special attention to trees species. So far, the research on plant terpenoids has mainly focused on herbaceous species. However, given that the bulk of volatiles released to the atmosphere is believed to come from woody species, clearly there is a pressing need to gain more insight into biochemical and genetic regulation of terpenoid biosynthesis in woody species.

3.2 Terpenoid Biosynthesis

The huge chemical diversity of plant isoprenoids is formed by an extensive array of enzymes that can be divided among three groups. The first group of enzymes serves as the interface between primary and secondary metabolism, being responsible for channeling of metabolites to terpenoid synthesis pathways (Modolo et al. 2009). The second group of enzymes forms the terpenoid molecule scaffolds in terpenoid pathways (Modolo et al. 2009). The third group of enzymes alters the terpenoid backbones, resulting in new molecules with different biological activities (Modolo et al. 2009), e.g., by hydroxylation, epoxidation, arylmigration, glycosylation, methylation, sulfation, acylation, prenylation, oxidation, and reduction (Gowan et al. 1995; Ro et al. 2005; Modolo et al. 2009). Here we briefly outline the basic isoprenoid synthesis pathways. More detailed summary of terpenoid synthesis pathways is provided by Rosenkranz and Schnitzler (2013) and Li and Sharkey (2013b).

3.2.1 Main Biosynthesis Pathways: MVA and MEP/DOXP

All terpenoid compounds are synthesized from the same precursors: isopentenyl diphosphate (IDP) and its isomer dimethylallyl diphosphate (DMADP) (Wanke et al. 2001; Crozier et al. 2006; Bohlmann and Keeling 2008; Modolo et al. 2009). These precursors are synthesized by two different pathways. The cytosolic mevalonic acid (MVA) pathway is present in most eukaryotes and is responsible for the synthesis of C15 (sesquiterpenoids) and C30 (triterpenoids such as sterols) terpenoid compounds in plants (Gershenzon and Croteau 1993). The second recently discovered 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway) is present in several prokaryotes and in plastids of eukaryotic organisms (Rohmer et al. 1993; Eisenreich et al. 2004; Crozier et al. 2006). The MEP/DOXP pathway is responsible for the synthesis of isoprene (C5), mono- (C10), di- (C20) and tetraterpenoids (C40) in plants (Lichtenthaler et al. 1997a, b; Modolo et al. 2009). The two pathways operate almost independently, although there is a certain cross-talk among the two pathways at the level of IDP (e.g., Hemmerlin et al. 2003; Laule et al. 2003).

Isoprenoid synthesis through MVA pathway starts with condensation of three molecules of acetyl coenzyme A (acetyl-CoA) producing 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA is further reduced by HMG-CoA reductase to mevalonic acid (MVA), which is phosphorylated by two kinases forming mevalonate 5-diphosphate (MVADP). MVADP is converted into the terpenoid precursor, IDP, by mevalonate diphosphate carboxylase (Gershenzon and Croteau 1993; Eisenreich et al. 2004; Crozier et al. 2006). The cytosolic enzyme isopentenyl diphosphate isomerase (IDI) further catalyzes the reversible conversion between IDP and its isomer DMADP.

The plastidic MEP/DOXP pathway starts with the condensation of the substrates pyruvate and glyceraldehyde 3-phosphate (GAP) to DOXP. Carbon skeleton rearrangements and dehydration steps result in formation of 2-C-methyl-D-erythritol 4-phosphate (MEP). MEP is further converted to 2-C-methyl-D-erythritol 2,4-cyclodiphosphate and to 4-hydroxy-3-methylbut-2-enyl diphosphate (HMBDP). Finally, HMBDP is converted both to IDP and DMADP by HMBDP reductase, and the pool sizes of IDP and DMADP are further modulated by plastidic isopentenyl diphosphate isomerase (Eisenreich et al. 2004; Crozier et al. 2006; Li and Sharkey 2013b).

In the past years, major progress has been made in characterizing the enzymes of MEP/DOXP pathway in plants, but kinetic characteristics of all enzymes are still not available, although the available evidence suggests that they resemble those in bacterial counterparts (Harrison et al. 2013; Li and Sharkey 2013b). However, differently from bacteria, MEP/DOXP pathway in plants can directly accept electrons from photosynthetic electron transport chain. In particular, HMBDP synthase and HMBDP reductase can accept electrons from ferredoxin (Seemann et al. 2006; Seemann and Rohmer 2007), possibly explaining the tight coupling of MEP/DOXP pathway to light reactions of photosynthesis in the chloroplasts. Overall, synthesis of highly reduced terpenoids is energetically costly with synthesis of one isoprenoid C5 residue needing fixation of 6 molecules of CO_2, and consuming 20 ATP, and 14 NADPH molecules (Sharkey et al. 2008), underscoring the need for high plastidic ATP and NADPH status for DOXP/MEP pathway (Rasulov et al. 2011; Li and Sharkey 2013a, b).

3.2.2 From Precursors to Terpenoids

DMADP is the substrate for the synthesis of the smallest isoprenoids, the hemiterpenes isoprene and 2-methyl-3-buten-2-ol (MBO). IDP and DMADP are further substrates for short-chain prenyltransferases (Bohlmann and Keeling 2008; Modolo et al. 2009). The assembly of geranyl diphosphate, the backbone for monoterpenes, by head-to-head addition of IDP and DMADP is catalyzed by GDP synthase. Further farnesyl diphosphate (FDP), the backbone for sesquiterpenes, is formed by condensing GDP and IDP by FDP synthase. Geranylgeranyl diphosphate (GGDP) that is the substrate for diterpene synthesis is formed by condensing FDP and IDP by GGDP synthase. Further, tri- and tetraterpenoids are made by head-to-head condensation of two FDP and two GGDP molecules, respectively (Bohlmann and Keeling 2008; Modolo et al. 2009). The resulting terpenoid polymers are used as precursors by terpene synthases/cyclases and enter into synthesis of primary terpenoid compounds such as sterols, phytyl-chain of chlorophyll and carotenoids (Modolo et al. 2009). Terpenes can further be modified by hydroxylation and oxidization by cytochrome P450-dependent enzymes (Ro et al. 2005; Keeling and Bohlmann 2006a).

3.3 Terpenoid Synthases

Terpenoid synthases form a diverse class of enzymes catalyzing formation of molecules with different chain length, including hemiterpene synthases (C5), monoterpene synthases (C10), sesquiterpene synthases (C15), and diterpene synthases (C20). Being often the end-points of the pathway, they are the key terminal flux-controlling enzymes. So far, over 60,000 members of the terpenoid family are recognized, and a broad grouping of structures has been clarified (http://dnp.chemnetbase.com/) (Xie et al. 2012). There has been a major progress in understanding the function and structure of terpenoid synthases catalyzed by rapid developments in molecular biology techniques allowing for dissection of the structure of terpenoid synthase gene families and heterologous expression and study of recombinant terpenoid synthases (Degenhardt et al. 2009). A number of model terpene synthases has been characterized in detail (Hyatt et al. 2007; Köksal et al. 2010, 2011a, b; McAndrew et al. 2011; Zhou et al. 2012), but we are just starting to understand the size and structure of terpene synthase families in key organisms (Sect. 3.4). Furthermore, there is less information on tree terpenoid synthases than on synthases in herbaceous species, except perhaps for gymnosperms (Degenhardt et al. 2009). Nevertheless, the progress has been amazingly rapid as new biotechnology techniques are providing strong tools to understand the complex biochemical function and regulation of genes involved in the terpenoid pathways. The emergence of new high throughput techniques such as deep sequencing together with developments in computational bioinformatics is allowing shedding light on previously hidden aspects of genomes, transcriptomes, proteomes, metabolomes and finally terpenomes with unprecedented detail (Christianson 2008; Cane and Ishida 2012). Here we introduce the basic contemporary methods to study terpenoid synthases and analyse the basic functional structure of terpenoid synthases with emphasis on tree enzymes.

3.3.1 Identification and Analysis of the Functional Activity of Terpenoid Synthases

Due to simultaneous expression of multiple terpenoid synthases in plant tissues and relatively low product specificity of most synthases (Sect. 3.3.3.4), functional analysis of terpenoid synthases based on enzyme purification from crude leaf extracts is difficult, although a lot of pioneering work has been conducted using partially purified enzymes extracted from plants (e.g., Croteau and Karp 1977; Croteau et al. 1978; Dehal and Croteau 1988). Development of molecular biology techniques for identification and isolation of individual terpenoid synthases has opened completely new vistas for studying biochemistry, structure, genetics and evolution of terpenoid synthases. Development of RNA sequencing platforms in recent years using deep-sequencing technologies have changed the transcriptomics world and sometimes

predicate the death of micro-array and other transcriptome analysis technologies like serial analysis of gene expression (SAGE, Velculescu et al. 1995), cap analysis gene expression (CAGE, Shiraki et al. 2003), and massively parallel signature sequencing (MPSS, Brenner et al. 2000). However, the high throughput technologies have some disadvantages like high cost, inability to detect transcripts for isoforms and splice variation (Wang et al. 2009; Myllykangas et al. 2012).

The workflow for functional characterization of given terpene synthase typically consists of determination of the sequence of terpenoid synthase genes either on the basis of mRNA or genomic DNA, heterologous expression in a host system of the sequenced gene and functional characterization of the recombinant protein. Ultimately, the function can be further studied in a transgenic plant model system. Here these basic steps are briefly outlined.

3.3.1.1 Identification of Terpenoid Synthase Genes

In the infancy of terpenoid molecular and functional studies, identification of terpene synthase genes was a highly tedious task due to lack of information on homologous sequences for degenerate primer construction. Now, as more and more synthases have been sequenced, the rich existing genetic information allows for more rapid progress, albeit identification of terpenoid synthases with low level of homogeneity with those described so far, especially in organisms with little genome coverage is still difficult (Cane and Ishida 2012). By now, 51 genomes of higher plants (38 published) have been fully sequenced (as of Sept. 4, 2012, http://genomevolution.org/wiki/index.php/Sequenced_plant_genomes), making it possible to identifiy putative terpenoid synthases by genome mining. Genome sequence analysis of *Arabidopsis thaliana* has identified about 30 terpene synthase genes in this model organism (Aubourg et al. 2002), but in several vascular plant species much larger terpene synthase families have been detected (Sect. 3.4.2, Martin et al. 2010; Li et al. 2012), especially by using the widest range of terpenoid synthase sequences possible in homology searches (Li et al. 2012). In particular, information about the size and structure of tree terpene synthase gene families has been exponentially increasing since the first tree, *Populus trichocarpa* (Tuskan et al. 2006), genome completion, followed by other tree genome projects including *Carica papaya* (Ming et al. 2008), *Malus domestica* (Velasco et al. 2010), *Phoenix dactylifera* (Al-Dous et al. 2011), and *Pyrus bretschneideri* (Wu et al. 2013). New bioinformatics tools such as the use of profile-based hidden Markov models rather than pairwise searchers makes it possible to identify terpene synthase genes carrying remote sequence homology, thereby identifying putative terpene synthases with potential structural similarity of basic conserved functional domains (Gough et al. 2001; Wilson et al. 2009; Cane and Ishida 2012).

Although the number of fully sequenced genomes is rapidly increasing, the genome of only 11 tree species has been sequenced, most of them tree crops. Among sequenced trees, only *Populus trichocarpa* and *Eucalyptus grandis* can be conditionally considered as ‘wild plants’. Thus, techniques alternative to genome

mining need to be applied to most tree species. New high throughput techniques (e.g., next generation sequencing, Liu et al. 2012) have opened up possibilities for fast characterization of transcriptome, and identification of expressed terpenoid synthases by transcriptome mining. Conifers are characterized by a particularly rich blend of terpene volatiles, suggesting a highly diverse terpenoid family, but conifer genome is especially complex, and full sequences of first conifer genomes are unlikely in the near future, although a number of genome projects has started (see e.g., http://pinegenome.org/). Thus, relatively few terpenoid synthases have been functionally characterized in conifer species so far, although more than in angiosperm trees (e.g., Wildung and Croteau 1996; Bohlmann et al. 1997, 1999; Hall et al. 2011). In these pioneering studies, different techniques were used to identify conifer terpene synthases, such as cDNA library screening and similarity-based PCR (Bohlmann et al. 1998a). Expressed sequence tag (EST) libraries have been available lately for loblolly pine (*Pinus taeda*) (Allona et al. 1998), Japanese cedar (*Cryptomeria japonica*) (Ujino-Ihara et al. 2000), white spruce (*Picea glauca*), interior spruce (*P. glauca* × *P. engelmannii*), and Sitka spruce (*Picea sitchensis*) (Holliday et al. 2008). Moreover, lots of attempts are currently in progress, trying to identify and characterize more terpene synthases in conifers using a combination of targeted cDNA cloning, large amounts of ESTs and full-length cDNA mining (Byun McKay et al. 2003; Miller et al. 2005; Keeling et al. 2011) as well as using other techniques such as bacterial artificial chromosome (BAC) technique (Hamberger et al. 2009). Use of these new methods has allowed identification of large terpenoid synthase families in several tree species, e.g., 69 actively expressed Tps genes (including monoterpene, sesquiterpene and diterpene synthases) have been identified in *Picea* species (Keeling et al. 2011).

3.3.1.2 Heterologous Expression of Tree Terpenoid Synthases in *E. coli* and in Plants

Functional characterization of terpene synthases is usually carried out by heterologous expression in *Escherichia coli* (Table 3.1 for a selected list of tree terpenoid synthases expressed in *E. coli*). However, expression of terpene synthase genes in *E. coli* carries potential problems. Some terpene synthase proteins are produced in cytosol, but are targeted to chloroplastic compartment, therefore having chloroplastic signal peptides. These transient sequences should be removed before cloning and expression in the host for sufficiently high expression of recombinant protein (Phillips et al. 2003). On the other hand, codon usage of eukaryotic genes is different from prokaryotic host, and the eucaryotic genome also contains rare codons (Fig. 3.1). The comparison between codon usages in the angiosperm *Salix discolor* and *Escherichia coli* shows the mean difference close to 30 % in codon usage, whereas the difference between the angiosperm *Populus trichocarpa* and the gymnosperm *Abies grandis* is about 10 %. Thus, for high level of expression, co-transformation of a plasmid encoding the rare tRNAs (e.g., for Arg) is needed (Hohn 1999; Martin et al. 2004). The codon optimization for gene expression of tree Tps

Table 3.1 List of selected tree terpenoid synthases expressed and characterized in *Escherichia coli* host system

Terpenoid synthase	Gene family[a]	UniProtKB/ Swiss-Prot entry	Species	Expression host	Substrate	Terpenoid products[b]	References
Hemiterpenes (C_5)							
Isoprene synthase	Tps-b	Q50L36	*Populus alba*	*E. coli* origami B (DE3)	DMADP	Isoprene (100 %)	Sasaki et al. (2005)
Isoprene synthase	Tps-b	Q9AR86	*Populus x canescens*	*E. coli* TG1	DMADP	Isoprene (100 %)	Miller et al. (2001)
2-Methyl-3-buten-2-ol (MBO) synthase	Tps-d1	F5CJS6	*Pinus sabiniana*	*E. coli* BL21 (DE3)-RIL	DMADP	Methylbutenol (99 %), isoprene (1 %)	Gray et al. (2011)
Monoterpenes (C_{10})							
(−)-Camphene synthase	Tps-d1	Q948Z0	*Abies grandis*	*E. coli* BL21(DE3)	GDP	(−)-camphene (54 %), (−)-α-pinene (32 %), (−)-limonene	Bohlmann et al. (1999)
(+)-Carene synthase 1	Tps-d1	F1CKI6	*Picea sitchensis*	*E. coli*	GDP	(+)-3-carene (42 %), α-terpinolene (27 %), (+)-sabinene (11 %), (−)-β-phellandrene, myrcene, α-terpinene, γ-terpinene, (−)-α-pinene, α-thujene	Hall et al. (2011)
(+)-Carene synthase 2	Tps-d1	F1CKI8	*Picea sitchensis*	*E. coli*	GDP	(+)-3-carene (64 %), α-terpinolene (15 %), (+)-sabinene (7 %), (−)-β-phellandrene, α-terpinene, γ-terpinene, myrcene	Hall et al. (2011)
(−)-Limonene synthase	Tps-d1	O22340	*Abies grandis*	*E. coli* BL21(DE3)	GDP	(−)-limonene (>90 %), (−)-α-pinene, (−)-β-pinene	Bohlmann et al. (1997)

(continued)

Table 3.1 (continued)

Terpenoid synthase	Gene family[a]	UniProtKB/ Swiss-Prot entry	Species	Expression host	Substrate	Terpenoid products[b]	References
(−)-Limonene/(−)-α-pinene synthase	Tps-d1	Q9M7C9	*Abies grandis*	*E. coli* BL21(DE3)	GDP	(−)-limonene (35 %), (−)-α-pinene (24 %), (−)-β-phellandrene (20 %), (−)-β-pinene (11 %), (−)-sabinene (10 %)	Bohlmann et al. (1999)
(+)-Limonene synthase	Tps-b	Q8L5K1	*Citrus limon*	*E. coli* BL21-CodonPlus-RIL	GDP	(+)-limonene (99 %)	Lücker et al. (2002)
(+)-Limonene synthase	Tps-b	Q8L5K3	*Citrus limon*	*E. coli* BL21-CodonPlus-RIL	GDP	(+)-limonene (99 %)	Lücker et al. (2002)
Limonene synthase	Tps-d1	Q675L1	*Picea abies*	*E. coli* BL21-CodonPlus	GDP	(−)-limonene (88 %), myrcene, (−)-α-pinene, (+)-limonene	Martin et al. (2004)
Linalool synthase	Tps-d1	Q675L2	*Picea abies*	*E. coli* BL21-CodonPlus	GDP	(−)-linalool (97 %), (+)-linalool, *E*-β-ocimene	Martin et al. (2004)
Myrcene synthase	Tps-d1	O24474	*Abies grandis*	*E. coli* XL1-Blue	GDP	Myrcene (100 %)	Bohlmann et al. (1997)
Myrcene synthase	Tps-b	Q93X23	*Quercus ilex*	*E. coli* TG1	GDP	Myrcene (97 %), limonene, β-pinene	Fischbach et al. (2001)
Myrcene synthase	Tps-d1	Q675K9	*Picea abies*	*E. coli* BL21-CodonPlus	GDP	Myrcene (100 %)	Martin et al. (2004)
(−)-β-Phellandrene synthase	Tps-d1	Q9M7D1	*Abies grandis*	*E. coli* BL21(DE3)	GDP	(−)-β-phellandrene (52 %), (−)-β-pinene (34 %), (−)-α-pinene (9 %), (−)-limonene	Bohlmann et al. (1999)
(+)-α-Pinene synthase	Tps-d1	Q84KL3	*Pinus taeda*	*E. coli* BL21(DE3)	GDP	(+)-α-pinene (97 %), (−)-α-pinene	Phillips et al. (2003)

(−)-α-Pinene synthase	Tps-d1	Q84KL6	*Pinus taeda*	*E. coli* BL21(DE3)	GDP	(−)-α-pinene (79 %), (−)-β-pinene, (−)-limonene, (+)-limonene, (+)-camphene, (−)-camphene, (+)-α-pinene, (+)-β-pinene	Phillips et al. (2003)
(−)-β-Pinene synthase	Tps-b	Q8L5K2	*Citrus limon*	*E. coli* BL21-CodonPlus-RIL	GDP	(−)-β-pinene (81 %), sabinene (11 %), (−)-α-pinene, (−)-limonene	Lücker et al. (2002)
(−)α/β-Pinene synthase	Tps-d1	Q675L3	*Picea abies*	*E. coli* BL21(DE3)	GDP	(−)-α-pinene (57 %), (−)-β-pinene (27 %), β-phellandrene (11 %), myrcene	Martin et al. (2004)
Pinene synthase	Tps-d1	Q6XDB5	*Picea sitchensis*	*E. coli* BL21-CodonPlus (DE3)	GDP	(−)-α-pinene (65 %) (−)-β-pinene (18 %),	Byun McKay et al. (2003)
(−)-Pinene synthase	Tps-d1	O24475	*Abies grandis*	*E. coli* XL1-Blue/*E. coli* XLOLR	GDP	(−)-α-pinene (42 %), (−)-β-pinene (58 %)	Bohlmann et al. (1997)
(+)-Sabinene synthase	Tps-d1	F1CKJ1	*Picea sitchensis*	*E. coli*	GDP	(+)-sabinene (54 %), α-terpinolene (31 %), (−)-α-pinene, α-terpinene, (−)-β-phellandrene, γ-terpinene, myrcene, (+)-3-carene	Hall et al. (2011)

(continued)

Table 3.1 (continued)

Terpenoid synthase	Gene family[a]	UniProtKB/ Swiss-Prot entry	Species	Expression host	Substrate	Terpenoid products[b]	References
γ-Terpinene synthase	Tps-b	Q8L5K4	*Citrus limon*	*E. coli* BL21-CodonPlus-RIL	GDP	γ-terpinene (71 %), (−)-limonene (9 %), (−)-α-pinene, (+)-β-pinene, terpinolene, α-thujene, (+)-limonene, α-terpinene	Lücker et al. (2002)
Terpinolene synthase	Tps-d1	Q9M7D0	*Abies grandis*	*E. coli* BL21(DE3)	GDP	Terpinolene (42 %), (−)-α-pinene (18 %), (−)-limonene (11 %), (−)-β-pinene (10 %)	Bohlmann et al. (1999)
(−)-α-Terpineol synthase	Tps-d1	Q84KL4	*Pinus taeda*	*E. coli* BL21(DE3)	GDP	α-terpineol (57 %), limonene (28 %), terpinolene (8 %), β-pinene, α-pinene, myrcene[c]	Phillips et al. (2003)
(+)-α-Terpineol synthase	Tps-b	B5A434	*Santalum album*	*E. coli* C41	GDP	(+)-α-terpineol (44 %), (−)-limonene (33.6 %), E-geraniol, linalool, myrcene, (−)-α-pinene, (+)-sabinene, α-terpinolene	Jones et al. (2008)
Sesquiterpenes (C_{15})							
α-Bisabolene synthase	Tps-d3	O81086	*Abies grandis*	*E. coli* XL1-Blue	FDP	*E*-α-bisabolene (>99 %)	Bohlmann et al. (1998a)
					GDP	(+)-limonene (main product)	
E-α-Bisabolene synthase	Tps-d3	Q675L6	*Picea abies*	*E. coli* BL21-CodonPlus	FDP	*E*-α-bisabolene (100 %)	Martin et al. (2004)

α-Farnesene synthase	Tps-b	Q84LB2[d]	*Malus domestica*	*E. coli* DH5α	FDP	*E*,*E*-α-farnesene (>99 %), *Z*,*E*-α-farnesene (<1 %)	Pechous and Whitaker (2004)
					GDP	E-β-ocimene (68 %), (Z)-β-ocimene (32 %)	Pechous and Whitaker (2004)
α-Farnesene synthase	Tps-b	Q84LB2[e]	*Malus domestica*	*E. coli* BL21-CodonPlus-RIL	FDP	*E*,*E*-α-farnesene (96 %), *Z*,*E*-α-Farnesene (<1 %)	Green et al. (2007)
					GDP	*E*-β-ocimene (90 %), linalool, myrcene	Green et al. (2007)
E,*E*-α-Farnesene synthase	Tps-d1	Q675K8	*Picea abies*	*E. coli* BL21-CodonPlus	FDP	*E*,*E*-α-farnesene (100 %)	Martin et al. (2004)
(−)-Germacrene D synthase	Tps-a	Q64K29	*Populus trichocarpa x P. deltoides*	*E. coli* BL-21-CodonPlus	FDP	(−)-germacrene D (79 %), β-caryophyllene, α-humulene, alloaromadendrene and 4 non-identified terpenoids	Arimura et al. (2004)
γ-Humulene synthase	Tps-d2	O64405	*Abies grandis*	*E. coli* XL1-Blue	FDP	γ-humulene (dominant), sibirene, longifolene, β-himachalene, γ-himachalene, α-himachalene	Steele et al. (1998)
Longifolene synthase	Tps-d2	Q675L0	*Picea abies*	*E. coli* BL21-CodonPlus	FDP	Longifolene (61 %), α-longipinene (15 %), longicyclene, *E*-β-farnesene, longiborneol, cyclosativene, β-longipinene	Martin et al. (2004)

(continued)

Table 3.1 (continued)

Terpenoid synthase	Gene family[a]	UniProtKB/ Swiss-Prot entry	Species	Expression host	Substrate	Terpenoid products[b]	References
Diterpenes (C_{20})							
Abietadiene synthase	Tps-d3	Q38710	*Abies grandis*	*E. coli* BLR/*E. coli* XL1-Blue	GGDP	Abietadiene (mixture of double bond isomers)	Peters and Croteau (2002) and Stofer Vogel et al. (1996)
Isopimaradiene synthase	Tps-d3	Q675L5	*Picea abies*	*E. coli* BL21-CodonPlus	GGDP	Isopimara-7,15-diene (100 %)	Martin et al. (2004)
Levopimaradiene synthase	Tps-d3	Q947C4	*Ginkgo biloba*	*E. coli*	GGDP	Levopimaradiene (main product)	Schepmann et al. (2001)
Levopimaradiene synthase	Tps-d3	Q675L4	*Picea abies*	*E. coli* BL21-CodonPlus	GGDP	Levopimaradiene (37 %), abietadiene (32 %), neoabietadiene (23 %), palustradiene	Martin et al. (2004)
Taxadiene synthase		Q41594	*Taxus brevifolia*	*E. coli* JM109/ *E. coli* BL21(DE3)	GGDP	Taxadiene (mixture of double bond isomers)	Wildung and Croteau (1996), Williams et al. (2000) and Jin et al. (2005)

[a]According to Bohlmann et al. (1998b), Byun McKay et al. (2003), and Martin et al. 2004

[b]Only minor products ≥1 % are demonstrated

[c]Dominated by (−) isomer, except for α-pinene (100 % (+)-α-pinene)

[d]GenBank accession number AY182241.2, expressed with C-terminal Myc-tag (Pechous and Whitaker 2004)

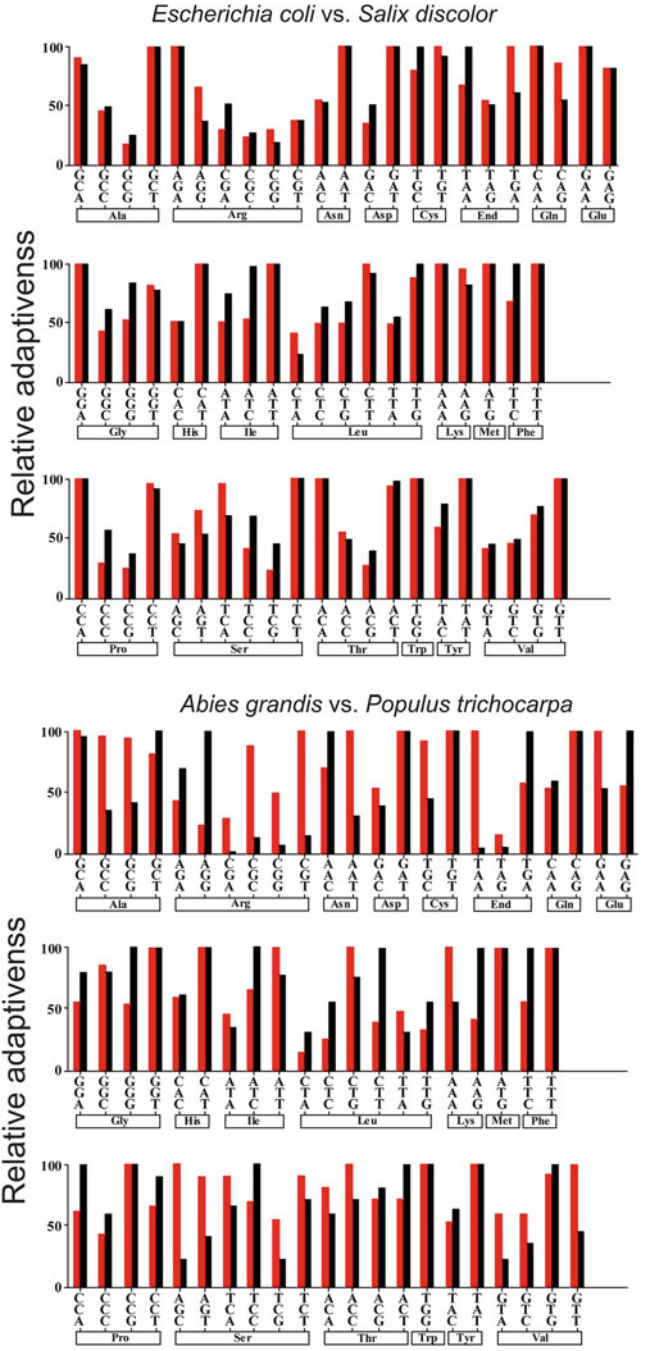
[e]GenBank accession number AY787633, expressed with N-terminal His-tag (Green et al. 2007)

genes can also be achieved by using suitable host strains. Rosetta host strains are BL21 derivatives designed to enhance the expression of eukaryotic proteins that contain rare codons (like tree codons) which are seldom used in *E. coli*. This strategy can facilitate overexpression and characterization of different tree Tps genes in *E. coli* (Kane 1995).

The heterologous expression of Tps synthase in *E. coli* is followed by disruption of cellular contents of *E. coli* cultures carrying the transgenic construct, enzyme purification whenever needed, and assay of enzymatic activity (Martin et al. 2004; Sasaki et al. 2005; Majdi et al. 2011). Typically, the functional characterization of the protein involves incubation of the soluble recombinant enzyme with the substrate GDP, FDP, or GGDP in the presence of Mg^{2+} and/or Mn^{2+}, and analysis of the product profiles by gas chromatography – mass spectrometry (GC-MS) and identification of terpenoids by authentic standards (Bohlmann et al. 1997; Martin et al. 2004; Falara et al. 2011). Typically, an overlay of pentane or other hydrophobic solvent is used to trap the hydrophobic reaction products formed in the aqueous reaction mixture (e.g., Martin et al. 2004), or volatiles can also be sampled in the head-space using a solid-phase micro extraction (SPME) fiber (e.g., Falara et al. 2011) or using sample air from the headspace with a GC preconcentration trap (e.g., Fischbach et al. 2001).

Although heterologous expression offers an unique opportunity to work with the isolated protein without other potentially interfering proteins, the kinetic characteristics of the recombinant enzyme can differ from the native enzyme, but not necessarily substantially, e.g., as demonstrated by similar K_m values for GGDP for the diterpene taxadiene native and recombinant enzyme (Williams et al. 2000). Also, enzyme characteristics can depend on the transgenic construct, e.g., farnesene synthase expressed with C-terminal Myc-tag (Pechous and Whitaker 2004) and N-terminal His-tag (Green et al. 2007) had somewhat different product profiles (Table 3.1). Expression of poplar isoprene synthase either with N-terminal or C-terminal His-tag resulted in altered pH and temperature dependence and substrate specificity (Schnitzler et al. 2005). Thus, some caution is warranted when making inferences on the performance of native enzyme on the basis of measurements with recombinant protein.

Heterologous expression in *E. coli* can be followed by characterization of the functional role of the protein in plants as discussed in detail by Rosenkranz and Schnitzler (2013). For example, transgenic *Arabidopsis* model systems expressing isoprene synthase from white poplar (*Populus alba*) (Sasaki et al. 2007), grey poplar (*P. x canescens*) (Loivamäki et al. 2007a, 2008) and kudzu (*Pueraria lobata*) (Velikova et al. 2011), and transgenic tobacco (*Nicotiana tabacum*) expressing *P. alba* isoprene synthase gene (Vickers et al. 2009b, 2011) are available. Also, herbaceous model systems overexpressing certain mono-, sesqui- and diterpenes have been constructed (El Tamer et al. 2003; Besumbes et al. 2004; Wu et al. 2006). However, no such model systems were yet available for trees. In this book, Rosenkranz and Schnitzler (2013) first time describe successful introduction of poplar isoprene synthase gene into silver birch (*Betula pendula*), providing a new exciting model to test the role of isoprene synthase in plants.


Escherichia coli vs. Salix discolor
Relative adaptivenss
100
50
0
Ala
Arg
Asn
Asp
Cys
End
Gln
Glu
Gly
His
Ile
Leu
Lys
Met
Phe
Pro
Ser
Thr
Trp
Tyr
Val
Abies grandis vs. Populus trichocarpa
Relative adaptivenss

3.3.2 *Conserved Motifs and Functional Domains of Terpenoid Synthases*

3.3.2.1 Conserved Motifs

According to the mechanism of catalysis, terpenoid synthases can be separated among two major classes. In the case of class I enzymes, the catalysis is initiated by metal-triggered ionization of the substrate diphosphate group, while for class II enzymes, the catalysis is initiated by protonation of an epoxide ring or carbon–carbon double bond (Christianson 2006, 2008; Aaron and Christianson 2010; Cao et al. 2010). In both cases, a highly reactive carbocation is formed that enters into isomerization and cyclization steps until the catalysis is terminated by either proton elimination or nucleophilic capture from the final carbocation (Aaron and Christianson 2010; Cao et al. 2010; Köksal et al. 2011a). Thus, the primary difference among the class I and class II terpenoid synthases is the initial step of the catalysis. These differences in catalytic mechanism also reflect different origin of terpenoid synthases with all type I synthases sharing the 'type I synthase fold', an α-helical structure, containing a core of bundled anti-parallel α-helices, while type II enzymes have a characteristic "α-barrel" structure (Aaron and Christianson 2010; Cao et al. 2010).

These differences in the catalytic mechanism are reflected in differences in conserved motifs and functional domains. Class I terpenoid synthases are characterized by the aspartate (D)-rich DDXXD and (N/D)DXX(S/T)XXXE (N is aspargine, S is serine and T is threonine, X can be any amino acid) motifs (Fig. 3.2) that are responsible for binding of divalent metal ions, in particular Mg^{2+} or Mn^{2+}; these metal ions are responsible for diphosphate elimination from the substrate, resulting in carbocation formation (Starks et al. 1997; Degenhardt et al. 2009; McAndrew et al. 2011). The DDXXD motif is typically located at the entrance position of the catalytic site and plays a prominent role in positioning the substrate for catalytic reaction, while (N/D)DXX(S/T)XXXE motif is located at the opposite site of the active site entry, with the underlined amino acids coordinating the metal cations (Little and Croteau 2002; Degenhardt et al. 2009; Cao et al. 2010; Köksal et al. 2011a). Mutational analysis of these motifs in diterpene abietadiene synthase

Fig. 3.1 Comparison of codon usage between (*upper panels*) the bacterium *E. coli* (*red bars*) and the angiosperm *Salix discolor* (*black bars*) and between (*lower panels*) the gymnosperm *Abies grandis* (*red bars*) and the angiosperm *Populus trichocarpa* (*black bars*). Relative adaptiveness is an index of usage of synonymous codons that scales the frequency of use of given codon relative to the optimal codon (most frequently used codon with the greatest translation efficiency) (Sharp and Li 1987). This index is calculated for a given codon *j* and for given amino acid *i*, w_{ij}, as x_{ij}/x_{imax}, where x_{ij} is the frequency of the use of the given codon and $x_{i,max}$ is that for the optimal codon (Sharp and Li 1987). w_{ij} facilitates comparison of the codon usage in different proteins and among different organisms. Codon frequency is based on NCBI GenBank (www.kazusa.or.jp/codon/). The data was extracted and analysed by graphical codon usage analyzer (http://gcua.schoedl.de/)

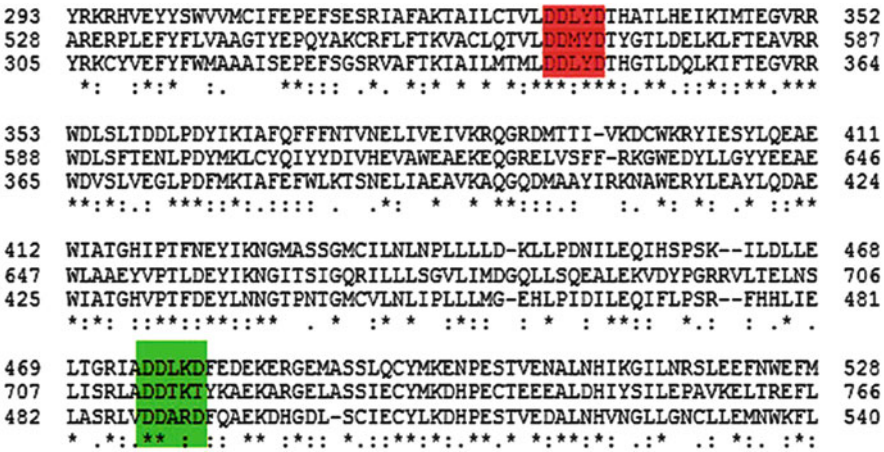


Fig. 3.2 Two DDXXD metal binding motifs in sesquiterpene synthases in the gymnosperm grand fir (*Abies grandis*). The first sequence is for δ-selinene synthase (ag4, UniProtKB/Swiss-Prot entry O64404), the second for α-bisabolene synthase (ag1, O81086) and the third for γ-humulene synthase (ag5, O64405) (The sequences were aligned using UniProt protein sequence database, http://www.uniprot.org/align/)

in *Abies grandis* demonstrated that mutations in metal-coordinating amino acids strongly reduced the enzyme catalytic activity (Zhou and Peters 2009). However, for some terpene synthases, e.g., the sesquiterpene synthases γ-humulene synthase (ag5) and δ-selinene synthase (ag4) from *Abies grandis*, the sequence information indicates that (N/D)DXX(S/T)XXXE motif is replaced by another DDXXD motif (Back and Chappell 1996; Steele et al. 1998).

Class II terpene synthases contain a conserved DXDD motif which is responsible for protonation of the double bond in the initial reaction step (Cao et al. 2010; Köksal et al. 2011a; Zhou et al. 2012). Mutations in the central aspartic acid render the active site completely non-functional (Christianson 2006; McAndrew et al. 2011). As the diphosphate cleaving activity is missing in class II terpenoid cyclases, they commonly yield diphosphorylated products such as the diterpenoid copalyl diphosphate (CDP) formed by CDP synthase (Keeling et al. 2010; Köksal et al. 2011a) unless they use non-diphosphorylated substrates such as the bacterial squalene-hopene synthase (Wendt et al. 1997). Exceptionally, class I monoterpene bornyl diphosphate synthase from *Salvia officinalis* forms a diphosphorylated product, but in this unusual reaction mechanism, diphosphate residue is first cleaved and then again reincorporated (Whittington et al. 2002).

3.3.2.2 Functional Domains of Terpenoid Synthases

Terpenoid synthases consist of up to three functional domains, α-, β and γ-domain. α-domain bears class I terpene synthase activity, β-domain class II terpene synthase

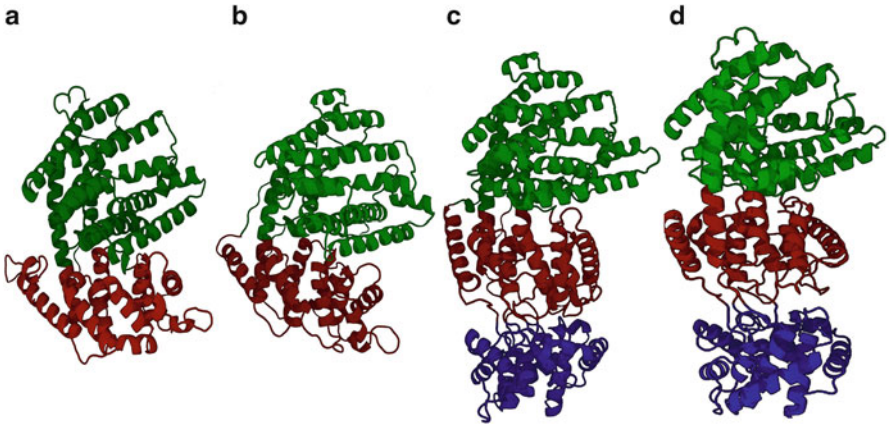


Fig. 3.3 Structure of selected tree terpenoid synthases: (**a**) hemiterpene isoprene synthase from *Populus* x *canescens* (Protein Data Bank, http://www.rcsb.org/pdb, PDB ID: 3N0F) (Köksal et al. 2010), (**b**) sesquiterpene δ-cadinene synthase from *Gossypium arboreum* (PDB ID: 3G4F, Gennadios et al. 2009), (**c**) sesquiterpene α-bisabolene synthase from *Abies grandis* (PDB ID: 3SAE, McAndrew et al. 2011), and (**d**) diterpene abietadiene synthase from *Abies grandis* (PDB ID: 3S9V, Zhou et al. 2012). Different colours correspond to α- (green, C-terminus), β- (red, N-terminus) and γ-domain (blue). The active site for the terpenoid synthases in (**a**–**c**) is in the α-domain. The bifunctional abietadiene synthase has two active sites, one in the α-domain and the other in the β-domain. The illustrations were generated by Protein Homology/analogY Recognition Engine 2.0 (PHYRE 2.0, http://www.sbg.bio.ic.ac.uk/phyre2) (Kelley and Sternberg 2009) that uses the protein structure library stored in Protein Data Bank (http://www.rcsb.org)

activity, while γ-domain does not have any known catalytic site (Aaron and Christianson 2010; Cao et al. 2010; Köksal et al. 2011a). Although the domains form clearly separate folds in protein tertiary structure (Fig. 3.3), in the protein amino acid sequences, γ-domain sequence is typically embedded within the β-domain sequence (Wendt et al. 1997; Zhou et al. 2012). In nature, α-domain synthases and β-γ-domain synthases can be found in several organisms such as bacteria and fungi. For example, sesquiterpene pentalenene synthase from the actinobacterium *Streptomyces* (Cane et al. 1994; Lesburg et al. 1997; Caruthers et al. 2000), sesquiterpene aristolochene synthase from the fungus *Penicillium roqueforti* (Caruthers et al. 2000) and sesquiterpene trichodiene synthase from the fungus *Fusarium sporotrichioides* (Rynkiewicz et al. 2001) are α-domain only class I terpenoid synthases, whereas triterpenoid squalene-hopene cyclase from the firmicute *Alicyclobacillus acidocaldarius* (Wendt et al. 1997; Siedenburg and Jendrossek 2011) and diterpene tuberculosinol diphosphate synthase from the actinobacterium *Mycobacterium tuberculosis* (Nakano and Hoshino 2009) are β-γ-domain terpenoid synthases. To our knowledge, none of the plant species possesses β-γ-domain synthases, and most of the plant terpene synthases either contain α-β-domains or all the three domains, α-β-γ (Cao et al. 2010; Hillwig et al. 2011). These plant terpenoid synthases have been postulated to originate from a fusion of an α-domain

type and a β-γ-domain type terpenoid synthase in an ancient progenitor (Morrone et al. 2009; Cao et al. 2010, Sect. 3.4.1), resulting in formation of α-β-γ domain synthases followed by γ-domain loss, yielding α-β domain synthases (Hillwig et al. 2011).

Until recently, it was thought that plants do not possess single, α-domain, synthases. However, it was just discovered that phylogenetically old spikemoss *Selaginella muellendorffii* has both "plant-type" α-β-γ- and α-β-type terpenoid synthases, and "microbial-type" α-domain only terpenoid synthases, fundamentally altering our understanding of the structure of plant terpene synthase families (Li et al. 2012).

All proteins can be classified based on the functional domains. Structural Classification of Proteins (SCOP, http://scop.mrc-lmb.cam.ac.uk/scop/) database provides an hierarchical way to systematize proteins among classes, folds, superfamilies and families (Murzin et al. 2001; Andreeva et al. 2008). A terpenoid synthase superfamily embraces all terpenoid synthase protein domains sharing a common evolutionary origin (Wilson et al. 2009). As α-domain and β- or β-γ-domains of terpenoid synthases have different evolutionary origin (Morrone et al. 2009; Cao et al. 2010, Sect. 3.4.1), plant terpenoid synthase protein domains are divided between two superfamilies, the superfamily *Terpenoid synthases* that includes the α-domains of terpenoid synthases (for most plant terpenoid synthases the relevant domain family is 'Terpenoid cyclase C-terminal domain') and superfamily *Terpenoid cyclases/protein prenyltransferases* that includes β- or β-γ-domains of terpenoid synthases (family: 'Terpenoid cyclase N-terminal domain'). As plant terpenoids commonly consist of either α-β- or α-β-γ-domains, they typically belong simultaneously to both superfamilies. γ-domain, sequence of which is embedded within the β-domain sequence, is classified together with β-domain in SCOP (Gough et al. 2001), The superfamily can be highly diverse at the level of amino acid sequences, but the structures of terpenoid synthases within given superfamily are broadly similar (Fig. 3.4, Sect. 3.3.2.3).

3.3.2.3 Structural Alignment of Terpenoid Synthases in Trees

The three-dimensional protein structure reveals ultimate level of structural information directly related to its function. It is possible that any two proteins with low amino acid sequence similarity still have close structural homology, suggesting similar functional activity. In fact, for terpenoid synthases this appears to be the case. The terpenoid synthases are present in multiple domains of life, and the sequence homology can be quite low even within the same domain, and in particular, across the domains of life (Bohlmann et al. 1998b; Chen et al. 2011; Cane and Ishida 2012; Li et al. 2012). As noted in Sect. 3.3.1.1, genomes can be screened for proteins sharing even remote homology with powerful bioinformatics computational algorithms, identifying new terpenoid synthases (see also Sect. 3.3.2.2). This way, the spikemoss "microbial-type" terpenoid gene family has been recently discovered (Li et al. 2012).

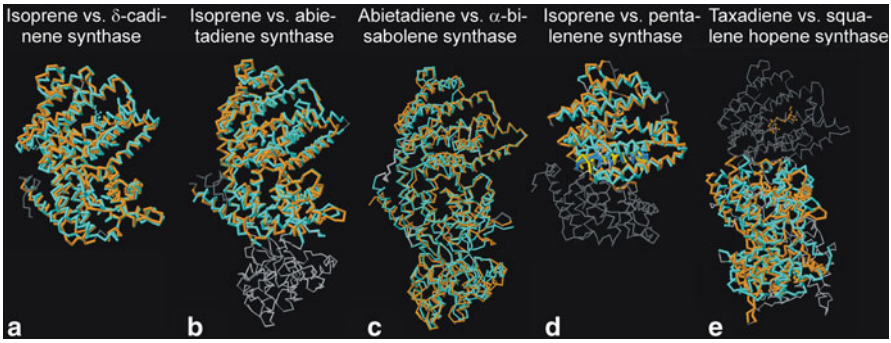


Fig. 3.4 Pairwise structural alignment of tree terpenoid synthases (**a**–**c**) and alignment of proteins structures across different domains of life (**d**, **e**). The proteins aligned in (**a**–**c**) are as described in Fig. 3.3. The sesquiterpene pentalenene synthase in (**d**) is from the actinobacterium *Streptomyces* sp. UC5319 (PDB ID: 1PS1, Lesburg et al. 1997), the diterpene taxadiene synthase in (**e**) is from the gymnosperm *Taxus brevifolia* (PDB ID: 3P5R, Köksal et al. 2011b), and the triterpenoid squalene-hopene cyclase is from the firmicute *Alicyclobacillus acidocaldarius* (PDB ID: 1SQC, Wendt et al. 1997). The alignment was conducted with the Protein Data Bank alignment tool (http://www.rcsb.org) (Berman et al. 2000; Prlic et al. 2010). Pentalenene synthase has only α-domain, isoprene and δ-cadinene synthases have α-β-domains, squalene-hopene cyclase has β-γ-domains and α-bisabolene, abietadiene and taxadiene synthases have α-β-γ-domains. The proteins are oriented as in Fig. 3.3 with the α-domain at the top and β- or γ-domain at the bottom. The olive colour is for the first aligned synthase and cyan for the second, and the grey parts stand for non-aligned sequence components. In (**c**) the sequence similarity is 52, and 97 % of δ-cadinene synthase (sequence length 515 amino acids, AA) is structurally aligned with isoprene synthase (531 AA). In (**b**), the sequence similarity is (36 %) and 95 % of isoprene synthase is aligned with abietadiene synthase (755 AA). In (**c**), the sequence similarity is 48 and 100 % of abietadiene synthase is aligned with α-bisabolene synthase (780 AA). In (**d**), the sequence similarity is 22, and 92 % of pentalenene synthase (304 AA) is aligned with isoprene synthase. In (**e**), the sequence similarity is 17 and 67 % of squalene-hopene synthase is aligned with taxadiene synthase (750 AA)

At present, only a few X-ray crystal structures of plant Tps proteins are available (Starks et al. 1997; Kampranis et al. 2007; Degenhardt et al. 2009; Gennadios et al. 2009; Köksal et al. 2011a, b). In the case of trees, crystal structures are available for isoprene synthase from grey poplar (*P. x canescens*) (Köksal et al. 2010), α-β-domain sesquiterpene synthase δ-cadinene synthase from tree cotton (*Gossypium arboreum*) (Gennadios et al. 2009) and α-β-γ-domain sesquiterpene α-bisabolene synthase from *Abies grandis* (McAndrew et al. 2011), and two α-β-γ-domain diterpene synthases, bifunctional (class I and class II) abietadiene synthase from *A. grandis* (Zhou et al. 2012) and monofunctional (class I) taxadiene synthase from *Taxus brevifolia* (Köksal et al. 2011b) (Fig. 3.3). The three gymnosperm synthases are all from terpene synthase family Tps-d1, while poplar isoprene synthase belongs to Tps-b family and tree cotton δ-cadinene synthase to Tps-a family (Li and Sharkey 2013b for discussion of classification of terpenes into gene families). For α-β-domain monoterpene synthases, the crystal structures are available only for herbs, including Tps-b family monoterpene limonene (Hyatt et al. 2007) and bornyl diphosphate (Whittington et al. 2002) synthases.

Despite limited coverage, available crystal structures have provided major insight into the structure of catalytically active sites, metal binding motifs, substrate recognition and subsequently the function of proteins. Data on protein three-dimensional structure can be employed for protein structural alignment. Structural alignment is a robust tool to compare proteins' tertiary structure and reveal hidden evolutionary relationships, especially for proteins with high evolutionary distance, and consequently with little similarity in their nucleic acid or amino acid sequences. In such cases, the evolutionary relationships between proteins cannot be easily detected by standard sequence alignment techniques, but structural alignment can be used to gain insight into functional relationships among proteins with low sequence homology. For example, despite low level of sequence similarity, poplar isoprene synthase (Tps-b family) and tree cotton δ-cadinene synthase (Tps-a family) exhibit high structural similarity (Fig. 3.4a). High structural similarity is also evident among poplar isoprene synthase and grand fir abietadiene synthase (Tps-d family) α-β-domains (Fig. 3.4b). Furthermore, significant structural similarity is evident between plant and microbial terpenoid synthases. Albeit the sequence similarity is only about 20 % (Fig. 3.4d, e), α-domain alignment of poplar isoprene synthase and bacterial pentalenene synthase, and β-γ-domain alignment of Pacific yew (*Taxus brevifolia*) taxadiene synthase and bacterial squalene-hopene cyclase are remarkably good. Overall, this evidence again emphasizes the strong structural similarity within given domains of terpene synthases across plants and even across the kingdoms of life (Aaron and Christianson 2010; Cao et al. 2010; Cane and Ishida 2012).

3.3.3 Characteristics of Key Plant Terpenoid Synthases

3.3.3.1 Isoprene Synthase

Isoprene synthase (IspS) is the terminal enzyme completing chloroplastic isoprene synthesis through MEP/DOXP pathway (Sharkey and Yeh 2001; Sharkey et al. 2008). IspS crystal structure for recombinant protein from grey poplar (*P. x canescens*) was recently characterized, and it was demonstrated that it is a classic two domain, α-β, terpenoid synthase. The C-terminal class I terpenoid synthase fold (α-domain) possesses the catalytic activity, while the N-terminal class II terpenoid synthase domain (β-domain) possesses no known catalytic activity (Köksal et al. 2010). Formation of isoprene from DMADP occurs through a syn-periplanar elimination mechanism via an allylic carbocation intermediate as in other class I terpenoid synthases (Köksal et al. 2010). The enzyme requires Mg^{2+} for catalytic activity and has a relatively broad alkaline pH optimum between 7 and 8.5, and a temperature optimum between 40 and 45 °C (Monson et al. 1992; Sasaki et al. 2005; Schnitzler et al. 2005). IspS has a high K_m value for DMADP in vivo of ca. 0.3 mM (Rasulov et al. 2009), and there is evidence of allosteric regulation (Schnitzler et al. 2005) and competitive inhibition by GDP, the substrate for monoterpenoid synthases

(Köksal et al. 2010). Multiple isoprene synthase genes have been demonstrated in some poplar species, but the role of these paralogous genes is not yet clear (Vickers et al. 2010). Further details of isoprene synthase are provided in Rosenkranz and Schnitzler (2013) and Li and Sharkey (2013b).

All of the sequenced IspS synthase genes so far have suggested to posses specific chloroplastic signal sequences (Miller et al. 2001; Sasaki et al. 2005; Sharkey et al. 2005; Fortunati et al. 2008; Vickers et al. 2009a, 2010). Localization of IspS to chloroplasts in constitutive emitters has also been confirmed by chloroplast extractions (Wildermuth and Fall 1996; Wildermuth and Fall 1998), immunogold-labelling (Schnitzler et al. 2005), and chloroplast-allocation of green fluorescent protein fused with isoprene synthase in transformed constructs (Sasaki et al. 2005). In fact, transgenic tobacco (*Nicotiana tabacum*) expressing isoprene synthase in cytosol appeared to be essentially void of isoprene emission (Vickers et al. 2011).

3.3.3.2 Terpene Synthases

Terpenes are synthesized by terpene synthases from one of three common prenyl diphosphate precursors formed by the fusion of DMADP with one or more isopentenyl diphosphate (IDP) molecules, catalyzed by prenyltransferases (Chappell 1995; Koyama and Ogura 1999; Dewick 2002).

Typically, tree mono- and sesquiterpene synthases are α-β-domain proteins with only the α-domain (class I) terpene synthase active site functional (Aaron and Christianson 2010; Cao et al. 2010). These terpene synthases are ca. 550–650 amino acids long with sesquiterpene synthases functionally active in the cytosol being characteristically 50–70 amino acids shorter than hemi- and monoterpene synthases that possess a N-terminal plastid-targeting sequence (Bohlmann et al. 1998a; Degenhardt et al. 2009).

Most diterpene synthases and some sesquiterpene synthases are three-domain proteins, 800–870 amino acids long. Again, diterpene synthases are longer than three-domain sesquiterpene synthases due to N-terminal plastid-targeting sequence (Bohlmann et al. 1998a), but some of these three-domain sesquiterpene synthases might contain N-terminal signal sequence (Bohlmann et al. 1998b; Martin et al. 2004). Despite having three domains, only class I terpene synthase active site in the α-domain is functional in sesquiterpenes due to lack of DXDD motif as in α-bisabolene synthase in *Abies grandis* (McAndrew et al. 2011). In the case of diterpenes, commonly either only class I or class II terpene synthase activity is present and the other active site is rendered non-functional (Keeling et al. 2010). Among the diterpenes, taxadiene synthase in Pacific yew (*Taxus brevifolia*) has class I terpene synthase activity (Köksal et al. 2011b), while *ent*-copalyl diphosphate (CDP) synthase in *Arabidopsis* has class II terpene synthase activity (Köksal et al. 2011a).

A few bifunctional diterpene synthases having both class I and class II terpene synthase activities have been reported to date in trees, including abietadiene

synthase from *Abies grandis* (Vogel et al. 1996; Peters et al. 2001; Zhou et al. 2012), *cis*-abienol synthase from *Abies balsamea* (Zerbe et al. 2012b), levopimaradiene synthases from *Gingko biloba* (Schepmann et al. 2001), and *Picea abies* (Martin et al. 2004) and abietadiene/levopimaradiene synthase from *Pinus taeda* (Ro and Bohlmann 2006). In bifunctional diterpene synthases, β-domain with class II activity typically forms a diphosphorylated diterpenoid intermediate (e.g., CDP) that freely diffuses to the second, class I active site in the α-domain, where the diphosphate group is cleaved and given diterpenoid (or typically, a spectrum of terpenoids) is formed (Zhou et al. 2012).

While it is generally thought that mono- and diterpene synthases are functional in plastids, and sesquiterpene synthases in cytosol, there is surprisingly little information on subcellular location of terpene synthases other than the presence or absence of plastid targeting sequences in the expressed proteins (Nagegowda 2010). Available evidence does suggest that monoterpenes are functionally active in plastids (Nagegowda 2010), but α-terpineol synthase in *Magnolia* appeared to be targeted both to chloroplasts and mitochondria (Lee and Chappell 2008), and there are terpene synthases, capable of formation of both mono- and sesquiterpenes depending on substrate (Sect. 3.3.3.3), that can be localized in the cytosol or in the plastids (Aharoni et al. 2004; Nagegowda et al. 2008). On the other hand, while sesquiterpene synthases are generally located in the cytosol, presence of N-terminal residue in some three-domain sesquiterpene synthases in conifers *Abies grandis* and *Picea abies* (Bohlmann et al. 1998b; Martin et al. 2004) suggests that they might be targeted to chloroplasts. Recently, three-domain sesquiterpene santalene/bergamotene synthase from wild tomato (*Solanum habrochaite*s) was shown to be targeted to chloroplasts, suggesting that sesquiterpene synthesis can occur via MEP/DOXP pathway (Sallaud et al. 2009). On the other hand, further biochemical modifications in plastid-synthesized terpenoids, e.g., by cytochrome P450-dependent oxidases typically take place in cytosol (Haudenschild et al. 2000; Ro and Bohlmann 2006; Hamberger et al. 2009). Cleary, more work is needed to gain insight into subcellular location of various terpene synthases, but the evidence suggests that three-domain sesquiterpene synthases with strong homology to diterpenoid synthases could be located in plastids.

In general, terpene synthases have much higher substrate affinity than isoprene synthase with K_m values as low as 0.9 μM (linalool synthase, Pichersky et al. 1995), 7.6 μM (linalool/nerolidol synthases, Nagegowda et al. 2008) to 84 μM (myrcene synthase, Fischbach et al. 2001) for GDP and 1.4 μM (santalene synthase, Jones et al. 2011) – 23 μM (linalool/nerolidol synthases, Nagegowda et al. 2008) for FDP, and 3–10 μM for GGDP (taxadiene synthase, Hezari et al. 1995; Williams et al. 2000). Typically, the pH optimum of terpene synthases is neutral to somewhat alkaline between 6 and 7.5, but the optimum tends to be sharper than that for isoprene synthase (Cori and Rojas 1985; Alonso and Croteau 1993; Bohlmann et al. 1998b). The temperature optimum of terpene synthases seems to be lower than that for isoprene synthase with reported values around 40 °C for conifer *Picea abies* and broad-leaved evergreen angiosperm *Quercus ilex* (Fischbach et al. 2000, 2001).

3.3.3.3 Substrate Specificity of Terpenoid Synthases

Typically, monoterpene synthases use geranyl diphosphate (GDP), sesquiterpene synthases farnesyl diphosphate (FDP) and diterpene synthases geranylgeranyl diphosphate (GGDP) as the only substrate (e.g., Fischbach et al. 2001; Martin et al. 2004 for the tests of multiple substrates). However, a few plant terpene synthases have reported to form terpenoids of different chain length depending on substrate. Among these multifunctional enzymes, α-bisabolene synthase from the gymnosperm tree *Abies grandis* forms sesquiterpene *E*-α-bisabolene with FDP as substrate and monoterpene (+)-limonene with GDP (Bohlmann et al. 1998a, Table 3.1). α-Farnesene synthase from the angiosperm tree *Malus domestica* forms sesquiterpene *E*,*E*-α-farnesene with FDP and *E*-β-ocimene with GDP (Pechous and Whitaker 2004; Green et al. 2007, Table 3.1). For both sesquiterpene synthases, the enzymes preferably form sesquiterpenes when FDP and GDP are supplied simultaneously (Pechous and Whitaker 2004; Green et al. 2007). Sesquiterpene santalene synthase from *Santalum* species can form a spectrum of monoterpenes when incubated with GDP, but the affinity to GDP is much less than to FDP (Jones et al. 2011). In snapdragon (*Antirrhinum majus*) (Nagegowda et al. 2008) and strawberry (*Fragaria ananassa*) (Aharoni et al. 2004) nerolidol/linalool synthases were shown to form monoterpenes linalool and other acyclic monoterpenes with GDP and nerolidol with FDP. Recently a myrcene/isoprene synthase was characterized in *Humulus lupulus* that formed myrcene with GDP, and isoprene with DMADP (Sharkey et al. 2013).

Capacity to form different products depending on substrate has also been reported for bacterial terpenoid synthases and seems to be widespread in nature (Hamano et al. 2002; Siedenburg and Jendrossek 2011). So far, the available evidence suggests that this trait is rare among plants, but clearly it is recommended to routinely use multiple substrates to test for broad-spectrum substrate use capacity in functional characterization of terpene synthases.

3.3.3.4 Product Specificity of Terpenoid Synthases

Plants produce a huge variety of terpenoids with diverse composition among and within species. This high variety reflects expression of several terpene synthases (Sects. 3.3.3.2 and 3.4.2) as well as multiple products of single terpene synthases. For example, γ-humulene synthase from *Abies grandis* has been shown to produce 52 sesquiterpene olefins (Steele et al. 1998). However, all synthases have a certain specificity to form some main products with greater probability. Typically, the synthases also are stereospecific, forming preferably certain stereoisomers (Table 3.1, Croteau 1987; Prosser et al. 2004; Christianson 2006, 2008; Degenhardt et al. 2009). In fact, the capacity to produce multiple products is not universal, and some terpenoid synthases are almost completely specific, forming only one product or nearly so (Table 3.1). For example, isoprene synthases tend to form only isoprene, with the only known exception being a myrcene/isoprene synthase that can form

acyclic monoterpenes when GDP is the substrate (Sect. 3.3.3.3, Sharkey et al. 2013), but there are also several highly specific mono-, sesqui- and diterpene synthases (Table 3.1).

Predicting product profiles based on the terpenoid synthase full amino acid sequences is currently not possible as the correlative patterns are weak (Degenhardt et al. 2009). Even with the genes having high sequence homology, some enzymes might produce multiple products while others form one single product; highly homologous enzymes forming multiple products might produce strongly different product spectra (Degenhardt et al. 2009). Depending on the reaction mechanism (Markovnikov vs. anti-Markovnikov addition to a double bond), more or less stable carbocation can be formed in the initial reaction steps, thereby affecting the potential range of products formed (Christianson 2006). Also, active site structure, presence of certain fixed and protected dipoles that can stabilise the carbocation, and steric limitations play an important role in the product formation (Christianson 2006, 2008). Clearly, more X-ray crystal structures of tree terpene synthases are needed to gain further insight into the reaction mechanisms in the highly diverse terpenoid synthase family in trees.

3.4 Origin and Size of Terpenoid Synthase Gene Families

The vast array of different terpenoid compounds in nature results both from the low product specificity of many terpenoid synthases (Sect. 3.3.3.4) and from large number of terpenoid synthases present in most plant species studied. Presence of a vast number of terpenoid synthase genes can reflect divergent evolutionary paths for catalytic activities of terpenoid synthase genes leading to variations in terpenoid compounds formed (Bohlmann et al. 1998a; Aubourg et al. 2002; Dornelas and Mazzafera 2007), and resulting in novel functions that increase species resistance to insects, pathogens, and herbivores (Trapp and Croteau 2001) as well as to abiotic stresses (Owen and Peñuelas 2005; Fineschi et al. 2013; Possell and Loreto 2013), overall increasing species fitness. On the other hand, synthesis of similar compounds by phylogenetically widely distant plant species, e.g., among angiosperms and gymnosperms possessing terpenoid synthases with low homogeneity, also indicates convergent evolution of terpenoid synthases, and in some cases, multiple events of evolution and loss of capacity to form the given compound such as isoprene (Bohlmann et al. 1998a; Aubourg et al. 2002; Dornelas and Mazzafera 2007; Monson et al. 2013). Here we analyse the evidence of the origin of terpenoid synthases in plants and the size of terpenoid synthase gene families with emphasis on tree terpenoid synthases. Evolution of terpenoid synthases, including classification of plant terpenoid synthase gene families, is addressed in other chapters of the book (Fineschi et al. 2013; Li and Sharkey 2013b).

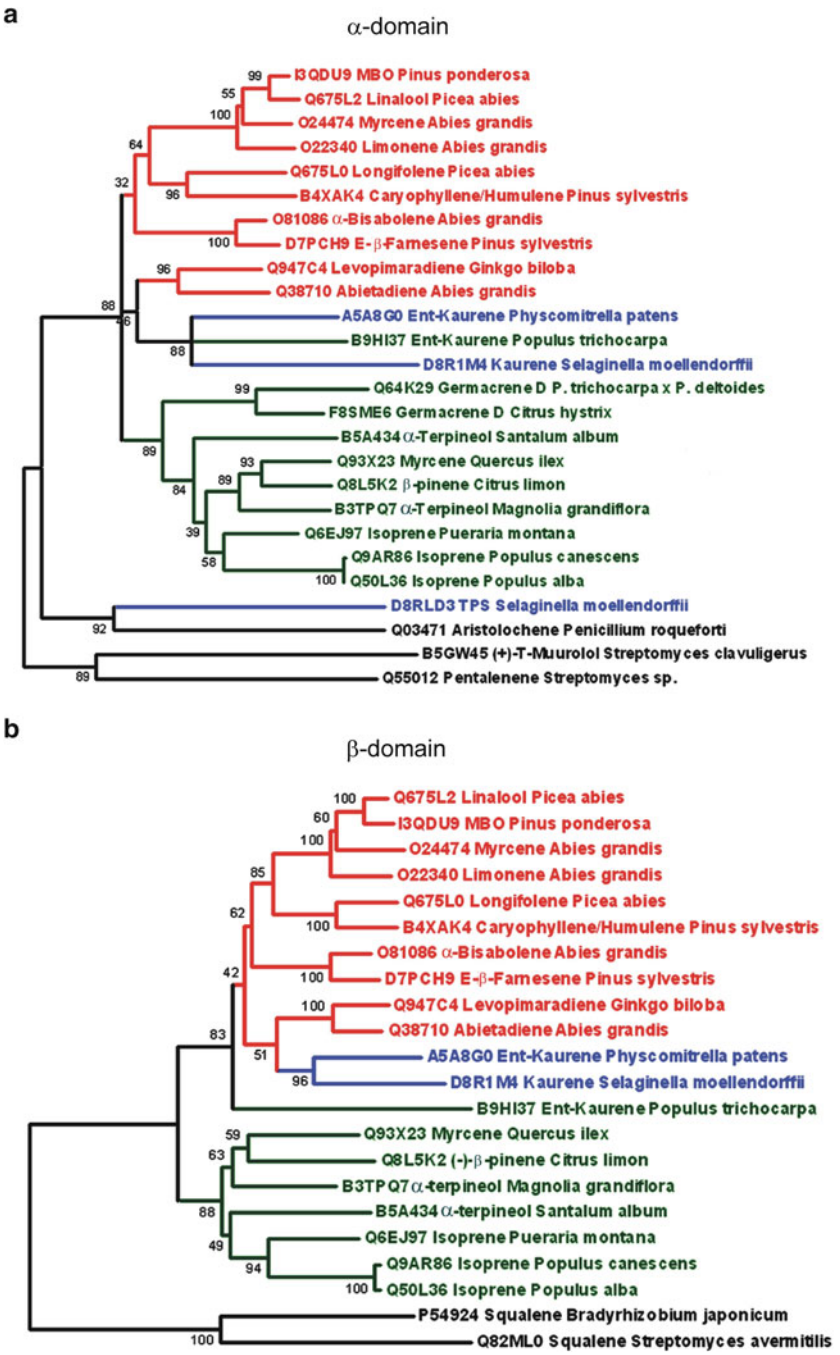
3.4.1 Origin of Plant Terpenoid Synthase Gene Families

It has been suggested that all modern plant terpenoid synthases originate from a common three-domain, α-β-γ-diterpene synthase in an ancient progenitor, followed by independent gene duplications and evolution of new genes in different organisms. As such an ancestor gene in plants, a bifunctional *ent*-copalyl diphosphate synthase/*ent*-kaurene synthase (PpCPS/KS) of the moss *Physcomitrella patens* has been suggested (Hayashi et al. 2006). This enzyme produces *ent*-kaurene which is a common precursor of gibberellins and endogenous diterpenes derived from this compound (Hayashi et al. 2010). The active site in the β-domain has class II terpene synthase activity and is responsible for *ent*-copalyl diphosphate synthesis from GGDP, while the active site in the α-domain forms *ent*-kaurene from *ent*-copalyl diphosphate. Additional bifunctional kaurene synthases have been discovered in the phylogenetically old species liverwort *Jungermannia subulata* (Kawaide et al. 2011) and spikemoss *Selaginella muellendorffii* (Li et al. 2012).

In phylogenetically younger gymnosperms and angiosperms, *ent*-kaurene is produced by two distinct mono-functional enzymes, *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS) (Keeling et al. 2010; Zerbe et al. 2012a). Monofunctional diterpene synthases still have α-β-γ three-domain structure, but the active site in either α- or β-domain has lost the functional activity. As noted in Sect. 3.3.3.2, such three-domain structure is characteristic also to some sesquiterpene synthases, but most sesquiterpene, and all mono- and hemiterpene synthases have lost γ-domain sequence and have α-β domain configuration. Phylogenetic analyses suggest that the formation of α-β-domain terpenoid synthases via loss of the γ-domain has occurred several times during evolution (Hillwig et al. 2011).

An interesting question is what are the immediate ancestors for the α-domain and β-γ-domain in the common higher plant terpenoid α-β-γ ancestor? Analysis of the phylogenetic signals separately for α- and β-domains across a broad-spectrum of plant terpenoid synthases actually does not confirm the postulated origin of all terpenoid synthases from the moss *Physcomitrella patens* diterpene synthase gene (Fig. 3.5). If this were the ancestor of all multidomain plant terpenoid synthases, phylogenetic signals in α- and β-domains should reflect this, but the sequence homologies rather suggest that there must have been another common ancestor for moss, liverwort, spikemoss, gymnosperm and angiosperm terpene synthases (Fig. 3.5). Interestingly, *ent*-copalyl diphosphate and *ent*-kaurene synthases have been discovered in the bacterium *Bradyrhizobium japonicum* (Morrone et al. 2009). These terpenoid synthases share some homology with plant terpenoid synthases, and it has been suggested that there might be even an ancient progenitor for modern terpenoid synthases across the domains of life (Morrone et al. 2009).

Particularly interesting is the position of recently discovered spikemoss *Selaginella muellendorffii* α-domain only 'microbial'-type terpene synthases (Li et al. 2012). These sequences carry homology with both 'modern plant'


a
α-domain
I3QDU9 MBO Pinus ponderosa
Q675L2 Linalool Picea abies
O24474 Myrcene Abies grandis
O22340 Limonene Abies grandis
Q675L0 Longifolene Picea abies
B4XAK4 Caryophyllene/Humulene Pinus sylvestris
O81086 α-Bisabolene Abies grandis
D7PCH9 E-β-Farnesene Pinus sylvestris
Q947C4 Levopimaradiene Ginkgo biloba
Q38710 Abietadiene Abies grandis
A5A8G0 Ent-Kaurene Physcomitrella patens
B9HI37 Ent-Kaurene Populus trichocarpa
D8R1M4 Kaurene Selaginella moellendorffii
Q64K29 Germacrene D P. trichocarpa x P. deltoides
F8SME6 Germacrene D Citrus hystrix
B5A434 α-Terpineol Santalum album
Q93X23 Myrcene Quercus ilex
Q8L5K2 β-pinene Citrus limon
B3TPQ7 α-Terpineol Magnolia grandiflora
Q6EJ97 Isoprene Pueraria montana
Q9AR86 Isoprene Populus canescens
Q50L36 Isoprene Populus alba
D8RLD3 TPS Selaginella moellendorffii
Q03471 Aristolochene Penicillium roqueforti
B5GW45 (+)-T-Muurolol Streptomyces clavuligerus
Q55012 Pentalenene Streptomyces sp.
b
β-domain
Q675L2 Linalool Picea abies
I3QDU9 MBO Pinus ponderosa
O24474 Myrcene Abies grandis
O22340 Limonene Abies grandis
Q675L0 Longifolene Picea abies
B4XAK4 Caryophyllene/Humulene Pinus sylvestris
O81086 α-Bisabolene Abies grandis
D7PCH9 E-β-Farnesene Pinus sylvestris
Q947C4 Levopimaradiene Ginkgo biloba
Q38710 Abietadiene Abies grandis
A5A8G0 Ent-Kaurene Physcomitrella patens
D8R1M4 Kaurene Selaginella moellendorffii
B9HI37 Ent-Kaurene Populus trichocarpa
Q93X23 Myrcene Quercus ilex
Q8L5K2 (-)-β-pinene Citrus limon
B3TPQ7 α-terpineol Magnolia grandiflora
B5A434 α-terpineol Santalum album
Q6EJ97 Isoprene Pueraria montana
Q9AR86 Isoprene Populus canescens
Q50L36 Isoprene Populus alba
P54924 Squalene Bradyrhizobium japonicum
Q82ML0 Squalene Streptomyces avermitilis

multi-domain terpenoid synthases and microbial α-domain only synthases, possibly providing the missing link to modern plant terpenoid origin. As more information of genomes becomes available, we will be possibly able to more clearly identify the origin of terpenoid synthases in plants.

3.4.2 Size of Terpenoid Synthase Gene Families

Given the possible monophyletic origin of 'higher plant type terpene synthases', continuous and extensive gene duplication followed by functional and structural specialization is responsible for the high diversity of terpenoid synthase gene families in plants (Fryxell 1996; Bohlmann and Keeling 2008; Chang and Duda 2012). The Viridiplantae have the largest terpenoid synthase families among living organisms (Fig. 3.6), but the size of terpenoid gene families varies strongly among plant species. According to genome mining by profile-based hidden Markov models, the size of terpenoid gene family varies from one in the moss *Physcomitrella patens* to 86 in the angiosperm tree *Eucalyptus grandis* (Fig. 3.6). These estimates based on similarity screens of full genomes broadly agree with independent estimates in other studies (Bohlmann and Keeling 2008; Chen et al. 2011). However, it is difficult to detect genes with low level of homogeneity. For instance, in *Selaginella muellendorffii*, 16–18 terpene synthases have been suggested based on genome screens for higher plant, α-β- or α-β-γ-domain, terpene synthases (Chen et al. 2011),

Fig. 3.5 Phylogenetic trees of selected tree terpene synthases (*red font* corresponding to gymnosperms and *green font* to angiosperms) and representative plant (*blue font*, moss *Physcomitrella patens* and spikemoss *Selaginella muellendorffii*) and bacterial and fungal outgroups based on α-domain (C-terminal part of the sequence, (**a**) and β-domain (N-terminal part of the sequence, (**b**). It has been suggested that all modern plant terpenoids originate from a fusion of α-domain and β-γ-domain terpenoid synthases in an ancient progenitor (Morrone et al. 2009; Cao et al. 2010). Thus, α-domain and β-domain might carry somewhat different phylogenetic signals (Sharkey et al. 2013). UniProtKB/Swiss-Prot entry codes are also given with species name (http://www.uniprot.org/). Isoprene and MBO (2-methyl-3-buten-2-ol) are hemiterpenes; linalool, limonene, myrcene, β-pinene and α-terpineol are monoterpenes; aristolochene, α-bisabolene, caryophyllene, β-farnesene, germacrene D, humulene, longifolene, T-muurolol, and pentalenene are sesquiterpenes; abietadiene, levopimaradiene, and *ent*-kaurene are diterpenes; and hopene and squalene are triterpenes. The microbial synthases in (**a**) only have α-domain as the *S. muellendorffii* 'microbial type' terpene synthase D8LD3 (Li et al. 2012), while the microbial and fungal squalene/hopene synthases in (**b**) have β-γ-domains only. The domains were separated using *Abies grandis* three-domain abietadiene synthase domain structure (Q38710, Zhou et al. 2012) as a seed. After alignment, signal peptides were deleted, and whenever pertinent, the sequence part corresponding to γ-domain was deleted. α-domain and β-domain parts of the sequence were separately aligned, truncated after alignment to a common length, and the trees were constructed by MEGA5 software using the maximum likelihood method (Tamura et al. 2011). The numbers next to the branches refer to the actual bootstrap values of branches and characterize the reliability of the branching (bootstrap consensus trees are demonstrated). The higher the score, the more reliable is the branching at that point (Tamura et al. 2011)

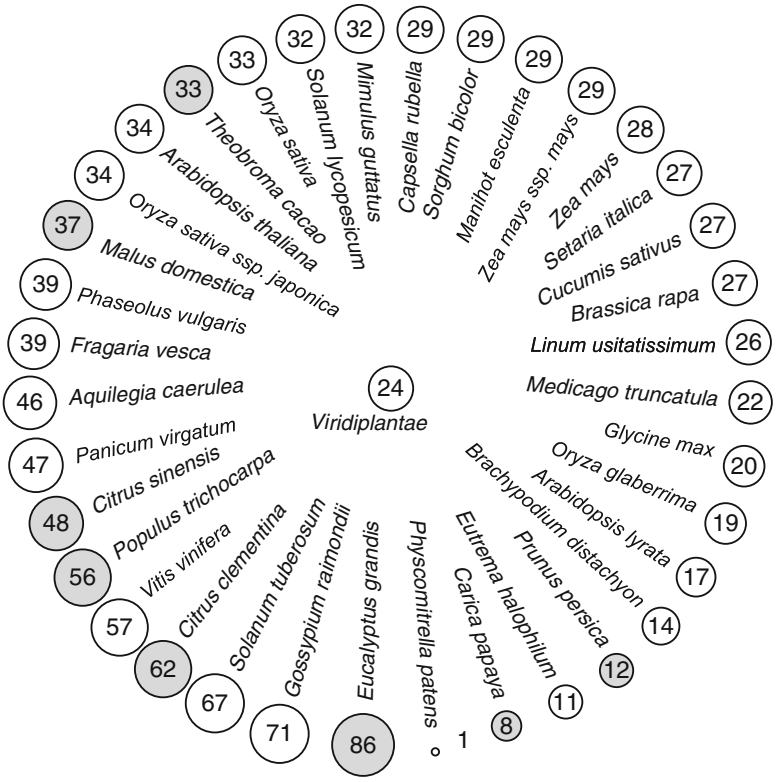


Fig. 3.6 Protein superfamily 'Terpenoid synthases' domains in Viridiplantae clade analysed using SUPERFAMILY 1.75 (http://supfam.cs.bris.ac.uk/SUPERFAMILY/) that includes essentially all protein domains from all available sequenced organisms, including 36 sequenced Viridiplantae genomes (Gough et al. 2001; Wilson et al. 2009). Superfamily is defined as a collection of domains (functional units of proteins) having structural and functional evidence of common evolutionary ancestor. Plant terpenoid synthases characteristically have either two (α and β) or three (α, β and γ) domains (Figs. 3.3 and 3.4, Hillwig et al. 2011; Köksal et al. 2011a, b). The homologous domains in SUPERFAMILY are identified by hidden Markov models, which is a profile-based method with high selectivity (Gough et al. 2001). Here the protein domains from the domain family of 'Terpenoid cyclase C-terminal domain' that contains the α-domain of the plant α-β- and α-β-γ-domain terpenoid synthases are demonstrated. In the figure, the radius of the circle reflects the size of the terpenoid synthase superfamily in logarithmic scale (numbers inside denote the number of terpenoid synthase domains in the given organism), the inner circle shows the average number of terpenoid synthase domains in Viridiplantae. Trees are denoted by *grey filling*

but recently 48 putative "bacterial" type, α-domain only synthases were identified in *Selaginella* genome, and functional activity of several of them was characterized (Li et al. 2012). Thus, we might have vastly underestimated the size of terpene gene families in plants.

The largest terpenoid synthase gene families among sequenced Viridiplantae are observed in trees (Fig. 3.6). Among top seven sequenced plants with the

greatest terpenoid synthase families, four, *Eucalyptus grandis*, *Citrus clementina*, *C. sinensis* and *Populus trichocarpa* are trees, and *Vitis vinifera* is a woody vine (Fig. 3.6, Martin et al. 2010). Furthermore, in addition to raw data generated by genome projects, functional characterization and analysis of terpenoid genes by transcriptome mining has highlighted large terpenoid gene families also in several gymnosperms (Martin and Bohlmann 2005; Chen et al. 2011; Keeling et al. 2011). However, some tree species such as *Carica papaya* have small terpenoid synthase gene families (Fig. 3.6). Currently, the functional implications of variations in the size of terpenoid synthase gene families have not yet been fully elucidated, especially in trees. However, it is reasonable to expect that presence of multiple terpenoid synthase genes forming different compounds and compound spectra and presence of paralogs with similar compound spectra, but potentially with differing regulatory promoter elements is associated with greater diversity of volatile product profiles and more diverse response patterns to biotic and abiotic stresses (Keeling et al. 2008; Bohlmann et al. 2011). Given that only minor modifications, at the level of single amino acid or a few amino acids, may be needed to change the terpenoid synthase function (Kampranis et al. 2007; Keeling et al. 2008; Hall et al. 2011), presence of duplicated genes can constitute an important gene pool for rapid adaptation to new biotic interactions, 'new' abiotic stresses in given plant habitat, and novel stress combinations or to changes in stress severities, thereby helping plants to adapt to their environment (Hall et al. 2011). In addition to variations among species, there is evidence of important within-species variation in the terpenoid gene family, in agreement with adaptive genomic modifications (Hall et al. 2011; Gonzales-Vigil et al. 2012).

3.5 Regulation of Terpenoid Synthesis

The quantities of metabolites ultimately produced are influenced by genetic features and environmental signals. So, the metabolite contents in plants are dynamic and controlled by internal and external stimuli. These changes are regulated by gene expression networks involved in targeting pathways to specific cells and organs and determining the overall expression level of the pathway (Grotewold 2008). Studying one or some genes is not enough for having a deep insight into such networks across a variety of tissues and treatments. Transcriptome and metabolome data provided by next generation technologies have opened new opportunities for understanding the biosynthesis and regulation of plant metabolites (Crispin and Wurtele 2013). Perhaps with this comprehensive data, we will be able to start unresolving the complex regulatory networks, not only focusing on the synthesis of terpenoid end-products, but addressing the entire cascade of events altering profoundly plant 'normal' metabolism, from stress signals to elicitation of pathways.

Substrate-level regulation of terpenoid synthesis has been addressed in the chapters of Li and Sharkey (2013b) and Monson (2013). Here we analyse the regulation of terpenoid synthesis driven by variations in gene expression. Terpenoid

synthase genes show a high variety of temporal and spatial expression patterns; some of these compounds are constitutively expressed (Steele et al. 1998), some of them induced by both biotic and abiotic stresses (Yin et al. 1997; Byun-McKay et al. 2006; Tholl 2006; Fares et al. 2008; Chen et al. 2011), some are expressed in leaf mesophyll and leaf surface trichomes (Köllner et al. 2004; van Schie et al. 2007; Falara et al. 2011), and others in flowers (Chen et al. 2003; Dudareva et al. 2003; Falara et al. 2011). This level of control has been termed as 'genetic control' (Monson 2013).

3.5.1 'Constitutively' Expressed Synthases

Regulation of terpenoid synthase gene expression is controlled by the regulatory elements in the gene promoter region that interact with a variety of transcription factors. Rosenkranz and Schnitzler (2013) provide an in-depth overview of the poplar isoprene synthase promoter region (*PcIspS*). Isoprene synthase in emitting species is a constitutively expressed gene. However, *PcIspS* contains circadian, heat and stress-dependent regulatory elements (Loivamäki et al. 2007b; Cinege et al. 2009) and thus, expression of isoprene synthase varies during the day, during the season and is responsive to changes in temperature (Mayrhofer et al. 2005; Sasaki et al. 2005; Wiberley et al. 2008, 2009). So far, it is however, unclear how isoprene synthase activity is regulated in plants grown under different atmospheric CO_2 concentrations (Wilkinson et al. 2009; Sun et al. 2012).

There is much less information available on regulation of expression of terpene synthases. In particular, there are several constitutively expressed terpene synthase genes analogous to isoprene synthase, such as myrcene synthase in broad-leaved evergreen Mediterranean oaks (Fischbach et al. 2001), which seem to be regulated similarly to isoprene synthase, i.e., responding to ambient temperature and light conditions (Fischbach et al. 2002; Staudt et al. 2003), and might be responsive to CO_2 as well (Loreto et al. 2001a), but so far the promoter regions of these enzymes have not been characterized.

In most conifers, there are also several constitutively expressed enzymes for oleoresin formation. Oleoresin in these species is accumulated in specific resin ducts or galls in the bark, sapwood, and needles, and serves as a major physico-chemical defence barrier against insects and pathogens (Bohlmann and Croteau 1999; Trapp and Croteau 2001; Martin and Bohlmann 2005; Raffa et al. 2005). Release of oleoresin after insect attack repels and deters insects; emissions of mono- and sesquiterpenes provide indirect defence against herbivores, but diterpenes and terpene oxidation products provide direct defence by forming a physical barrier at the place of insect attack (Martin et al. 2003; Miller et al. 2005; Raffa et al. 2005). The promoter regions of constitutively expressed terpenoid synthases in conifers have not yet been characterized. In herbs, there has been some progress in identifying promoters targeting the gene expression to glandular trichomes (Tissier 2012), but again, terpenoid-specific promoters have not yet been identified.

Information about terpenoid-specific transcription factors is currently also very limited (Tholl 2006; Chen et al. 2011), and obviously understanding terpenoid synthase regulation should constitute a priority for future studies.

3.5.2 Stress-Induced Terpene Synthases

Emissions of a large number of mono- and sesquiterpenes are elicited in response to numerous biotic stresses such as herbivory, oviposition and fungal inoculation, but also to abiotic stresses through activation of signalling responses triggered by reactive oxygen species. Stress-induced production of traumatic resin plays a prominent role as a physical and toxic direct defence to insects and pathogens (Martin et al. 2002; Miller et al. 2005; Raffa et al. 2005), while volatile terpenoid emissions can serve as repellents to herbivores and attractants to enemies of herbivores (Thaler 2002; Hilker et al. 2005; Miller et al. 2005; Raffa et al. 2005; Dicke et al. 2009). There is a plethora of examples of stress-elicited modifications in terpenoid synthesis. For detailed consideration of these responses, we refer to the chapters of Trowbridge and Stoy (2013) and Holopainen et al. (2013) and the review of Keeling and Bohlmann (2006b) and only briefly mention a few of these elicited responses. Feeding by the white pine weevil (*Pissodes strobi*) on Sitka spruce (*P. sitchensis*) induced traumatic resin accumulation in stems and also induced expression of several terpene synthase transcripts, such as the (−)-pinene synthase (Byun McKay et al. 2003; Miller et al. 2005). In addition, (−)-linalool synthase activity increased after weevil feeding, resulting in linalool emissions (Miller et al. 2005). Analogously, monoterpene synthase activities in needles of ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and white fir (*Abies concolor*) were enhanced after feeding by tiger moth (*Halisdota ingens*) larvae (Litvak and Monson 1998). Treatments with methyl jasmonate (MeJA), elicitor causing responses similar to herbivore attacks, have been widely used to study influence of biotic stress effects on terpenoid synthesis (Keeling and Bohlmann 2006b; Bohlmann 2008). In several conifers, MeJA treatment has shown to result in formation of traumatic resin ducts, terpenoid accumulation, and induction of prenyltransferase and terpene synthase activities, and altered volatile terpenoid emission profiles (e.g., Martin et al. 2002, 2003; Miller et al. 2005).

There are different patterns of timing of elicitation of various terpenoid synthases. In response to wounding in the gymnosperm tree *Abies grandis*, monoterpene synthase genes were elicited first, followed by sesqui- and diterpene synthases (Steele et al. 1998). In the angiosperm tree *Alnus glutinosa*, feeding by common white wave (*Cabera pusaria*) larvae resulted in simultaneous elicitation of emissions of mono-, sesqui and homoterpenes, but sesquiterpene emissions were characterized by biphasic emission kinetics and the level of elicitation differed for different volatile terpenes (Copolovici et al. 2011). Such variations in temporal patterns can be simulated based on the theory of recursive action of regulators on the target gene(s) over time (Vu and Vohradsky 2007) as demonstrated in the

chapter of Grote et al. (2013). In addition, there are also genotypic differences in the degree of elicitation of given synthases (Byun-McKay et al. 2006), suggesting that modifications in gene regulation patterns can constitute a further important adaptive mechanism.

Overall, the phenomenological evidence consistently indicates the biotic stress-dependent enhancement of terpenoid synthesis pathway and modifications of terpenoid profiles. Although there has been a significant progress in biochemical and molecular characterization of the induced terpenoid responses (Huber et al. 2004; Martin and Bohlmann 2005; Keeling and Bohlmann 2006b; Tholl 2006; Chen et al. 2011), regulation of stress-induced terpene synthesis is still poorly known. In white spruce (*Picea glauca*), putative *cis*-acting elements such as MeJA- and wound-response elements, and promoter-enhancing sequences have been identified for monoterpene 3-carene synthase by BAC cloning (Hamberger et al. 2009), but the inducible terpenoid promoters have not yet been functionally tested. Potato (*Solanum tuberosum*) wound- and insect-inducible promoter of proteinase inhibitor protein was used to demonstrate local elicitation of terpenoid synthase activity in transgenic *Picea glauca* after mechanical wounding (Godard et al. 2007). This transgenic system provides encouraging platform for proof-of-concept studies, and possibly will also help to elucidate the regulation of inducible terpenoid synthases in native systems.

3.6 Conclusions

More than 60,000 terpenoid compounds have been described in living organisms. This huge chemical diversity results from a large number of terpenoid synthases and relatively low product specificity of many terpenoid synthases. High genetic richness of terpenoid synthases present in many plant species constitutes an important gene pool for adaptation. Especially, given that the product profiles of several terpenoid synthases can be altered with only minor modifications in active center structure, gene duplication of ancient progenitor terpenoid synthases has catalyzed the vast evolutionary adjustment in terpenoid profiles (Sect. 3.4.2). On the other hand, millions years of evolution have led to low sequence similarity among terpenoid synthases in distant organisms. However, sequence similarity is not necessarily associated with protein function, as the classic class I and class II terpene synthase tertiary structures are remarkably similar even across the domains of life. In fact, widely divergent terpene synthases at sequence level can make the same products and have similar product profiles. Thus, there is evidence of convergent evolution in terpenoid synthases among many plant groups.

So far, terpenoid synthase gene family structure is available only for a few tree species, with particularly limited information for key forest species, albeit highly detailed information has been gained by next generation transcriptome sequencing techniques and several full genome sequences for important tree species have become available (Sect. 3.4.2). As more genomic data become available, we will be

able to gain more conclusive insight into the variations in terpenoid synthase gene family size and diversity. Even among the sequenced organisms, recent evidence of polyphyletic origin of terpenoid synthases has been found, suggesting that genetic diversity of plant terpenoid synthases may be even larger than we have thought before (Li et al. 2012).

There is rich phenomenological evidence of changes in constitutively expressed synthase activities and induction of non-constitutively expressed terpenoid synthases by different abiotic and biotic stresses for many tree species (Sect. 3.5). However, the information on regulatory elements of terpenoid synthases is currently very limited, and clearly more work is needed to gain insight into regulation of the expression of terpenoid synthases as driven by environmental variability and stress.

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Chapter 4
Genetic Engineering of BVOC Emissions from Trees

Maaria Rosenkranz and Jörg-Peter Schnitzler

Abstract In recent years, it has become possible to inhibit constitutive production of biogenic volatile organic compounds (BVOC) in trees by genetic engineering. In addition, the trait for constitutive emissions has been introduced in several previously non-emitting herbaceous model organisms and crops. Research on these genetically engineered organisms has demonstrated that eliminating terpenes (syn. terpenoids or isoprenoids) emission reduces stress tolerance, while enhancing emissions often increases abiotic and biotic stress tolerance. In this chapter, the progress in terpene engineering work is reviewed, and the advantages of, changes in and obstacles related to genetically modified (GM) trees are discussed. We start by introducing the reader to terpene biosynthesis and the efforts undertaken to manipulate that process, we further review past attempts to repress and overexpress terpene synthases in herbs and trees, and finally describe the current achievements and suggest future possibilities in the field of terpene emission engineering.

4.1 Introduction

During the last few decades, it has become possible to transfer specific traits into different organisms, silence the expression of specific genes or analyse tissue-specific activation and regulation of promoter elements. The first genetically modified (GM) plant was a tobacco (*Nicotiana tabacum*) with a chimeric antibiotic resistance gene (Bevan et al. 1983). A few years later, the first GM tree, a hybrid poplar (*Populus alba* x *P. grandidentata*) carrying a gene for herbicide resistance, was developed (Fillatti et al. 1987). Genetic modifications have the potential for a wide range of applications (Leroy et al. 2000). GM trees have multiple socioeconomic benefits,

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including faster growth (Kawaoka et al. 2003; Jing et al. 2004), resistance to cold (Jin et al. 2009), herbivorous insects (Wang et al. 1996), and viruses (Gonsalves et al. 2004), as well as soil phytoremediation (Peuke and Rennenberg 2005). Given the central role of wood pulping and paper production, one of the main foci of GM tree research involves altering the metabolic flux of lignin biosynthesis pathways (Chen and Dixon 2007; Jouanin and Lapierre 2012; Meyermans et al. 2000; Pilate et al. 2004). Trees with reduced lignocellulose content need less extensive treatment with hot sulfuric acid during pulp production, and therefore reduce energy inputs, and the use of chemicals and white-water pollution (Herschbach and Kopriva 2002).

GM trees carry particular traits that make them novel to ecosystems (e.g., commercially available Bt-poplars in China (Wang 2004) or papaya (*Carica papaya*) trees resistant to papaya ringspot virus (PRVS) in Hawaii (Gonsalves et al. 2004)). The potential impacts of GM trees on native ecosystems are mainly ecological, as these trees could influence the fitness of other species, population dynamics, ecological roles, interspecies interactions, and air chemistry.

Because forests – the "lungs" of the Earth – consume CO_2 and other gases, they occupy a central role in maintaining tropospheric chemistry (Schulze and Caldwell 1995). In addition to oxygen, forests emit a wide variety of biogenic volatile organic compounds (BVOCs). Forests are globally the major source of BVOC emissions (Boissard et al. 2001). Isoprene (worldwide 44 % of total emissions) dominates BVOC emissions (Guenther et al. 1995). Due to its high reactive capacity in the atmosphere, it strongly modifies atmospheric reactivity, in particular, causing ozone formation together with NOx (Fuentes et al. 2000). Monoterpenes (11 %) constitute the second most important reactive class of BVOCs emitted from plants (Guenther et al. 1995; Fuentes et al. 2000). Moreover, the temperate and tropical forests are known to emit approximately 60 different chemicals in significant quantities (Kesselmeier and Staudt 1999), making it especially difficult to target specific modulations of BVOC emissions. It is unfortunate that most tree species used in agroforest plantations, including poplars (*Populus* spp.), willows (*Salix* spp.), eucalypts (*Eucalyptus* spp.) and oil palm (*Elaeis guineensis*) emit BVOCs, and particularly isoprene, in large quantities (Kesselmeier and Staudt 1999; Owen et al. 2013). These emissions tend to promote the production of atmospheric oxidants in urban and suburban regions (Chameides et al. 1988; Owen et al. 2013), especially in atmospheres with abundant NOx due to human activities (Hewitt et al. 2009). Thus, from an air quality perspective, low or non isoprene-emitting trees are desirable, and a reduction in the isoprene emissions from these trees would decrease the isoprene fluxes from plantations.

In addition to the efforts to modify tree wood quality, growth and resistance to stress, there are also attempts to create novel emission profiles in plants. GM plants with modified emission profiles (mainly with regard to terpenoids) are widely used to investigate plant-insect and plant-microbe interactions (e.g., Gershenzon and Dudareva 2007) and analyse 'upstream' metabolic processes, e.g., regulation of the mevalonate (MVA) pathway and 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway). Different approaches can be used to add or remove genes and enhance or decrease the existing emission activity.

Plants with engineered emission profiles will help to elucidate the physiological and ecological roles of these compounds. In addition, healthier plants with better resistance to environmental stresses or plants producing economically important compounds, e.g., pharmaceuticals, can be generated (Chang and Keasling 2006; Bouwmeester 2006).

There have been several attempts to modify terpene emissions in plants (e.g., Behnke et al. 2007; Kappers et al. 2005; Laothawornkitkul et al. 2008; Vickers et al. 2009; Lee et al. 2010). One of the main obstacles is the complex terpene biosynthesis regulatory system, which is not well understood. The successful modification of BVOC emissions requires an understanding of the different regulatory steps and feedback mechanisms in these pathways, and as these mechanisms differ between species, engineering work is even more challenging.

The biochemical terpene-world is complex; there are several different compounds with diverse structures (Gershenzon and Dudareva 2007). All of these compounds, including the non-volatile terpenes are produced from key intermediate compounds, isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) (McCaskill and Croteau 1998; Lichtenthaler 1999). Thus, metabolic elements downstream of the initial substrate are necessary to regulate the synthesis of different terpene compounds. More than once, the modification of a non-emitting species towards increased emissions has failed because there was not enough precursor substrate to produce moderate to high emission levels (Kappers et al. 2005; Sharkey et al. 2005; Schnee et al. 2006; Loivamäki et al. 2007a, b; Sasaki et al. 2007). Thus, increasing the substrate availability for terpene biosynthesis by boosting the terpene production pathway has provided promising results (Wu et al. 2006).

In this chapter, we first introduce the biosynthetic pathways for terpene production. We will give an overview of the efforts to engineer terpene-producing pathways that modify substrate availability and/or help to elucidate the pathway regulation systems. We continue by discussing the different approaches to the genetic engineering of BVOC profiles. Studies investigating the transcriptional regulation of emissions are discussed, and we outline the current work on the engineering of terpene emission profiles in trees and herbs. We concentrate on isoprene, the compound dominating the global BVOC emissions, but we also cover mono- and sesquiterpene emission engineering. Finally, we discuss the use of reporter lines that allow for monitoring activation of pathways and location of enzymes in BVOC studies.

4.2 Overview of BVOC Biosynthesis Pathways with Emphasis on Genetic Engineering

BVOC biosynthesis, regulatory pathways and emissions are complex. One of the problems related to genetically engineering BVOC emissions is substrate availability. Plant terpenoids are synthesized through condensation of the five-carbon

precursors IDP and DMADP, which can be synthesized in two pathways localized in two separate cell compartments. The availability of precursors is an important issue in order to succeed in gene engineering.

Kinetic characteristics of the two terpenoid synthesis pathway are discussed in Li and Sharkey (2013). In the present chapter the two pathways and the metabolic cross-talk between them are briefly reviewed for the purpose of a global understanding of the complex regulation of the emission profiles. This is done mainly by discussing the various attempts to genetically engineer or chemically manipulate flux through the pathways in order to gain better understanding of the regulation processes and analyse what is specific in plant species with high rate of terpene synthesis compared with plants with low terpene synthesis rate. Also the recently discovered terpene precursor biosynthesis pathway in mitochondria is discussed. Although volatile terpenoids are often considered secondary metabolites, many terpenoids also participate in primary metabolism, including plant hormones gibberellins, cytokinins, and abscisic acid and components of the photosynthetic machinery such as plastoquinol, phytol residue of chlorophyll and carotenoids. Thus, it is often difficult to separate "primary" and "secondary" metabolism (Fineschi et al. 2013), further emphasizing the difficulties with engineering terpene metabolism.

4.2.1 Cytosolic Terpene Production

The cytosolic mevalonate (MVA) or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) pathway is well understood (Agranoff et al. 1960). This pathway is required for the synthesis of cytokinins, sesquiterpenes (C15), and triterpenes (C30, e.g., sterols and brassinosteroids) (Suzuki et al. 2004). This pathway also provides precursors for ubiquinone synthesis in mitochondria (Disch et al. 1998). The MVA pathway is found in most eukaryotic organisms including animals, fungi and plants, but also in Archaea and in some bacteria (Lombard and Moreira 2011; Chappell et al. 1995). The MVA pathway starts with production of acetoacetyl-CoA from two molecules of acetyl-CoA. This step is catalyzed by the enzyme AcAc-CoA thiolase 2 (AACT2) (Vollack 1996; Ahumada et al. 2008). In the second step, HMG-CoA synthase condenses acetyl-CoA and AcAc-CoA to form HMG-CoA. The next step is one of the most studied enzymatic reactions in terpene biosynthesis (McCaskill and Croteau 1998; Rodríguez-Concepción 2006). HMG-CoA reductase catalyses the synthesis of mevalonic acid from HMG-CoA. The HMG-CoA reductase is encoded by a multigene family whose size varies from plant to plant (Stermer et al. 1994), and its expression is tightly controlled at the transcriptional and post-transcriptional levels (reviewed in Rodríguez-Concepción 2006). The HMG-CoA reductase can be activated by multiple different promoters (Lumbreras et al. 1995). This complex regulatory system underlines the importance of the enzyme. HMG-CoA reductase controls the flux of the MVA pathway (Rodríguez-Concepción 2006)

and, consequently, it is also the cytosolic enzyme upstream from IDP that has been targeted to gene engineering in order to enhance terpene emissions (Chappell et al. 1995). Overexpression of the gene in tobacco (*Nicotiana tabacum*) did, however, not alter the sesquiterpene emission, but sterol levels were enhanced (Rodríguez-Concepción 2006). HMG-CoA can be, in addition, chemically blocked by mevinolin or lovastatin. By this means the exchange of precursors between the cytosolic MVA and chloroplastic MEP pathways can be examined (e.g., Rodríguez-Concepción et al. 2004; Laule et al. 2003) (see below for more information about the exchange).

In the next step, mevalonate kinase phosphorylates mevalonate to form mevalonate 5-phosphate, which is further phosphorylated by phosphomevalonate kinase to mevalonate 5-diphosphate. Finally, mevalonate diphosphate carboxylase catalyses the decarboxylation of mevalonate 5-diphosphate to IDP. These last steps are well studied in yeast (*Saccharomyces cerevisiae*) and mammals but not yet in plants (Nagegowda 2010).

Isopentenyl diphosphate isomerase (IDI) is an enzyme that forms DMADP from IDP and vice versa, and this reaction can occur in both cytosol and plastids (Ramos-Valdivia et al. 1997; Brüggemann and Schnitzler 2002a), albeit the transfer of isoprenoid precursors among compartments (and among pathways) can be limited (Sect. 4.2.3). DMADP is the direct precursor of the hemiterpene isoprene in chloroplasts, whereas IDP is used to form higher terpene precursors. For higher terpene synthesis, the two diphosphorylated C5-units, DMADP and IDP, condense in a head-to-tail reaction to produce geranyl diphosphate (GDP; C10), which is the immediate precursor of monoterpenes. Condensation of GDP and IDP produces farnesyl diphosphate (FDP; C15), which is an immediate precursor of sesquiterpenes, while condensation of FDP and IDP results in formation of geranylgeranyl diphosphate (GGDP; C20), a diterpene precursor (Ramos-Valdivia et al. 1997).

Chen et al. (2000) showed that overexpression of FDP in sweet sagewort (*Artemisia annua* L.) can lead to a slightly higher production of the sesquiterpene artemisinin. More recently Wu et al. (2006) achieved high sesquiterpene emission levels by introducing a novel construct, in which an avian FDP synthase was combined with the sesquiterpene patchoulol synthase, into tobacco plants. Although sesquiterpenes are primarily synthesized via the MVA pathway, small sesquiterpene emissions have been reported also from plastidic FDP pools (Aharoni et al. 2003; Wu et al. 2006), and sesquiterpene synthesis could be engineered by this way both to cytosolic and chloroplastic compartments of tobacco (Wu et al. 2006).

4.2.2 Plastidic Terpenoid Production

DMADP in chloroplasts is synthesized through the MEP/DOXP pathway (Rohmer et al. 1993; Lichtenthaler et al. 1997; Fig. 4.1; Phillips et al. 2008). Previously, it was assumed that all isoprenoids are synthesized exclusively through the MVA pathway, but by now, the MEP pathway has also been found in Eubacteria, Chromalveolata,

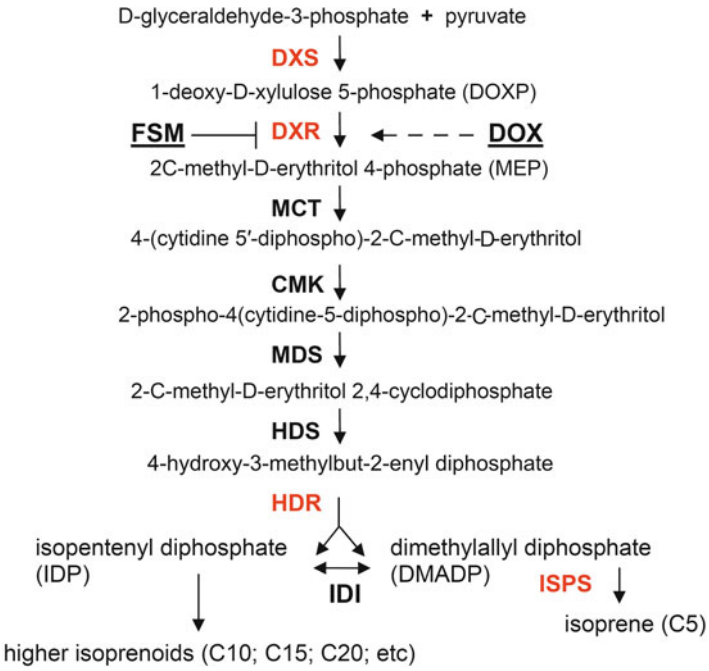

Fig. 4.1 Diagram of the plastidic 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DOXP) pathway for isoprenoid synthesis. The possible regulatory steps, shown to correlate with the end-product level, are *marked red*. The individual steps are discussed in the Sect. 4.2.2. The enzymes catalyzing the reactions are shown in *bold font* as follows: 1-deoxy-D-xylulose 5-phosphate synthase (DXS); 1-deoxy-D-xylulose 5-preductoisomerase (DXR); 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (MCT); 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase (CMK); 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS); 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS), 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR); isopentenyl diphosphate isomerase (IDI); isoprene synthase (IspS). Shown are also ways of chemical manipulation of the pathway by e.g., blocking with fosmidomycin (FSM) or feeding with di-deuterated deoxyxylulose (DOX)

and algae (Eoh et al. 2007; Cassera et al. 2004; Grauvogel and Petersen 2007; Okada and Hase 2005; Massé et al. 2004). This pathway produces IDP and its isomer DMADP, which are the building units of isoprene (C5), monoterpenes (C10), diterpenes (C20), carotenoids (C40), phytol, tocopherols, phylloquinones, and some sesquiterpene (C15) (Lichtenthaler 1999). In isoprene emitters, the major product of the MEP pathway goes into isoprene emission (Wolfertz et al. 2004; Li and Sharkey 2013), but the importance of the production of higher terpenoids should not be underestimated.

Terpene products from the MEP pathway are directly coupled to photosynthesis through the C3 metabolites glyceraldehyde 3-phosphate (GAP) and pyruvate that are the substrates for the first MEP pathway reaction. In this first step of the MEP pathway, a C2 unit from pyruvate is transferred to GAP by 1-deoxy-D-

xylulose 5-phosphate synthase (DXS) forming 1-deoxy-D-xylulose 5-phosphate (Lichtenthaler 1999). GAP is a product of the Calvin cycle, but the source of pyruvate in chloroplasts is not fully clear. Chloroplasts may import phosphoenol pyruvate (PEP) from cytosolic glycolysis that is further converted to pyruvate (Affek and Yakir 2003; Trowbridge et al. 2012), but pyruvate might also originate from chloroplastic metabolism (Sharkey and Yeh 2001; Rasulov et al. 2011; Li and Sharkey 2013). Under certain environmental conditions, carbon from stored sources can be mobilized to keep the MEP pathway active (Schnitzler et al. 2004).

DXS as the first enzyme in the MEP pathway may have a regulatory role, as its expression positively correlates with the concentration of several isoprenoid end products (Lois et al. 2000; Estévez et al. 2001; Muñoz-Bertomeu et al. 2006). Estévez et al. (2001) showed that overexpression of *DXS* in *Arabidopsis thaliana* leads to a moderate increase of carotenoid and abscisic acid (ABA) levels. Similar results were obtained for tomato (*Lycopersicon esculentum*) (Lois et al. 2000) and in lavender (*Lavandula latifolia*) (Muñoz-Bertomeu et al. 2006). DXS forms 1-deoxy-D-xylulose 5-phosphate (DOXP), which is converted to MEP by 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR). DOXP is not only the first intermediate of the MEP pathway, but it is also involved in the thiamine/shikimate pathway (Julliard and Douce 1991; Belanger et al. 1995). It has been assumed that DXR rather than DXS plays a regulatory role in the MEP pathway (Mayrhofer et al. 2005). The transformation of peppermint (*Mentha* x *piperita*) to enhance DXR activity increased the plant's essential oil yield (Mahmoud and Croteau 2001). Moreover, Carretero-Paulet et al. (2002; 2006) showed that overexpression of *DXR* in *Arabidopsis thaliana* increased the concentration of isoprenoid end-products. Additionally, Mahmoud and Croteau (2001) showed that altered DXR levels alter the flow through the MEP pathway in *Arabidopsis*, which supports the role of DXR as a regulatory step. Because *DXR* gene expression does not correlate with the rhythm of isoprene emission (Mayrhofer et al. 2005; Loivamäki et al. 2007b), the extent of its regulatory role remains unknown. On the other hand, reports by Estévez et al. (2001) and Enfissi et al. (2005) on tomato plants with altered *DXS* expression strongly suggested that DXS is the main regulator of the MEP pathway. These authors proved that changes in DXS levels result in similarly changed end-product profiles (Estévez et al. 2001; Enfissi et al. 2005). Feeding with an external substrate, di-deuterated deoxyxylulose (DOX) produces similar results (Loivamäki et al. 2007a). DOX is rapidly incorporated into the MEP pathway and can be used downstream from DXS to enhance the flow through the pathway (Fig. 4.1). In *Eucalyptus globulus*, however, isoprene emission was not changed by DOX-feeding, indicating that some intermediates of the MEP pathway contribute to a negative feedback loop (Wolfertz et al. 2004; Monson 2013).

The MEP pathway can be blocked with fosmidomycin, a DXR reductoisomerase inhibitor (Kuzyama et al. 1998; Fig. 4.1). Fosmidomycin has been used to study different aspects of isoprene biosynthesis and its physiological significance (Sharkey et al. 2001; Loreto and Velikova 2001; Velikova and Loreto 2005; Rasulov et al. 2009a; Rasulov et al. 2011). Because the inhibitor is not specific to isoprene and monoterpene biosynthesis - it also decreases synthesis of carotenoids and other

MEP pathway end-products – longer-term changes in plant physiological status after suppression of isoprene emission by fosmidomycin-feeding (e.g., altered response to heat or oxidative stress, Possell and Loreto 2013) cannot be exclusively attributed to isoprene depletion. Recent study showed that fosmidomycin treatment could lead to higher susceptibility to photoinhibition and photodamage at high light intensities (Possell et al. 2010), which might be partly due to inhibition of biosynthesis of carotenoids.

In the next steps of plastidic terpenoid synthesis, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (MCT) catalyzes the production of 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol (Eisenreich et al. 2001; Phillips et al. 2008). This intermediate is further converted to 2-C-methyl-D-erythritol 2,4-cyclodiphosphate by 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase (CMK) and 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS). In the next step, 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS) converts 2-C-methyl-D-erythritol 2,4-cyclodiphosphate to 4-hydroxy-3-methylbut-2-enyl diphosphate. The last step in the MEP pathway is catalyzed by the enzyme 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR), which converts 4-hydroxy-3-methylbut-2-enyl diphosphate to DMADP and IDP. HDR may have an additional regulatory role in the MEP pathway, as its enhanced expression results in higher carotenoid concentrations in *Arabidopsis* (Botella-Pavía et al. 2004). Thus, this evidence suggests that plastidic terpenoid production may be regulated at various points in the pathway (Fig. 4.1).

Wiberley et al. (2009) cloned all the genes from *Populus trichocarpa* and measured their expression levels under various environmental conditions. They showed that the *DXS* and *HDR* genes are regulated by circadian rhythms and the expression of *DXS*, *DXR* and *CMK* genes was temperature-dependent (Wiberley et al. 2009), similarly to the regulation of the isoprene synthase (*IspS*) gene (Loivamäki et al. 2007a, Sect. 4.3.1). Thus, multiple enzymes, including DXS, could have a regulatory role in the MEP pathway of *P. trichocarpa* (Wiberley et al. 2009).

As in the MVA pathway (Sect. 4.2.1), chloroplastic IDI converts IDP (the precursor of higher terpenoids) to DMADP (the immediate precursor of isoprene) and vice versa. IspS converts chloroplastic DMADP to isoprene. In addition to isoprene biosynthesis, monoterpene biosynthesis takes place in chloroplasts. GDP that is formed by head-to-tail condensation of IDP and DMADP is the precursor for various monoterpenes, whose synthesis is catalyzed by different monoterpene synthases (Lichtenthaler 1999), each of which often synthesizes multiple monoterpene species (Tholl 2006; Rajabi Memari et al. 2013). The regulation of emissions at transcription level is discussed in Sect 4.3 (see also Monson 2013) and by environmental drivers in other chapters of this book (Grote et al. 2013; Li and Sharkey 2013; Monson 2013).

Terpene precursors are present in chloroplasts and mitochondria. Kappers et al. (2005) targeted an *Arabidopsis* terpene synthase (Tps) to mitochondria and measured low amounts of nerolidol and linalool, which are terpenes that do not naturally occur in *Arabidopsis* BVOC emissions. Because FDP is present in mitochondria, the introduced sesquiterpene synthase resulted in terpene emission. The presence

of FDP could be expected because the cytosolic IDP precursor is transported to mitochondria, where it is utilised for ubiquinone biosynthesis (Disch et al. 1998). It is still unclear whether terpene precursors have functions in the mitochondria in other plant species.

4.2.3 Interactions Between MEP and MVA Pathways

The interactions between the plastidic MEP and cytosolic MVA pathways also need clarification (Lichtenthaler 1999; Laule et al. 2003; Rodríguez-Concepción et al. 2004). There may be a cross-talk between the two pathways in some species and plant tissues, such that MVA derived precursors can be used in plastids for isoprenoid biosynthesis and vice versa (Lichtenthaler 1999). Laule et al. (2003) showed that blocking the MVA pathway with lovastatin causes changes in chloroplastic carotenoid and chlorophyll contents and in transcript levels of the rate-controlling enzymes, whereas cytosolic sterol levels remained constant after recovering from an initial drop. The authors suggested that the transport of IDP from chloroplast to cytosol was responsible for these effects.

Blocking the MEP pathway with fosmidomycin resulted in decreased concentrations of all the terpenoid metabolites and the cytosolic sterols (Kuzyama et al. 1998). Taken together with the data from Laule et al. (2003), these results suggest unidirectional transport of IDP from chloroplasts to the cytosol. This hypothesis is supported by work from Loreto et al. (2004), who used ^{13}C-labelling and fosmidomycin to show that a possible cross-talk between MEP and MVA pathways does not modulate isoprene emission levels when the MEP pathway is blocked at least for the time-period of the experiment. A certain recovery of isoprene emissions after fosmidomycin inhibition can be sometimes observed in longer-term experiments (Rasulov et al. 2011).

In contrast to these findings, Rodríguez-Concepción et al. (2004) isolated *Arabidopsis* mutants that survived when the MEP pathway was blocked. This result may have been due to enhanced uptake of MVA derived terpenoid precursors by plastids, but no definitive proof of an enhanced cytosol-to-plastid transport exists to our knowledge.

4.3 The Regulation of Emissions During and After Transcription

4.3.1 Isoprene Synthase

Before the discovery of IspS (Silver and Fall 1991), isoprene formation was thought to occur spontaneously, as the two phosphate residues can be lost and a double bond

formed at low rates in acidic conditions. The relevance of IspS in isoprene emissions was demonstrated in several species by the positive correlation between the enzyme activity and basal emissions (Lehning et al. 1999; Brüggemann and Schnitzler 2002c; Kuzma and Fall 1993; Scholefield et al. 2004; Mayrhofer et al. 2005).

IspS can exist in two isoforms. The soluble form is located in the chloroplast stroma, and the insoluble form is bound to thylakoid membranes, as observed in willow (*Salix discolor*) by Wildermuth and Fall (1996, 1998) and in other species (Wiberley et al. 2005; Schnitzler et al. 2005). All deduced IspS proteins have a transit peptide that targets them to chloroplasts, where isoprene synthesis takes place (Mgalobilishvili et al. 1979; Schnitzler et al. 2005). Most of the known plant *IspS* genes have been isolated from poplar species (Miller et al. 2001; Sasaki et al. 2005; Fortunati et al. 2008; Sharkey et al. 2005; Calfapietra et al. 2007). In addition, the presence of a *IspS* gene has been verified in *Eucalyptus globulus*, *Melaleuca alternifolia* and *Pueraria montana* (Sharkey 2013; Li and Sharkey 2013). Sharkey (2013) recently constructed a phylogenetic tree with all of the known angiosperm *IspS* genes, suggesting that they form a monophyletic clade within the rosids. This result suggests a single evolutionary event for *IspS* gene formation followed by recurrent losses of isoprene emission capacity within this group. However, there is evidence that the evolution of isoprene production capacity and loss has taken place independently in various taxonomic groups of higher plants, especially if one considers also mosses, ferns and gymnosperms (Monson et al. 2013).

Promoter studies elucidate gene regulation at the transcription level. Knowing the promoter region sequence, regulatory elements can be predicted by *in silico* studies. For example, computational analysis of the *P.* x *canescens* isoprene synthase gene promoter revealed many putative light responsive boxes, heat shock and circadian elements (Loivamäki et al. 2007b). Similar elements were also present in the promoter sequence of *P. trichocarpa IspS* gene (Sharkey et al. 2008). Even under constant light, *IspS* gene transcripts fluctuate diurnally, demonstrating the circadian clock's involvement in their regulation (Loivamäki et al. 2007b).

To study the gene activation *in vivo*, Cinege et al. (2009) cloned the *IspS* gene promoter upstream of two reporter genes, green fluorescence protein (*E-GFP*) and a β-glucuronidase (*GUS*). Analysis of these reporters and the *PcIspS* promoter in *Arabidopsis* and poplar confirmed that the *IspS* gene in *P.* x *canescens* is activated by light and elevated temperature (Cinege et al. 2009). This example demonstrates the usefulness of GM trees for studying gene activation in real time. The circadian regulation of isoprene emission from oil palm (*Elaeis guineensis*) and grey poplar (*P.* x *canescens*) was also demonstrated (Wilkinson et al. 2006; Loivamäki et al. 2007b). Moreover, the correlation between mean air temperature and *IspS* gene transcript levels was observed in *P. x canescens* during the vegetation period (Mayrhofer et al. 2005).

During leaf development, isoprene emission is transcriptionally controlled. *IspS* mRNA and protein appear at the same developmental stage, which can be days to weeks after the onset of photosynthesis depending on the temperature at which the leaves have developed (Mayrhofer et al. 2005; Wiberley et al. 2005). Similarly, the regulation of isoprene emission through *IspS* transcription and IspS protein

levels may take place under stress, as has been observed in quaking aspen (*Populus tremuloides*) grown under elevated [O_3] (Calfapietra et al. 2007, 2008, 2013).

Several regulatory steps other than gene transcription can adjust the availability of functional protein. While the potential post-transcriptional and post-translational modifications of terpenes are still unclear, there have been a few demonstrations of these types of regulation. Transcript and protein levels, enzyme activity and isoprene emission do not always correspond on a diurnal scale (Loivamäki et al. 2007b), during a period of a few weeks (Wiberley et al. 2009) or over the course of the growing season (Mayrhofer et al. 2005). *IspS* gene transcription in *P.* x *canescens* leaves reaches its maximum in summer (July, beginning of August), and maximal protein expression occurs in late summer and autumn. However, IspS activity drops dramatically at the end of September, suggesting a possible post-translational regulation of IspS (Mayrhofer et al. 2005). Similarly, the drought stress response affects IspS activity more than its protein or transcript levels, suggesting that there is tighter post-transcriptional control of isoprene emissions during these conditions (Brilli et al. 2007; Fortunati et al. 2008; Laothawornkitkul et al. 2008). The nature of the possible modifications is, however, not yet known. On the other hand, stress conditions and aging are also known to reduce the concentrations of DMADP further possibly reducing the emissions (Sun et al. 2012b; Li and Sharkey 2013).

4.3.2 Other Volatile Terpene Synthases

Terpenes are the largest group of plant natural products and are structurally very complex (Degenhardt et al. 2009). In the present chapter, we cannot include all of the reaction mechanisms and regulatory events that are responsible for the gene activation and biochemical regulation of monoterpene and sesquiterpene synthase activities. The recent reviews of these topics are very comprehensive (see e.g., Tholl 2006; Christianson 2006; Degenhardt et al. 2009; Rajabi Memari et al. 2013). While many terpene synthases (Tps) catalyze the formation of multiple products, some are very specific in generating only one hydrocarbon product (Tholl 2006; Christianson 2006; Rajabi Memari et al. 2013). Volatile terpenes are often emitted from very specific plant parts, such as flowers (Dudareva et al. 2003; Chen et al. 2003), fruits (Lücker et al. 2004), young leaves (Brilli et al. 2009) or roots (Chen et al. 2004), depending their function in plant metabolism.

Intracellular gene activation analyses in GM trees are possible with confocal laser scanning microscopy, which clarify the roles of various genes in specific cells and tissues. A good demonstration of this method is the work by Tholl et al. (2005), who showed detailed activation of specific *Tps* gene promoters in different parts of *Arabidopsis* flowers. For example, the *At5g23960* terpene gene is active in the stigma. The same authors showed that β-caryophyllene, the dominant product of *At5g23960*, protects the flowers from pathogens (Huang et al. 2012). This result demonstrated nicely that low-level, directed terpene emission is very efficient.

4.4 Genetic Engineering of BVOC Emissions – Present Aspects and Future Potentials

4.4.1 Overexpression of Terpene Synthases

4.4.1.1 Overexpression with Constitutive Promoters

Because many *Tps* genes are cloned and available for further research, there are new opportunities for engineering biogenic BVOC emissions (for a comprehensive list of isolated monoterpene and sesquiterpene synthases, see Degenhardt et al. 2009). However, overexpressing *Tps* genes is more difficult than expected. Although several publications describe a successful introduction of a new *Tps* gene to a plant, almost as many papers report only very low increases in terpene emissions (Hohn and Ohlrogge 1991; Zook et al. 1996; Aharoni et al. 2003; Kappers et al. 2005; Loivamäki et al. 2007a; Sasaki et al. 2007). The emissions have been so low that they hardly passed the technical limits of detection. Most of the work has been performed in *Arabidopsis*, and the problem may be the scarce substrate availability or the tight control of the substrate supply in this model organism (Aharoni et al. 2003; Besumbes et al. 2004; Loivamäki et al. 2007a).

There have been several attempts to transform *Arabidopsis thaliana* to an isoprene emitting plant (Sharkey et al. 2005; Loivamäki et al. 2007a; Sasaki et al. 2007; Fig. 4.2). In these studies, different isoprene synthase genes were used for transformation, but none resulted in high isoprene emission. As demonstrated by Loivamäki et al. (2007a), substrate scarcity might inhibit isoprene emissions. Because of its low emissions, *Arabidopsis* was, for long time thought to be completely incapable of volatile emissions (Van Poecke et al. 2001) or to have only a low capacity for terpene synthesis. However, even low terpene emissions can have ecological impacts, like demonstrated for natural *Arabidopsis* BVOC emissions by Van Poecke et al. (2001). Similarly, low emissions achieved by genetic engineering can also lead to ecophysiological changes. Loivamäki et al. (2008) showed that low isoprene emissions in *Arabidopsis* change the behavior of a parasitic wasp searching for its host. Similarly, Kappers et al. (2005), who targeted nerolidol/linalool synthase to *Arabidopsis* mitochondria, and Schnee et al. (2006), who introduced maize sesquiterpene synthase into *Arabidopsis*, showed the ecological consequences of low levels of emitted terpenes on insect behavior.

Constitutive isoprene emission could recently be introduced in silver birch (*Betula pendula*) overexpressing the *IspS* gene from *P.* x *canescens* under the control of the 35S promoter (Rosenkranz and Schnitzler, unpublished data, Fig. 4.2). Compared to wild type plants, some GM lines emit significant amounts of isoprene, making birch an interesting system to study isoprene functions, in particular, since the first birch genome from *Betula nana* was published recently (Wang et al. 2012).

A clear success story in BVOC overexpression is that of Vickers et al. (2009), who engineered tobacco (*Nicotiana tabacum*) to emit isoprene. These plants allowed for verification of several hypotheses about the physiological and ecological

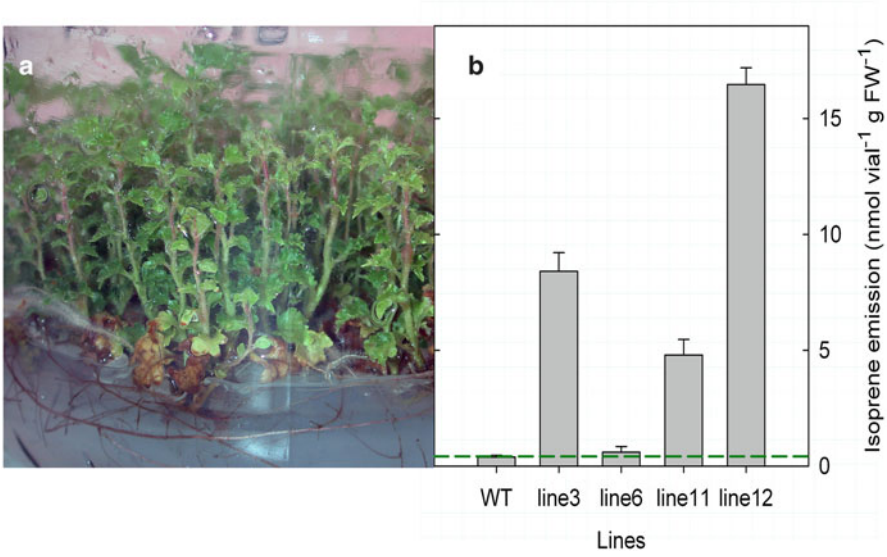

Fig. 4.2 Illustration of the tissue-cultured genetically modified silver birch (*Betula pendula*) overexpressing the *IspS* gene from grey poplar (*Populus* × *canescens*) under the control of the 35S promoter (**a**), and functional screening of birch leaves for isoprene emission (**b**). The screening was performed as described in Loivamäki et al. (2007a) (Rosenkranz and Schnitzler unpublished data)

functions of isoprene. For example, these plants were more resistant to oxidative stress, and they showed slightly enhanced thermotolerance (Vickers et al. 2009). Furthermore, the interactions between the plants and caterpillars of tobacco hornworm (*Manduca sexta*) were affected (Laothawornkitkul et al. 2008). The authors had to, however, screen a huge amount of plants to find a few high isoprene emitters (C. Vickers personal communication), which again emphasized the problems of overexpressing *IspS* and *Tps* genes.

A potentially successful approach to engineering high terpene emissions in trees could combine overexpression of a *Tps* gene and genes that regulate the MEP or MVA pathways. This approach was used by Wu et al. (2006) who combined FDP synthase with a sesquiterpene synthase in one construct and a GDP synthase with monoterpene synthase in another construct in order to engeneer tobacco plants. This work resulted in 1,000-fold higher sesquiterpene emissions and 10–30-fold higher monoterpene emissions than in wild-type tobacco plants. In another approach, Besumbes et al. (2004) used an inducible expression system to allow for more efficient use of substrates. The authors transformed *Arabidopsis* plants with diterpene taxadiene synthase from conifer *Taxus baccata* under tight regulation of the glucocorticoid system. The terpene yield was approximately 30-fold higher than in a constitutive expression system (Besumbes et al. 2004). This system should also be tested in trees.

In some studies, the up-regulation of gene expression was successful in trees. Genetic transformation has been used more often in fruit trees, principally to enhance their disease resistance. Additionally, marker-free GM trees and improved fruit quality are the common goals in engineering fruit trees (reviewed in Gambino and Gribaudo 2012). Another economically interesting and thus active line of investigation is for expansion of GM tree biomass production for use in second-generation biofuel development. The sustainability and biomass yield of different tree species has been enhanced by improving stress tolerance, wood properties, root formation, or phytoremediation (Harfouche et al. 2011; Osakabe et al. 2011).

It seems more problematic to overexpress a *Tps* gene in a species in which the function already exists. Thus, the approach has sometimes rather worked against itself than created higher emission levels (Mahmoud and Croteau 2001; Behnke et al. 2007). Behnke et al. (2007) attempted to create transgenic poplars emitting higher amounts of isoprene, but instead, native isoprene emission was repressed. Similarly, DXR overexpression in peppermint (*Mentha* x *piperita* L.) led to downregulation of MEP pathway products and chlorophyll deficient plants, indicating a co-suppression effect (Mahmoud and Croteau 2001).

4.4.1.2 Inducible Overexpression and Transient Overexpression

Most attempts to overexpress *Tps* genes used constitutive promoters, which might lead to high stress and energy consumption. Native isoprene emission is strictly regulated, and the emission level depends on many environmental conditions, such as leaf developmental stage, time of the day and the year (Brüggemann and Schnitzler 2002b; Kuzma and Fall 1993; Loivamäki et al. 2007b; Mayrhofer et al. 2005), plant past growth conditions (Wiberley et al. 2005; Sun et al. 2012a) and the level of abiotic stress (Loreto and Schnitzler 2010 for a review). In *Arabidopsis*, terpenes are emitted mainly from specific cells, which keeps emissions low, protects other plant parts and limits the costs of plant metabolism (Tholl et al. 2005; Huang et al. 2012). For BVOC emission engineering in trees, it might be interesting to use inducible or natural promoters to study the emission profiles in these conditions. If there were less profound changes to plant physiology, the possible effects of overexpression of *Tps* genes could become clearer.

Although widely used in other areas of plant science, especially in plant pathology, transient overexpression in herbaceous and woody plants has not been included in BVOC studies (Chrisholm et al. 2005; Grosskinsky et al. 2011). The short time interval and the local treatment are disadvantages that limit the use of transient transformation for BVOC expression. We have tried to transiently overexpress *IspS* gene in poplar protoplasts, but with little success (Rosenkranz and Schnitzler unpublished). In some cases, transient transformation is used to test the functionality of constructs (Meyer et al. 2004) before the initiation of labor- and time-intensive procedures required to produce transgenic trees (personal communication, A. Schmidt, MPI-ICE, Jena Germany).

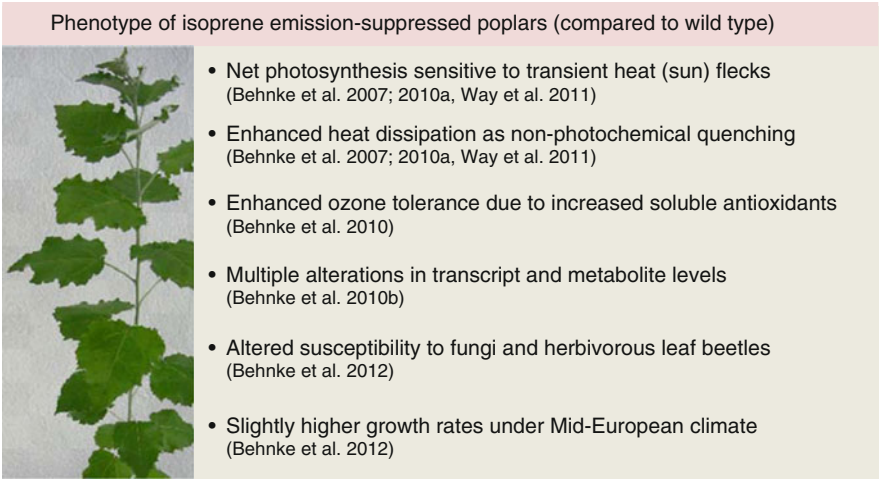

Fig. 4.3 Overview on phenotypic and chemotypic changes in genetically modified hybrid poplar (*Populus* × *canescens*) due to the repression of isoprene emission

4.4.2 Repression of Terpenoid Synthases

To engineer BVOC emissions in trees, single gene knock-downs or knock-outs may be more efficient than overexpression. In transgenic non-isoprene emitting poplars, isoprene emissions are almost fully repressed (Behnke et al. 2007). Isoprene emissions can be controlled by both IspS activity and substrate levels. Emissions from immature leaves in different poplar species depend on gene activation and IspS levels, whereas emissions in mature leaves depend both on substrate availability and IspS activity (Ghirardo et al. 2010; Vickers et al. 2010; Rasulov et al. 2009b; Rasulov et al. 2010). Therefore, suppression of *IspS* could reduce isoprene emissions from trees.

Currently, the only GM trees with reduced isoprene emission are grey poplars (*P. x canescens*) (Behnke et al. 2007; Schnitzler et al. 2010). In these lines, *IspS* is effectively suppressed by RNA interference (RNAi). Studies with these trees clearly demonstrate that isoprene is important for CO_2 assimilation and photosynthetic electron transport (Behnke et al. 2007, 2010a; Way et al. 2011, Fig. 4.3). A recent study (Behnke et al. 2012) documents the growth performance and fitness of these GM lines over two growing seasons in ambient, near-natural conditions in Göttingen, Germany. In the moderate climate of middle Europe, the trees' growth and biomass yield were not impaired but were instead temporarily enhanced in GM isoprene-suppressed lines compared to the natural isoprene emitters. However, the trees' susceptibility to biotic stress (herbivores and fungi) was altered. More field trials in various climatic and soil conditions are needed to clarify whether non-emitting bioenergy trees can prevent negative atmospheric impacts in regions with large wood tree plantations (e.g., Brazil, China, US, and Indonesia). A field trial with

isoprene emission-suppressed GM poplars has been started in 2012 in Oregon and Arizona (Schnitzler, Rosenstiel, Strauss, Monson, personal communication). That study will analyse whether the absence of isoprene emission impairs plant behavior in more extreme climates (warm/hot and dry summer) and the natural soil conditions of the northwestern and southwestern US.

4.5 Conclusion and Outlook

Since the first GM poplar was generated 25 years ago, numerous transformation protocols have been established for important forest trees such as pines (*Pinus* spp.), spruces (*Picea* spp.) and eucalypts (*Eucalyptus* spp.) (Harfouche et al. 2011; Osakabe et al. 2011), for commercially important fruit trees such as apple (*Malus domestica*), orange (*Citrus sinensis*), lemon (*Citrus limon*), plum (*Prunus domestica*), cherry (*Prunus avium*) and olive (*Olea europaea*) (Gambino and Gribaudo 2012); as well as woody perennials like grape (*Vitis vinifera*) (Jin et al. 2009). Many of these species are strong emitters of terpenes (Kesselmeier and Staudt 1999), making them interesting targets for transgenic approaches to terpene emission modifications. Terpene emissions can be modulated by the introduction of new *Tps* genes or knockdown of species-specific genes, as shown in poplar trees (Behnke et al. 2007). Moreover, these systems might be very useful for investigating terpenoid precursor biosynthesis via the MVA and MEP pathways in different tissues, such as resin ducts in wood and needles. Genetic modifications of trees could also be important for generating medically relevant terpenes. For example, the tea tree *Melaleuca alternifolia*, whose bark is very rich in bioactive terpenes and the volatiles terpinen-4-ol, α-terpineol and 1,8-cineole (Taga et al. 2012), would be an interesting target for genetic engineering. It is enticing to speculate that monoterpene biosynthesis in this tree could be further enhanced by changing the MEP pathway metabolic flux or by overexpressing the monoterpene ‘cineole cassette’ (Faehnrich et al. 2011). Taken together, the available GM trees and the resources for known genes of terpenes and the MVA and MEP pathways are versatile tools that can be used to test the biological and ecological functions of volatile terpenes in tree species that are important for forestry, horticulture, pharmaceutical and cosmetic industry.

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

Ziru Li and Thomas D. Sharkey

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

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5.1 Introduction

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

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

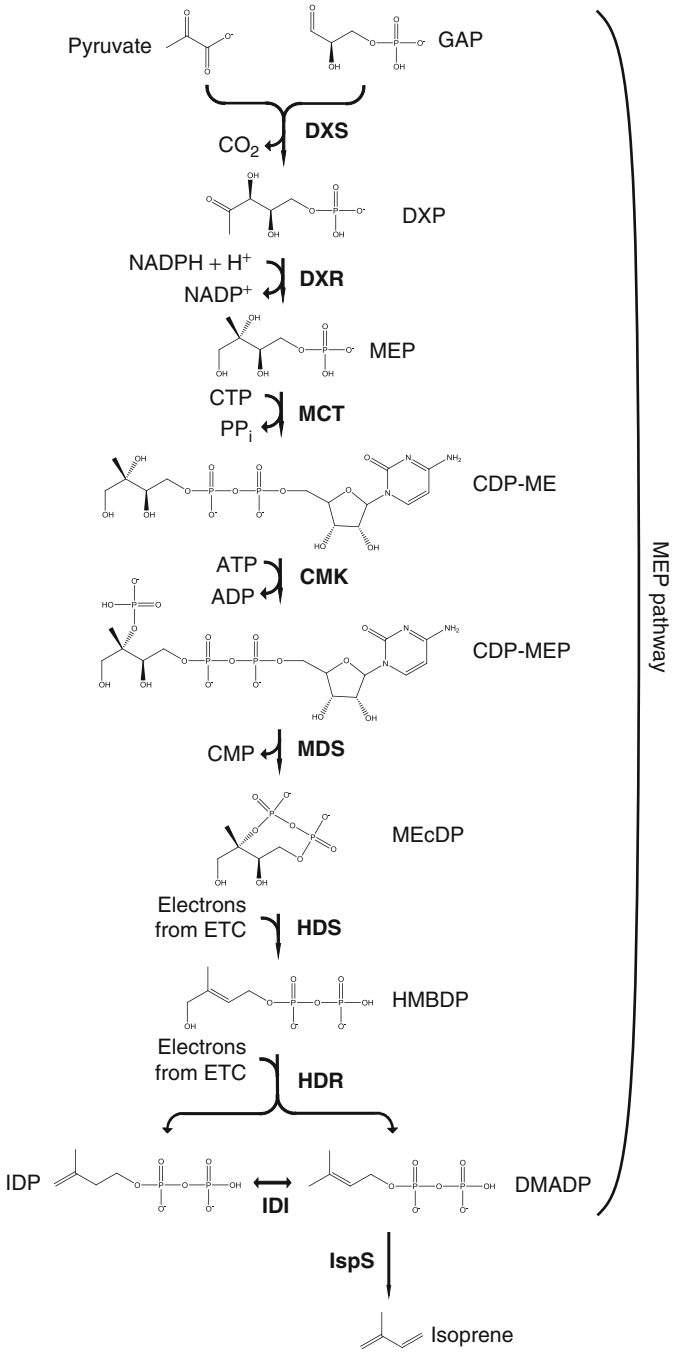
areas for future experimental research to fill the gaps in knowledge and aid towards development of fully mechanistic emission algorithms for plants stress studies and for biosphere models.

5.2 What Volatile Isoprenoids Are Emitted?

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

5.2.1 Hemiterpenes

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


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

5.2.2 Monoterpenes

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

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

5.2.3 Sesquiterpenes

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

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

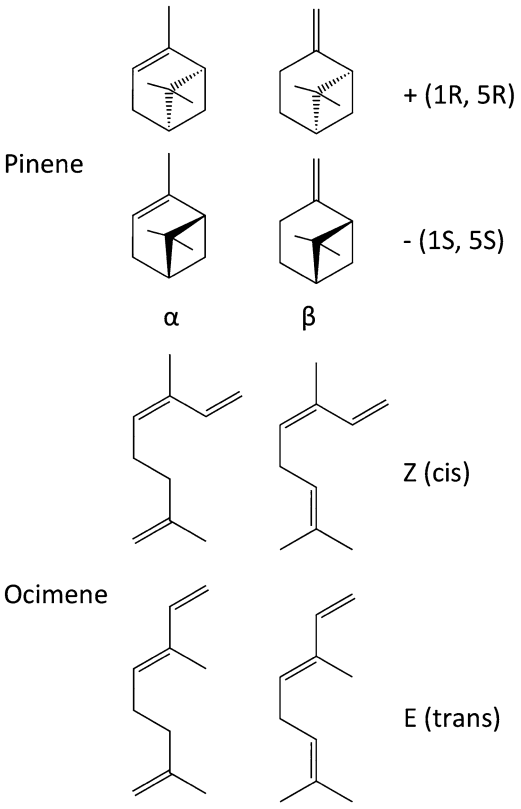


Fig. 5.2 Terpenes exist in many similar forms. Pinenes have two asymmetric carbon atoms and the double bond can be in either of two locations, resulting in different chiral (R/S or +/− or D/L) isomers. The acyclic ocimenes have a double bond that can be in either of two places and the orientation around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

5.3 Molecular and Pathway Controls of Volatile Isoprenoid Synthesis

5.3.1 The MEP Pathway

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

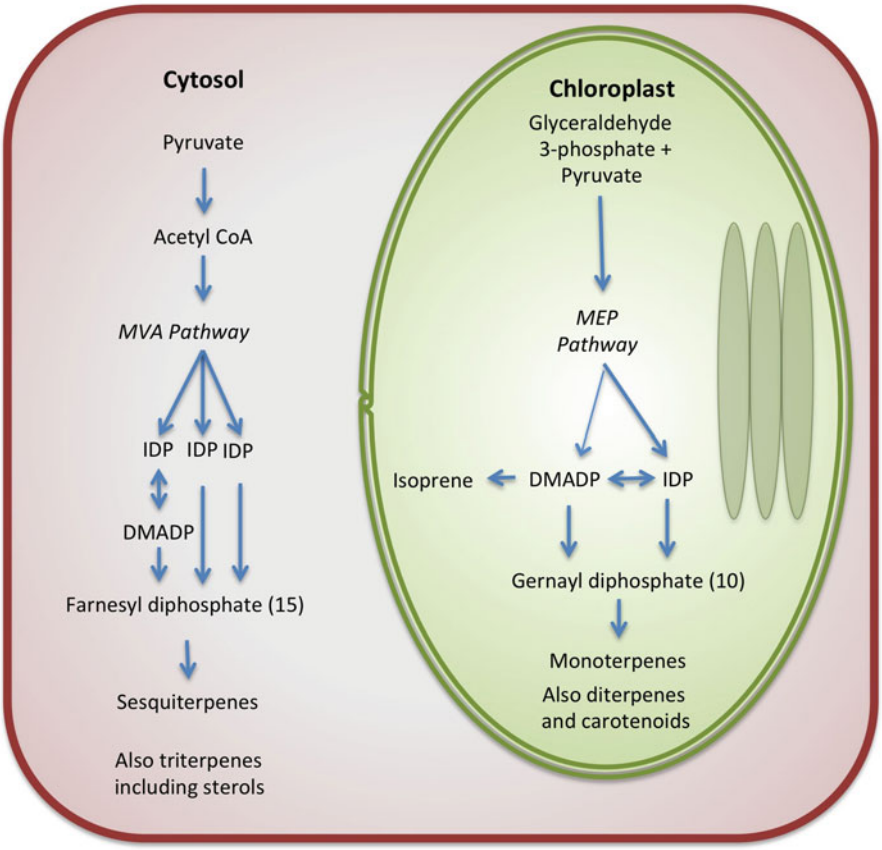


Fig. 5.3 Isoprenoid biogenic volatile organic compounds are made either in the cytosol by mevalonate (MVA) pathway (sesquiterpenes) or in the plastids by 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway (hemi- and monoterpenes). In the cytosol, the mevalonic acid (MVA) pathway converts acetyl-CoA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

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

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

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

5.3.2 Isoprenoid Synthases

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

5.3.2.1 Isoprene Synthase

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

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

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

Modified from Phillips et al. (2008)

Table 5.2 Available estimates of kinetic parameters of MEP pathway enzymes

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

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

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

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

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

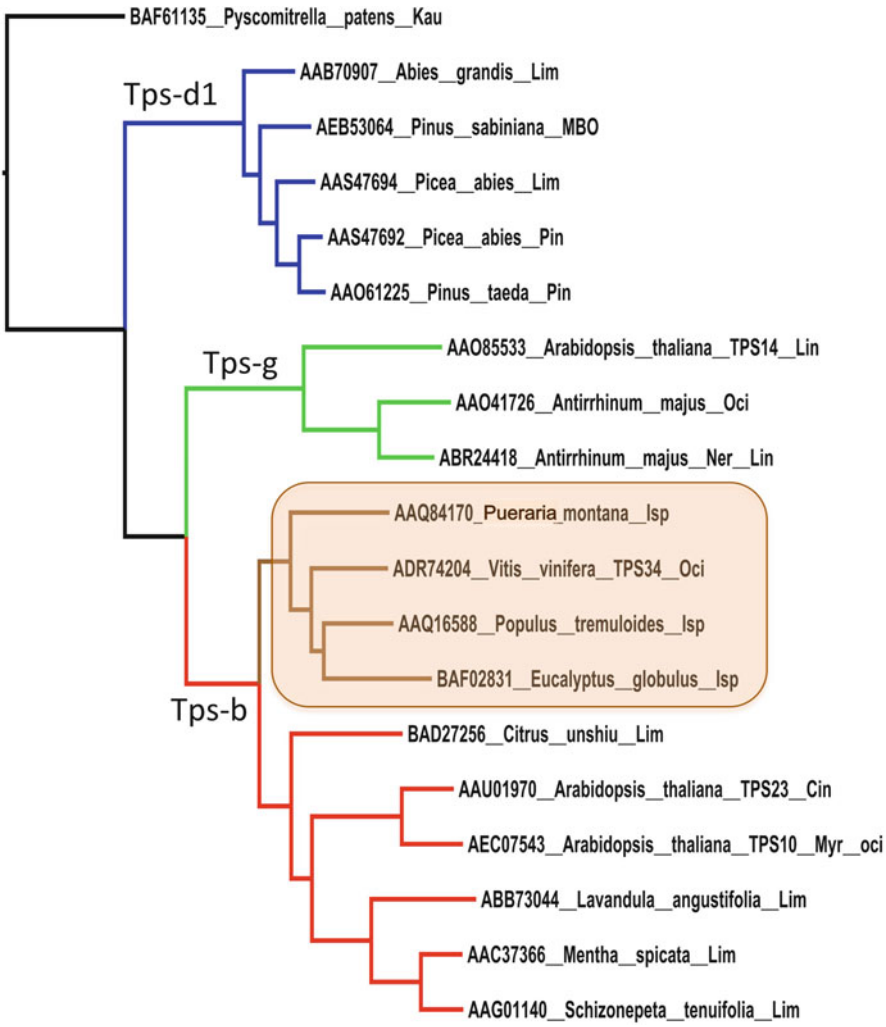


Fig. 5.4 Phylogenetic tree of a subset of genes making enzymes important for hydrocarbon emissions from trees, terpene synthases (Tps). The amino acid sequences of these genes were obtained from NCBI. The genes were selected to show the relationship of the Tps genes between gymnosperms (Tps-d) and angiosperms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

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

5.3.2.2 Methylbutenol Synthase

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

5.3.2.3 Monoterpene Synthases

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

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

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

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

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

5.3.2.4 Sesquiterpene Synthases

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

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

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

5.4 Environmental Regulation of Monoterpene and Isoprene Emissions

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

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

5.4.1 Short-Term Effects

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

5.4.1.1 Temperature

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

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

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

5.4.1.2 Light

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

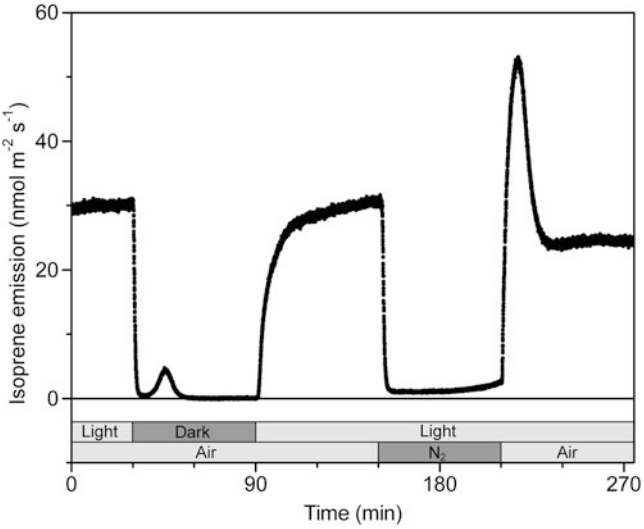


Fig. 5.5 Illustration of kinetic changes of isoprene emission from a leaf of hybrid poplar (*Populus tremula*) following light–dark transients and changes in ambient gas composition (Modified from Li and Sharkey 2013). When the light is turned off, isoprene emission from leaves falls rapidly but then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

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

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

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

5.4.1.3 CO_2 and O_2

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

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

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

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

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

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

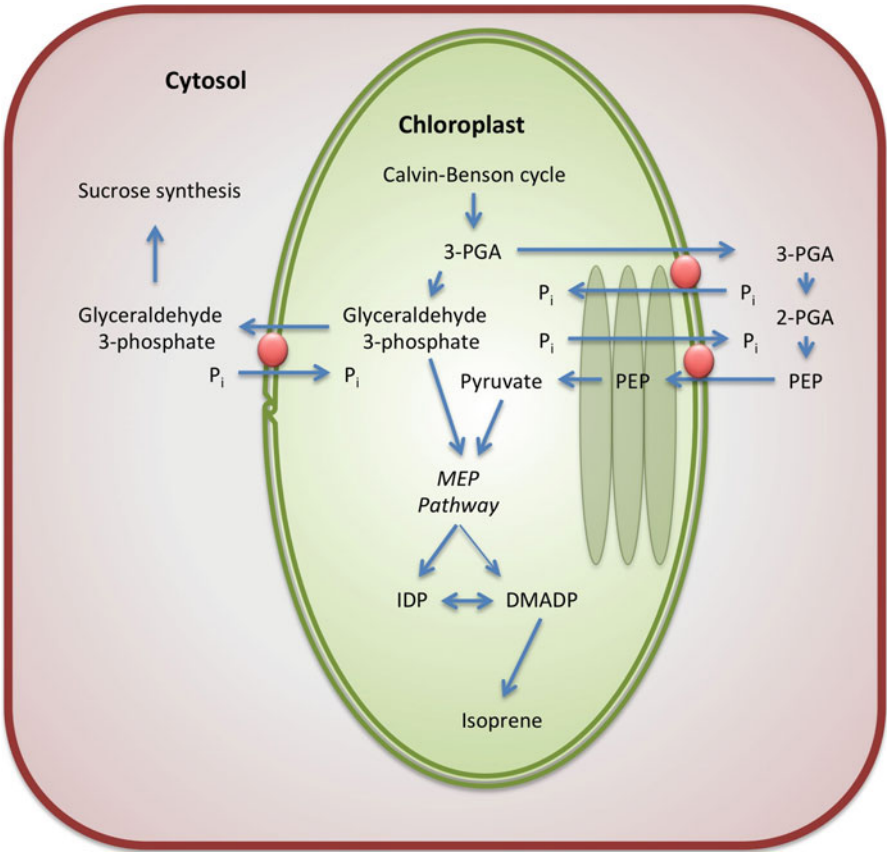


Fig. 5.6 Supply of carbon for isoprene synthesis from photosynthesis. The primary product of photosynthesis, glyceraldehyde 3-phosphate, can be used directly from the Calvin-Benson cycle, but chloroplasts generally do not have phosphoglucomutase to convert 3-phosphoglyceric acid (3-PGA) to 2-PGA. This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

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

5.4.2 *Long-Term Effects*

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

5.4.2.1 Circadian Effects on Isoprene Emission Capacity

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

5.4.2.2 Seasonality

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

5.4.2.3 Weather

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

5.4.2.4 Developmental Effects

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

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

5.4.2.5 Canopy Location

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

5.4.2.6 Ozone and CO_2

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

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

5.4.3 Isoprene Synthase Gene Expression

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

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

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

5.5 Conclusions and Future Perspectives

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

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

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Chapter 6
Metabolic and Gene Expression Controls on the Production of Biogenic Volatile Organic Compounds

Russell K. Monson

Abstract The emission of biogenic volatile organic compounds (BVOCs), including isoprenoid compounds, methanol and oxygenated organic compounds, is controlled by both the existing metabolic potential of a leaf and gene expression responses that modulate the existing metabolic potential to increase or decrease compound biosynthesis and emission rate. This capability to respond both instantaneously and in the long term to environmental variation provides plants with flexibility in their adaptions to biotic and abiotic stresses, which are also encountered in short and long-term time frames. This chapter reviews the mechanistic basis of the immediate controls of volatile BVOC emissions by light, temperature, and ambient CO_2 and O_2 concentrations, as well as the genetic responses that involve changes in gene expression patterns. Photosynthesis ultimately provides the carbon for BVOC production, though under non-stressed conditions the photosynthetic rate itself is rarely so low that it limits BVOC emissions. However, various metabolic pathways compete for substrates that are produced from photosynthate, including cytosolic pathways, such as the mevalonic acid (MVA) pathway and chloroplastic pathways such as the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP). Controls over the use of substrate are regulated among these pathways through feedback mechanisms, specificity in the transport of metabolites across organelle membranes, and the channeling of NADPH reductant and ATP to specific steps in the pathways. This chapter emphasizes that these interactive controls provide the major explanation for longer-term physiological controls of emissions. Emissions of several types of compounds are considered, including isoprenoids, methanol, and green leaf volatiles such as various aldehydes and ketones.

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6.1 Introduction

The broad diversity of biogenic volatile organic compounds (BVOCs) emitted by plants reflects the flow of carbon substrates through a diverse set of metabolic pathways (Gershenzon and Kreis 1999; Dudareva et al. 2005). This chemical diversity is required to provide plants with the ability to mitigate the influence of biotic and abiotic stresses and capture the resources required to maximize growth, competitive persistence, and ultimately, reproductive fitness (Fineschi et al. 2013). However, with access to a diversity of chemical options comes the challenge to regulate how that diversity is expressed in the face of a dynamic environment that changes across multiple timescales, and how to efficiently leverage existing processes and pathways to produce novel opportunities for plant adaptation as novel environments are encountered.

In the case of BVOCs, the evolutionary process has often co-opted and modified preexisting metabolic pathways as a means to produce compounds with novel structures and functions (Fineschi et al. 2013). Thus, many of the pathways that produce BVOCs are connected through mutual and parallel reliance on intermediate metabolites (pathway 'cross-talk') and the serial channeling of metabolites from one pathway to another (Lichtenthaler 1999; Bick and Lange 2003; Hampel et al. 2005; Hemmerlin et al. 2012). Control over the flux of metabolites through these pathways requires mechanisms for irreversibility in key catalyzed steps, feedbacks to regulate the activity of an entire pathway, and allosteric modification of specific enzymes to adjust the catalytic potential to process metabolites. Furthermore, many pathway products and intermediate metabolites are channeled among different cellular organelles, providing an even greater number of possibilities for the compartmentation and control over metabolic flux. In addition to these direct interactions among metabolic components of the cell, control over the production and emission of BVOCs is accomplished by constitutive and inducible genetic processes that regulate the timing and amount of enzyme catalysts produced. Many of these genetic controls are, in turn, regulated by cascades of metabolic effects that respond to ontogenetic or environmental cues (Keeling and Bohlmann 2006; Li and Sharkey 2013).

Taken together, these controls must be responsive to short-term (seconds-to-hours) and long-term (hours-to-weeks) changes in the environment, and they must reflect an interface between the perception of changing environmental cues and adjustments in the activity and expression of metabolic potential. In this chapter, I will focus on the mechanisms by which BVOC production and emission is controlled in short- and long-term scenarios at the cellular, leaf and plant scales. Most of the focus of my discussion will be on the production and emission of isoprenoid BVOCs, because most past research has focused on these compounds and the pathway controls are known to the greatest extent. I will also take up the issue of controls over the production and emission of methanol, acetaldehyde and other oxygenated BVOCs. I will focus on research that has concerned BVOC emissions from trees and forests, as once again this has been the subject of most past research.

6.2 The Short- and Long-Term Controls over BVOC Emissions with Emphasis on Isoprene

Conventionally, the emission of BVOCs has been defined according to the metabolic potential available for compound biosynthesis that can be influenced by instantaneous changes in the environment (the instantaneous emission rate) and the overall capacity for compound biosynthesis that is independent of instantaneous changes in the environment (the basal emission rate, or emission factor). This type of classification was historically developed for the case of isoprenoid BVOCs (Guenther et al. 1991, 1993). Partitioning of the BVOC emission rate into these two levels of control has not only served our conceptual understanding of cellular processes (the instantaneous rate is principally controlled by the availability of substrate or kinetic constraints of rate-limiting enzymes and the basal rate is principally controlled by gene expression and limiting enzyme activity), but it also provided a convenient structure by which to design BVOC emission models (Monson et al. 2007, 2012 for reviews).

Early in the writings on isoprenoid emissions the concepts of 'instantaneous and basal control' were equated to 'short-term and long-term' control, respectively (e.g., Monson et al. 1995). This conflation of 'types of control' with 'timing of control' was, in hindsight, unfortunate. We now know that changes in gene expression can under exceptional circumstances have an influence on BVOC emission rates on the timescale of hours (Wilkinson et al. 2006; Loivamäki et al. 2007; Wiberley et al. 2009) (traditionally considered 'long-term control'), i.e., almost the same timescales by which substrate limitations can develop (Magel et al. 2006; Li et al. 2011; Li and Sharkey 2013) (traditionally considered 'short-term control'). Thus, the traditional metrics used to differentially classify these responses are not reliable. While some responses, such as the light- and temperature dependence of isoprene emissions can clearly be differentiated into instantaneous (short-term) and basal (long-term) processes, classification along these lines has become more tenable than previously thought. In recognition of these difficulties, in this chapter, I will refer to ***metabolic responses*** as those that occur through dynamics to metabolite fluxes and pathway interactions (what might previously have been referred to as 'short-term responses') and I will refer to ***gene-expression responses*** as those reflected by a change in gene product levels (what might previously have been referred to as 'long-term responses') (Fig. 6.1).

Several examples serve to illustrate these distinctions further. The responses of isoprene emission rate to temperature (Magel et al. 2006; Rasulov et al. 2010), incident PPFD (Rasulov et al. 2009a) and changes in CO_2 concentration (Rosenstiel et al. 2003; Rasulov et al. 2009b; Trowbridge et al. 2012; Calfapietra et al. 2013) are due to dynamics in substrate concentration and perhaps the kinetic properties of the isoprene synthase enzyme. Similarly, studies that have transferred the isoprene synthase gene into otherwise non-emitting species have shown that the instantaneous emission rate is limited by the existing capacity to produce the immediate isoprene synthase substrate dimethylallyl diphosphate (DMADP) within

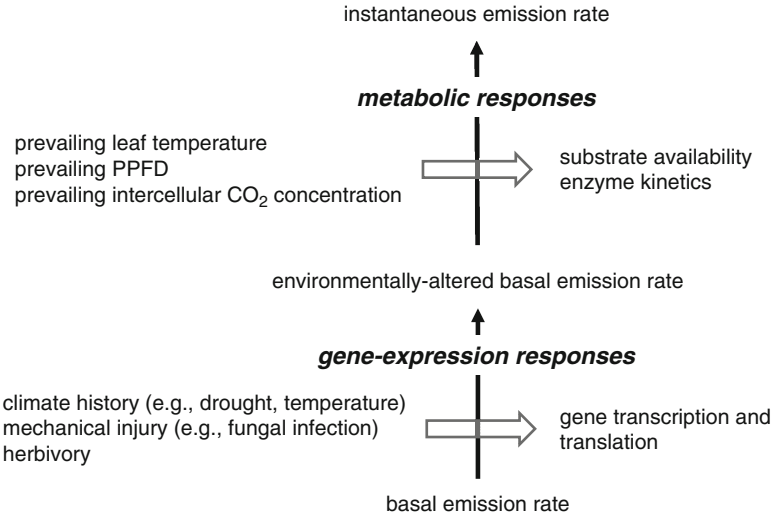

Fig. 6.1 A general scheme showing how gene-expression responses and metabolic responses combine to produce an instantaneous BVOC emission rate that is dependent on both longer-term and shorter-term cues and influences of the environment

the target plant (Sharkey et al. 2005; Vickers et al. 2011). In other words, transfer of the enzyme gene is not accompanied by an upregulation of the pathway that produces the substrate, and the resulting substrate limitation is a simple result of the transferred enzyme activity being greater than the existing potential for substrate production. All of these examples can be classified as ***control due to metabolic response***.

In contrast, ***control by gene expression response*** infers that past natural selection has favored control over the activity of specific genes in a way that causes a change in the metabolic potential to synthesize and emit compounds. For example, in the case of terpenes that deter herbivory or infection, biosynthesis potential may need to be upregulated in coordination with specific phenological phases of the plant (e.g., Huang et al. 2012) or after being induced by episodes of injury (Schiebe et al. 2012). These types of control often involve complex signalling pathways in the plant, leading to specific patterns of gene expression. These are all examples of what I classify as control by gene expression. Specific cases of this type of control have been described in the initiation of isoprenoid emission capacity during leaf maturation (Wiberley et al. 2005), shifts in emission capacity during acclimation to past temperature regimes (Cinege et al. 2009; Wiberley et al. 2009), modifications in emissions following exposure to ozone (Calfapietra et al. 2007; Fares et al. 2010; Loreto et al. 2004), increases in emissions in response to transitions from lower versus higher growth light regimes (Cinege et al. 2009).

6.2.1 Pathways and Controls for Substrate Channeling to Terpenoid BVOCs

Prior to 1997, a general consensus existed in the BVOC research community that carbohydrates produced through recent photosynthesis were channeled to the production of dimethylallyl diphosphate (DMADP) and its isomer, isopentenyl diphosphate (IDP) through the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase pathway, or mevalonic acid (MVA) pathway (also called the Bloch-Lynen pathway in the animal metabolism literature) in the cytosol of cells (Gray 1987 for a review). The incorporation of ^{13}C-labeled glucose into IDP was clearly observed in studies of cell-free extracts in mammals, fungi, bacteria and plants (Spurgeon and Porter 1981 for a review). In the MVA pathway, three molecules of acetyl-CoA are condensed to HMG-CoA, which is then chemically reduced to the six-carbon compound, mevalonic acid. Mevalonic acid, in turn, is converted to IDP with an energetic cost of 3 ATP molecules and loss of one CO_2 molecule.

In the mid-1990s, however, tracer studies in bacteria and algae revealed that not all taxa incorporated ^{14}C label into IDP in exactly the same way (Horbach et al. 1993; Rohmer et al. 1996; Schwender et al. 1996). Furthermore, it was difficult to reconcile observations that $^{13}CO_2$ that was fed to leaves was observed in some emitted BVOCs, like isoprene, over the timescale of seconds, yet metabolite pools in the MVA pathway turned over relatively slowly, on the timescale of minutes (Delwiche and Sharkey 1993). In early 1997, Lichtenthaler et al. proposed that two distinct pathways exist in plants for the synthesis of DMADP and IDP, with one being the traditional MVA pathway in the cytosol and one being a novel pathway, which is now recognized as the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP) pathway in the plastids (Li and Sharkey 2013; Rosenkranz and Schnitzler 2013). Studies using deuterium-labeled intermediates verified that isoprene is produced in the chloroplast with substrate provided by this novel pathway (Zeidler et al. 1997). Thus, within 3–4 years, a rapid series of discoveries allowed us to better understand how recent photosynthate is tightly coupled in time with the production of at least some terpenoid BVOCs, and to identify the types of controls that regulate carbon channeling during BVOC production (Fig. 6.2).

Discovery of the new, pathway, however, did not explain the origin of all BVOCs, as some, such as the C15 sesquiterpenes, could only be produced by terpene synthase enzymes localized in the cytosol; at the time of discovery of the MEP pathway there did not appear to be a mechanism for exporting IDP from the chloroplast (Gershenzon and Kreis 1999). Thus, sesquiterpene biosynthesis appears to be produced principally by substrate provided by the MVA pathway. We now recognize that there is a significant amount of metabolite exchange between both pathways, and that compensatory control mechanisms exist to balance the activities of the two pathways (Dudareva et al. 2005; Nagegowda 2010; Hemmerlin et al. 2012). Furthermore, distinct cytosolic and plastidial sesquiterpene synthases have been demonstrated (Nagegowda 2010; Rajabi Memari et al. 2013).

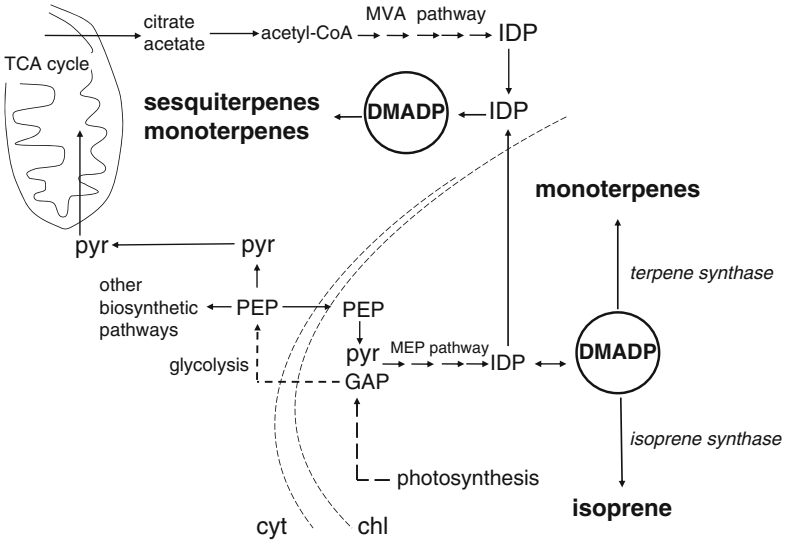

Fig. 6.2 The relationships between the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway) and mevalonate (MVA) pathway in synthesizing isoprene and monoterpenes in the chloroplast and sesquiterpenes and monoterpenes in the cytosol. The potential for 'cross talk' between the pathways occurs through exchange of the common metabolite isopentenyl diphosphate (IDP) between chloroplasts (chl) and the cytosol (cyt)

It is worth noting at this point that there is strong evidence, beyond tracer labelling studies, that isoprene is produced solely in the MEP pathway. A cytosolic form of isoprene synthase (IspS), the enzyme that catalyzes isoprene formation from DMADP, has not been discovered; leaving its possible production from MVA pathway metabolites undefined. Using immunogold-labelling with polyclonal antibodies generated against recombinant isoprene synthase protein, Schnitzler et al. (2005) showed that in poplar leaves this enzyme is located in the chloroplast stroma, with some fraction of the protein attached to the outside (stromal) surface of thylakoid membranes; this is consistent with previous studies showing both soluble and membrane-bound fractions of isoprene synthase in willow leaf extracts (Wildermuth and Fall 1998). Furthermore, in transgenic tobacco (*Nicotiana tabacum*) which has been engineered to express isoprene synthase in the cytosol, no significant amounts of isoprene are emitted (Vickers et al. 2011).

6.2.2 Cross-Talk Between the Two Pathways for Isoprenoid Biosynthesis in Plants

Plants are different than most organisms in that they utilise both the MEP and MVA pathways, in parallel, for the biosynthesis of isoprenoid molecules. Most Eubacteria

and Cyanobacteria utilise only the MEP pathway, whereas Archaea, and some other Eubacteria such as staphylococci, streptococci and enterococci belonging to Firmicutes utilise only the MVA pathway (Proteau 1998; Smit and Mushegian 2000; Trutko et al. 2004). *Streptomyces* spp. (Actinobacteria) appear to have operon genes for both pathways, and species in this group express both sets of genes, but in serial manner at different stages of growth (Seto et al. 1996); not simultaneously as in plants. Thus, one of the principal challenges faced by plant biologists has been to discover the means of proper coordination of activities in the two pathways.

Until the past decade or so, it was assumed that compartmentalization of isoprenoid biosynthesis was determined by the differential localization of terpene synthase enzymes with hemiterpene (isoprene), monoterpene and diterpene synthases occurring in the plastid, and sesquiterpene synthases occurring in the cytosol. There was little interaction suspected for the exchange of substrate between plastids and the cytosol. However, experiments in which deuterium-labeled 1-deoxy D-xylulose (an intermediate metabolite of the MEP pathway) or fosmidomycin (an inhibitor of the MEP pathway) were fed to cut snapdragon flowers, showed that the MEP pathway provides substrate in the form of IDP to both the plastidic and cytosolic synthesis of some BVOCs (Dudareva et al. 2005). The use of labeled metabolites allowed Dudareva et al. (2005) to distinguish a one-way transport of IDP from the plastid to the cytosol. The production of IDP to support BVOC emissions in both pathways may be under circadian control. A circadian dependency of MEP pathway activity has been demonstrated for the incorporation of labeled intermediates into monoterpenes and sesquiterpenes in flowers (Dudareva et al. 2005). Also some circadian effects were observed in the emission of isoprene from oil palm (*Elaeis guineensis*) leaves (Wilkinson et al. 2006) and in expression of isoprene synthase gene in hybrid poplar leaves (Loivamäki et al. 2007). Thus, a picture is emerging in which terpenoid BVOC emissions are synchronized by circadian rhythms to photosynthetic activity by upregulating both the pathway to produce substrate and the enzymes to produce a volatilized product. In a separate set of studies, a combination of pathway inhibitors was used to identify *Arabidopsis* mutants that could upregulate MVA pathway activity and survive in the presence of fosmidomycin (i.e., in the absence of MEP pathway activity) (Rodríguez-Concepción et al. 2004). One mutant was identified that appeared capable of importing substrate (presumably in the form of IDP) from the cytosol into the chloroplast for the production of carotenoids, chlorophylls and other critical primary components of the photosynthetic system. Thus, there is now good evidence that, while plants rely on two separate pathways for the production of BVOCs, they also synchronize activities of the pathways, depending on their differential capacities to produce IDP (Bick and Lange 2003). It has been argued that this type of interactive control provides advantages to plants in being able to maintain homeostasis in the face of different cellular demands on photosynthate and the isoprenoid precursors derived from photosynthate (Hemmerlin et al. 2012).

Cross-talk between the MEP and MVA pathways may be part of a general metabolic control that ensures the flow of substrate to pathways in the face of variable photosynthate production. For example, Rontein et al. (2002) observed

that in cultured tomato (*Solanum lycopersicum*) cells grown with a finite supply of glucose, glycolytic flux remained approximately constant as the available glucose was depleted from 70 to 40 % of the supply. This stability in glycolytic flux was not achieved by metabolic control within glycolysis, but rather by adjustments to intersecting pathways that utilise glycolytic intermediates. Thus, under conditions of replete carbohydrate, when glycolytic substrates were readily available, intersecting pathways were up-regulated to pull intermediate metabolites out of the central trunk of glycolysis, and increase the potential to synthesize secondary products. Conversely, as glucose supply decreased, intersecting pathways were down-regulated to preserve higher fluxes of intermediate metabolites through the main trunk of the pathway. This concept of 'network rigidity' has been proposed as a central tenet of metabolic adaptation (Stephanopoulos and Vallino 1991). In the case of glycolysis, a principal adjustment appears to occur in regulation of the availability of phosphoenol pyruvate (PEP), a glycolytic metabolite that also serves as the substrate for amino acid biosynthesis and for anapleurotic compensation of substrates in the tricarboxylic acid cycle. PEP is also a possible way through which cytosolic metabolites can enter chloroplastic isoprenoid synthesis pathway. Once transported to chloroplast, PEP can be converted to pyruvate, and thus enter the production of BVOCs from the MEP pathway. Within the context of these relations, it is reasonable to propose that photosynthetic activity and the availability of sugars can be a control point around which the flow of pyruvate to the MEP pathway is regulated.

6.2.3 Feedback Homeostasis in MEP Pathway Activity

6.2.3.1 Isoprene Synthase or Substrate Limitation?

One of the fundamental issues in need of clear understanding is the degree to which the rate of biosynthesis of isoprenoid BVOCs is controlled by the activity of terpene synthase enzymes or the availability of the two principal MEP pathway substrates – glyceraldehyde 3-phosphate (GAP) and pyruvate (Pyr). In a study of isoprene emission from kudzu (*Pueraria lobata*) leaves, Wolfertz et al. (2003) observed an inverse correlation between the isoprene emission rate and chloroplast DMADP concentration among 15 different leaves. This evidence was used to conclude that substrate concentration does not control the instantaneous isoprene emission rate in kudzu, but rather it is controlled by isoprene synthase activity. This conclusion is based on the reasoning that if DMADP limited isoprene emission rate, the leaves with the lowest emission rates should have also exhibited the lowest DMADP concentrations. The analysis provides a strong line of evidence for primacy in the role of isoprene synthase activity in controlling the isoprene emission rate. However, there is potential for weakness in certain conclusions derived from the analysis. The chloroplast DMADP concentrations used in the analysis were determined on a sample of all five leaves with the lowest versus highest emission rates. Thus,

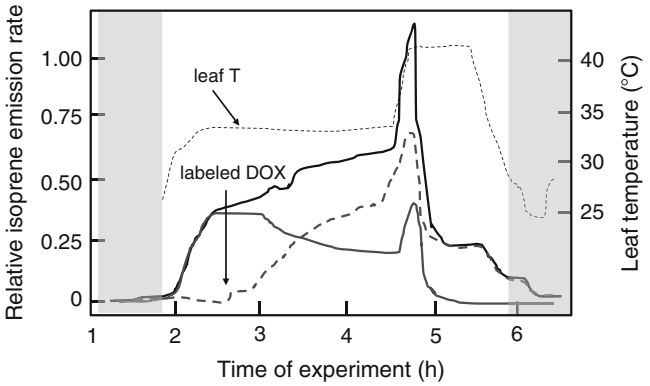

Fig. 6.3 Time-dependent labelling of emitted isoprene by di-deuterated deoxyxyulose (DOX), the first product formed in the MEP pathway. The *thin dashed line* shows an experimental progression of leaf temperature through a stepwise treatment. The *solid black line* is the total isoprene emission rate. The *solid grey line* is unlabeled emitted isoprene, and the *dashed grey line* is deuterium labeled emitted isoprene. The results show a compensatory tradeoff between labeled and unlabeled isoprene as a function of time since the application of the DOX label, suggesting feedback regulation of the channeling of DOX into isoprene in the MEP pathway. The *grey*-shaded boxes show periods of darkness before and after the experiment (Modified from Wolfertz et al. 2003)

it is not possible to assess whether the observed correlation was well distributed across the 15 leaf samples – outlier values for a more limited number of leaves may have over-influenced the correlation. There are also concerns about the accuracy of separating the cytosolic and chloroplastic DMADP pools in these types of analyses; the cytosolic pool of DMADP vastly exceeds the chloroplastic pool (Rasulov et al. 2009a), making accurate estimation of the latter difficult.

In an effort to better distinguish the relative levels of control by substrate availability versus enzyme activity, Wolfertz et al. (2003, 2004) fed leaves exogenous di-deuterated deoxyxylulose (DOX), the first metabolite produced in the MEP pathway (Fig. 6.3). Through this approach, these researchers tested the hypothesis that by artificially increasing the flow of carbon to DMADP, isoprene emission rate would be increased; this would support a role for substrate limitation. While it was shown that the labeled exogenous DOX replaced unlabeled endogenous DOX in the emitted isoprene, the overall rate of isoprene emission did not increase. Furthermore, at high levels of exogenous DOX supply, the incorporation of endogenous DOX into isoprene decreased to zero. The authors interpreted these results as indicating that DMADP supply is controlled by end-product (DMADP) feedback. Once again, these studies supported the lack of a role for substrate limitation in determining the isoprene emission rate. A similar result that is consistent with end-product inhibition was obtained by Ghirardo et al. (2010) using RNA interference (RNAi) techniques to knock out production of the isoprene synthase enzyme in selected poplar lines. These researchers observed reduced activities of deoxyxylulose phosphate synthase (DXS), the first enzyme in the MEP pathway, in RNAi trees, compared to wild type

trees. However, they did not measure leaf DMADP levels to validate a correlation between increases in end-product concentration and inhibition of DXS. In a separate study, Behnke et al. (2010) used the same RNAi lines of poplar and observed increases in leaf DMADP through the growing season in RNAi trees, compared to wild type trees. This study provides indirect evidence that the end-product inhibition hypothesized by Ghirardo et al. (2010) does indeed exist in the RNAi lines. In the Behnke et al. (2010) study there was no observed downregulation of genes in the initial steps of the MEP pathway. Thus, any end-product inhibition that might exist is likely due to post-translational allosteric modification of DXS.

6.2.3.2 Limitations at Different Temperatures

Returning to the experiments of Wolfertz et al. (2003), the addition of exogenous, labeled DOX did not result in significantly higher rates of labeled isoprene emission at moderate leaf temperatures (30 °C), but it significantly increased both the rate of isoprene emission and fraction of isoprene labelling at higher leaf temperatures (45 °C). These results led the authors to conclude that while substrate limitations may not be significant at moderate leaf temperatures, they are significant at higher leaf temperatures. Temperature dependence in the control over isoprene emission by substrate availability versus the kinetic constraints on isoprene synthase, as revealed by Wolfertz et al. (2003), may explain the results of Fortunati et al. (2008). In the latter study, poplar (*Populus alba*) trees which were exposed to extreme drought stress and then allowed to recover, lacked a significant response in isoprene emission rate when measured at 35 °C, compared to 25 °C. The kinetic response of isoprene synthase in recovered leaves should have produced significantly higher post-recovery emission rates; this result was not observed. Consistent with the results of Wolfertz et al. (2003), it is possible that limited substrate availability prevented isoprene synthase from reaching its potential catalytic activity at the higher leaf temperature (35 °C). Feedback control over the MEP pathway is shown within the context of the metabolic and gene expression controls over isoprene emission rate in Fig. 6.4.

A similar conclusion as to the role of differential temperature-dependent constraints on isoprene synthase activity has been reached in studies by Rasulov et al. (2010). Using a post-illumination burst of isoprene emission to model and quantify the chloroplast DMADP concentration, these researchers demonstrated that at temperatures up to 30 °C, the temperature response of isoprene emission rate was not limited by DMADP substrate availability, but rather by kinetic constraints on isoprene synthase activity. At temperatures greater than 30 °C, however, isoprene emission rate was influenced by both substrate availability and isoprene synthase activity. In other studies by Magel et al. (2006) and Li et al. (2011), DMADP was also observed to limit the temperature dependence of isoprene emission rate, but in these cases, only at temperatures above 35 °C and only after one hour.

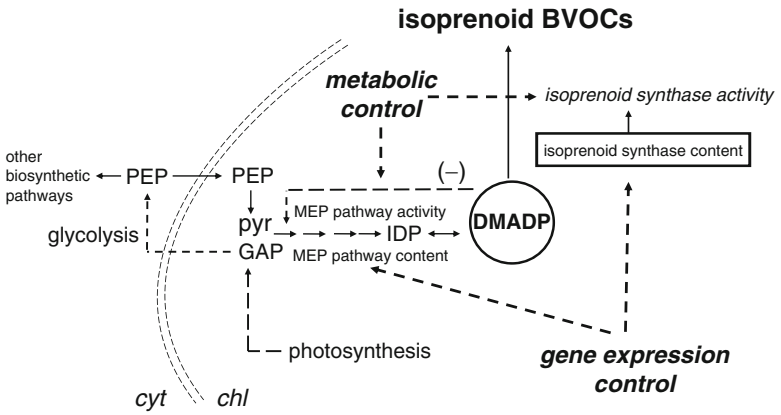

Fig. 6.4 Scheme showing end-product (DMADP) control over the flux of substrate through the MEP pathway within the context of gene-expression and metabolic controls over the isoprene emission rate from chloroplasts (*chl*) and interactions with processes in the cytosol (*cyt*)

Consistent with all of these past observations, studies using $^{13}CO_2$ as a tracer of recently-assimilated carbon in isoprene-emitting leaves have shown that while leaves at all temperatures rely to some extent on older, stored carbon for substrate, this reliance increases at higher leaf temperatures (Funk et al. 2004). In these conditions, the rate of DMADP depletion by isoprene synthase activity may exceed the rate by which photosynthesis can provide substrate for DMADP repletion. Thus, substrate limitations to isoprene emission rate appear to increase when CO_2 assimilation rate is also limited. Under these conditions, internal mobilization of stored carbon (or injection of exogenously added carbon) compensates for the substrate limitations.

The conclusion that the kinetic constraints on isoprene synthase limit the overall emission rate, except at higher temperatures is generally dependent on the balance between isoprene synthase activity and photosynthesis rate. When the catalytic capacity of isoprene synthase is high and the capacity for GAP production through photosynthesis is low (e.g., at high leaf temperatures or during drought), and in particular under low light (Rasulov et al. 2009b), then the availability of DMADP will have a greater role in limiting the emission rate. Similarly, when the capacity for GAP production is high and the catalytic capacity of isoprene synthase is low (either because of kinetic constraints or because of low isoprene synthase protein concentrations), then isoprene synthase activity will have a greater role in limiting the overall emission rate. This latter condition explains the inverse correlation between isoprene emission rate and DMADP concentration observed in the study of Wolfertz et al. (2003) that were discussed above.

6.2.3.3 Limitations at Different CO_2 Concentrations

These same relations cannot explain the potential role of DMADP substrate limitations when leaves are exposed to varying intercellular CO_2 concentrations (Sanadze 1964; Monson and Fall 1989; Loreto and Sharkey 1990; Rosenstiel et al. 2003; Centritto et al. 2004; Rapparini et al. 2004; Scholefield et al. 2004; Pegoraro et al. 2005; Wilkinson et al. 2009; Possell et al. 2005; Possell and Hewitt 2011). In that case, increases in intercellular CO_2 concentration, even at temperatures below 35 °C, cause in increase in the capacity to produce GAP through greater photosynthesis rates, but a decrease in the isoprene emission rate. There is no evidence to date of a CO_2-dependent, direct effect on isoprene synthase kinetics. However, leaf DMADP concentrations have been shown to decrease as intercellular CO_2 concentration increases (Rosenstiel et al. 2003; Rasulov et al. 2009b; Sun et al. 2012). Thus, DMADP substrate availability appears to be the primary cause of the CO_2-dependency of isoprene emission rate, despite a high ratio in the capacity to produce GAP substrate relative to the activity of isoprene synthase. In this case, the substrate limitation appears to be due to either the rate at which pyruvate is supplied to the chloroplast (Rosenstiel et al. 2003) or the rate at which GAP is converted to DMADP in the chloroplast (Rasulov et al. 2009b). In the past (Monson et al. 2012), I have favored the hypothesis offered by Rosenstiel et al. (2003) because of results obtained by Trowbridge et al. (2012). In the latter study, proton-transfer reaction mass spectrometry was used to detect the differential kinetics of ^{13}C incorporation into fragments of isoprene presumed to come from cytosolic versus chloroplastic sources (Fig. 6.5). The results during periods of low versus high intercellular CO_2 concentration suggested slower labelling in the fragment purported to come from cytosolic sources (which provides pyruvate to the chloroplast), and this fragment was more highly labeled in the presence of low CO_2, compared to that derived from GAP directly (which would not be expected if chloroplastic ATP limited the overall emission rate). These latter results can be interpreted as supporting the Rosenstiel et al. (2003) perspective more than the Rasulov et al. (2009b) perspective.

6.3 Short- and Long-Term Controls Over Emissions of Other BVOCs

6.3.1 Monoterpenes

Monoterpene emissions from leaves and needles are subject to both metabolic and gene expression controls. In coniferous species, monoterpenes tend to be stored as a component of oleoresin, a viscous solution of terpenes, acids and phenolic compounds that can be stored in individual cells, ‘blister-like’ structures, or continuous duct systems (Lewinsohn et al. 1991). In this chapter, I will focus on terpene emissions from resin ducts, which are most iconically represented in the genus *Pinus*. The terpene composition of oleoresin is largely determined

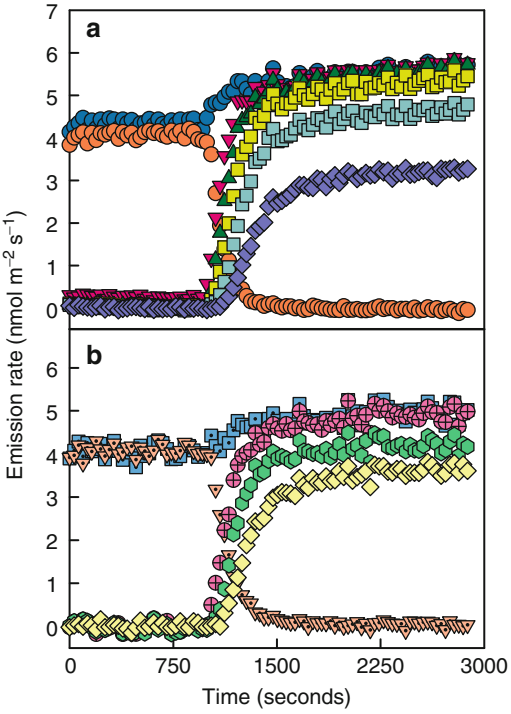

Fig. 6.5 Labelling of carbon atoms using assimilated $^{13}CO_2$ in mass 69^+ ($M69^+$) and mass 41^+ ($M41^+$) and their isotopomers through time. (**a**) $^{13}CO_2$-labelling of carbon atoms in trees grown and measured in ambient CO_2 conditions (400 μmol mol^{-1} CO_2) in the parent isoprene molecule, as characterized by a decrease in the $M69^+$ signal (*orange circles*) and simultaneous increase in its isotopomers (denoted as *sums*) as labeled carbons were successively incorporated through time. Total emission (*blue circles*), $M70^+$ (*red downward triangles*), $M71^+$ (*green triangles*), $M72^+$ (*yellow squares*), $M73^+$ (*sea green squares*), $M74^+$ (*purple diamonds*) are represented. (**b**) $^{13}CO_2$-labelling of carbon atoms in trees grown and measured at 30 °C in ambient CO_2 conditions (400 μmol mol^{-1} CO_2) in the 3-C-methyl-vinyl isoprene fragment, characterized by a decrease in the $M41^+$ signal (*light orange dotted downward triangles*) with a simultaneous increase in its labeled isotopomers (denoted as *sums*). Total emission (*blue dotted squares*), $M42^+$ (*pink crossed circles*), $M43^+$ (*green hexagons*), $M44^+$ (*yellow diamonds*) are represented. Before leaves were exposed to $^{13}CO_2$-labelling at 1,000 s, plants were exposed to the same $^{12}CO_2$ concentrations at which they were grown. The simultaneous labelling of the first carbon in the parent molecule ($M70^+$) and the fragment ($M42^+$) suggest that the first carbon contributing to the synthesis of isoprene comes from the $M41^+$ fragment. However, while all of the isoprene molecules show the next two carbons labeled shortly after ($M71^+$ and $M72^+$), the next two carbons on the $M41^+$ fragment ($M43^+$ and $M44^+$) are never fully labeled and may result from the incomplete labelling of pyruvate

by genetic control; however, environmental variation can also induce chemical variation (Nerg et al. 1994; Keeling and Bohlmann 2006; Nikolic et al. 2008). The actual biosynthesis of the terpene components of oleoresin occurs in epithelial cells that line the walls of the resin ducts. Monoterpenes can leave the liquid phase of

the oleoresin and diffuse in the vapour phase through the internal tissues of the needles to stomatal pores, where they can be emitted to the atmosphere. Metabolic controls are not significant in determining the rate of volatile terpene emission from conifer needles, although physiological controls over diffusive resistance, including stomatal resistance, and environmental controls such as temperature, can impose significant control (Lerdau 1991; Lerdau and Gray 2003). Interactions among needle temperature, volatility (the Henry's law constants and octanol/water partition coefficients) of the terpenes composing the oleoresin and diffusive resistance represent what have traditionally been referred to as 'short-term' controls in pine needle emission rates (Lerdau et al. 1994).

Needle herbivory imposes both physiological (diffusive) and genetic controls on the monoterpene emission rate. In terms of the shorter-term, physiological control, herbivory has the potential to make new breaks in the diffusive barriers to terpene evaporation to the atmosphere (Litvak and Monson 1998; Loreto et al. 2000), thus causing an immediate increase in the needle emission rate. In terms of genetic control, herbivory causes an increase in the expression of genes that encode terpene synthase enzymes in both needles (Litvak and Monson 1998; Martin et al. 2003) and stems (Schmidt et al. 2011). These genes are often induced by complex signalling webs involving plant hormones, such as jasmonic acid (van Poecke and Dicke 2004). In many species, mechanical injury by herbivory or fungal infection can induce the formation of additional 'traumatic' resin ducts in wood, a process that potentially contributes to increased terpene emission (Christiansen et al. 1999; Martin et al. 2002), though the emissions from stems and branches to the atmosphere have been poorly characterized. Many pine species also exude droplets of oleoresin at the juncture of needle follicles and cone bracts during the spring, when resin pressures are high. The exposure of oleoresin directly to atmosphere through exuded droplets has the potential to significantly affect canopy emission rates (Eller et al. 2013).

In the past, it was assumed that most monoterpene emissions from pine needles were from stored oleoresin pools. However, it has been shown using $^{13}CO_2$-labelling that in some species, such as *Pinus sylvestris* (Ghirardo et al. 2010; Shao et al. 2001) and *Pinus pinea* (Noe et al. 2006), 30–90 % of the observed monoterpene emissions were derived from recently assimilated CO_2. These emissions are light-dependent (Staudt et al. 1997; Niinemets et al. 2002), similar to light-dependent monoterpene emissions observed in some broad-leaved species (Lerdau and Gray 2003 for a review), and result from the channeling of carbon substrate through the MEP pathway. For this type of monoterpene emissions, the same metabolic and genetic controls that were discussed above for isoprene emissions are relevant (Owen et al. 2002).

6.3.2 Methanol

Leaf methanol emission is catalyzed by the enzyme pectin methylesterase (PME) which is most active in the cell wall domain of plant cells (Micheli 2001; Pelloux

et al. 2007). PMEs catalyze the demethylation and associated esterification of homogalacturonic acids (HGA), which are used to compose the polysaccharide matrix of the primary plant cell wall. The products of de-methylation include methanol and protons. The HGA substrate for PMEs is secreted into the cell wall domain and constitutes the pectin fraction of the cell walls. Short-term control over the rate of methanol emission is determined by the availability of HGA substrate (Oikawa et al. 2007) and interactions between the solubility of methanol in the aqueous phase of the leaf and stomatal diffusion resistances to leaf-atmosphere exchange (Niinemets and Reichstein 2003; Harley et al. 2007; Harley 2013). There is evidence for light-dependency of methanol emissions, but this appears to be due to the response of stomatal resistance to the incident photosynthetic photon flux density (PPFD), rather than a direct effect of PPFD on substrate availability (Oikawa et al. 2007; Harley et al. 2007). Several studies have shown light-dependency in the acidification of the primary cell walls of leaves, which in turn enhances leaf expansion rate through a variety of mechanisms (van Volkenburgh 1999). Acidification can occur in as quickly as in a few minutes following an increase in PPFD (Elzenga et al. 2000). The activity of PMEs in producing methanol is highly sensitive to cell wall pH (Catoire et al. 1998). Thus, it is possible that changes in PPFD could elicit changes in the rate of export of HGA from cells and/or changes in the activity of PMEs, and thus elicit changes in methanol emission; however, this response has not been detectable in recent experiments (Oikawa et al. 2007). Furthermore, nighttime acidification can occur in some species, and thus, a significant fraction of diurnal methanol emissions can be stored internally at night and released the following morning when stomata open (Hüve et al. 2007). Thus, for the present time, it appears that changes in stomatal resistance are the principal causes for light-dependency in the methanol emission rate, and even that level of control is due to slow equilibration between aqueous- and gas-phase methanol following a change in stomatal resistance (Harley 2013 in this volume).

Long-term controls over methanol emissions from leaves have not been explicitly studied. However, given the evidence to date that methanol emission is the result of PME activity and HGA availability, it is reasonable to propose that methanol emission capacity is controlled by levels of expression in the genes encoding the production of PMEs and the enzymes controlling the biosynthesis and/or export of HGA. Secretion of HGA is most active during leaf expansion (Pelloux et al. 2007). Thus, methanol emissions are the highest in maturing leaves (Nemecek-Marshall et al. 1995). Damage to leaves by feeding caterpillars increases the rate of methanol emission, and this response can be replicated by the treatment of cut leaves with oral secretions from the caterpillars (von Dahl et al. 2006). It was discovered that the oral secretions elicit a change in pH in the cell wall domain of damaged tissues, which in turn caused an increase in the transcript levels for specific PMEs. The possibility has been raised that methanol acts as an ecological signalling molecule that affects plant-to-plant communication (Dorokhov et al. 2012).

6.3.3 Acetaldehyde and the 'Green Leaf Volatiles'

The biosynthesis of a variety of oxygenated BVOCs in leaves has been linked to both metabolic imbalances in the flow of carbon between glycolysis and mitochondrial respiration, and wounding, which in turn induces the oxidation of fatty acids (Loreto and Schnitzler 2010). One of the most frequently identified oxygenated compounds emitted from leaves is acetaldehyde. Historically, studies on acetaldehyde emission have focused on anoxia in the root zone (Kimmerer and Macdonald 1987; Kreuzwieser and Rennenberg 2013). Under anoxia, aerobic respiration is inhibited, and the pyruvate formed through glycolysis is oxidized to ethanol plus CO_2 in a two-step type of 'fermentation' catalyzed in the first reaction by pyruvate decarboxylase (PDC) to form acetaldehyde plus CO_2, and catalyzed in the second reaction by alcohol dehydrogenase (ADH) to form ethanol. The ethanol is then transported through the plant xylem to the leaves, where it is oxidized in a two-step process, first to acetaldehyde in a reaction catalyzed by ADH (the reverse of the reaction in the root that produced the ethanol), then to acetate in a reaction catalyzed by aldehyde dehydrogenase (ALDH) (Kreuzwieser et al. 1999). It is presumed that relatively volatile acetaldehyde leaks from the leaf pool, between the steps of ADH and ALDH. In support of this hypothesis, Graus et al. (2004) fed disulfiram, an inhibitor of ALDH to leaves, and observed a marked increase in acetaldehyde emissions.

In a second process, it has been shown that acetaldehyde is emitted as emission bursts following rapid light–dark transitions in leaves, and this process is independent of the oxygen status of roots (Karl et al. 2002; Graus et al. 2004; Brilli et al. 2011; Jardine et al. 2012). Leaf mesophyll and vein tissues have constitutive activities of pyruvate decarboxylase (Kimmerer and MacDonald 1987; Nguyen et al. 2009), so it is feasible that acetaldehyde is formed directly from pyruvate. Karl et al. (2002) demonstrated that the observed bursts of acetaldehyde were not accompanied by ethanol emissions, suggesting it as a product of PDC acting alone or in sequence with ALDH (producing acetate as a final product). Tracer studies showed that recently assimilated $^{13}CO_2$ in leaves was transferred into emitted aldehyde, suggesting a process confined to the leaves (also see Jardine et al. 2012). Karl et al. (2002) proposed that the oxidation of pyruvate to acetaldehyde might function as an 'overflow' mechanism, allowing the cell to balance the production of pyruvate by glycolysis against the demands imposed by mitochondrial respiration. This balance may be necessary because of the highly regulated nature of pyruvate dehydrogenase (PDH), the 'bridge' between glycolytic production of pyruvate and the production of acetyl-CoA, which enters mitochondrial respiration. According to this hypothesis, during a rapid light-to-dark transition, leaf pyruvate concentrations increase due to adjustments in the partitioning of glycolytic products and intermediates to several pathways. The buildup of pyruvate presumably triggers an increase in the activity of PDC, producing a burst of acetaldehyde synthesis (Fig. 6.6).

This hypothesis was tested by Graus et al. (2004) in an experiment in which a combination of acetylphosphinate, an inhibitor of PDH, and disulfiram, an inhibitor

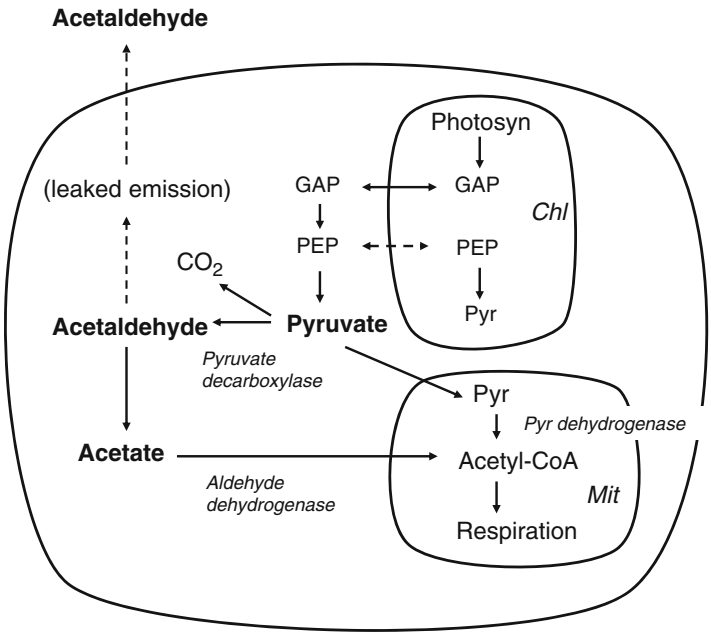

Fig. 6.6 A scheme showing the possible origin of emitted acetaldehyde as an 'overflow' from excess pyruvate that accumulates due to imbalances between its production in glycolysis and consumption in the mitochondrial tricarboxylic acid (TCA) cycle. *Chl* stands for chloroplast and *Mit* for mitochondrion

of ALDH, was fed to leaves of poplar (*P. x canescens*). Together, these inhibitors should lead to reduced consumption of pyruvate by PDH (i.e., reduced production of acetyl-CoA) and reduced consumption of acetaldehyde by ALDH, resulting in the channeling of even more pyruvate than normal to an enlarged acetaldehyde pool. In fact, this combination did indeed lead to an increase in acetaldehyde emissions from leaves. However, when they put leaves treated with disulfiram through a light–dark transition, they did not observe an increase in emissions relative to control leaves; in fact, they observed a large decrease (Graus et al. 2004). From these results, it was concluded that indeed elevated acetaldehyde pools in the leaf lead to increased acetaldehyde emissions from the leaf, but that this is not the cause of the previously observed light-to-dark bursts of emissions.

Instead, it was suggested that the acetaldehyde bursts observed during light-to-dark transitions could be linked to the wound-related oxidation of fatty acids, a process that releases so-called 'green leaf volatiles' (Graus et al. 2004). In fact, there is evidence that some of the wound-induced 'green-leaf' volatiles (GLVs), such as hexenyl acetate, are derived from pyruvate (Jardine et al. 2009; Jardine et al. 2012) and acetate units released from the breakdown of membrane fatty acids following wounding (Cojocariu et al. 2005; Loreto et al. 2006). The production of green leaf volatiles requires oxygen in a process catalyzed by lipoxygenase enzymes. In past

studies, the emissions of GLVs from leaves have been attributed to various biotic and abiotic stresses including ozone exposure (Heiden et al. 2003; Beauchamp et al. 2005), freeze-thaw episodes (Fall et al. 2001; Copolovici et al. 2012), high light and temperature extremes, as well as mechanical wounding (Loreto et al. 2006), and programmed cell death during senescence (Holopainen and Gershenzon 2010). Returning to the case of Graus et al. (2004) in explaining an alternative source of emitted acetaldehyde during light-to-dark transitions, it was proposed that acetyl-CoA reacts with GLVs, including C6 aldehydes, to form C6 acetates; the acetaldehyde was hypothesized to be leaking from the acetyl-CoA pool during this reaction. Thus, in the Graus et al. (2004) hypothesis, unlike the pyruvate overflow hypothesis, acetaldehyde emissions are linked to the wound-response production of GLVs during light-to-dark transitions.

There are other possible explanations of the results from Graus et al. (2004), however. As stated above, a novel high-affinity pyruvate decarboxylase has been identified in the vein tissues of leaves (Nguyen et al. 2009). It is possible that this enzyme is involved in the direct conversion of pyruvate to acetaldehyde plus CO_2. Increases in cytosolic pyruvate during inhibition of ALDH may force more of the pyruvate to the veins, where it is decarboxylated. Alternatively, the pyruvate may be emitted directly to the atmosphere, as has been observed by Jardine et al. (2010). In one study, Jardine et al. (2012) observed that leaves of mesquite (*Prosopis velutina*) emitted no C6 green leaf volatiles when exposed to light-to-dark transitions in anoxic atmospheric conditions, but did emit large amounts of acetaldehyde. In the study by Brilli et al. (2011), a burst of GLV emissions was observed after a light-to-dark transition in the grass *Dactylis glomerata*, but with no accompanying acetaldehyde emissions. Together, the results of these studies do not support the conclusion of Graus et al. (2004) that the acetaldehyde and green leaf volatile bursts following light-to-dark transitions are necessarily linked. There is clearly a lot of uncertainty that continues to surround the controls over acetaldehyde and GLV emissions from leaves, especially following light-to-dark transitions. There is a general consensus that the source of these emissions involves perturbations in the balance of carbon flows among different primary and secondary pathways, but the exact nature of those imbalances will likely need to be resolved through studies of processes in isolated organelles, metabolic-control modelling and re-construction of pathways through transgenic manipulation.

6.4 The Anthropocene and New Variables in the Control Over BVOC Emissions

The primary changes expected to the global environment over the next century due to continued societal and economic development include increases in the atmospheric CO_2 concentration, drier tropical forests, warmer temperatures at the mid-latitudes and a transition from native to managed forest ecosystems (IPCC 2007). Environmental changes such as these are likely to affect landscape BVOC

emission rates through existing potential for genetic control, as well as slower ecological changes in community composition. The latter type of change is beyond the scope of this chapter. With regard to the former type of change, we might expect BVOC emissions to generally increase in proportion to the frequency of extreme weather events. Episodes of extremely high temperature and drought tend to trigger mechanisms that improve tolerance of abiotic stress through BVOC production (Loreto and Schnitzler 2010).

Land-use change due to human activities has created a potential to change landscape surface albedo and influence the Earth's radiation budget. Forest ecosystems tend to have a lower surface albedo than pasturelands and grasslands. More recently, political and economic initiatives have included strategies to expand reliance on short-rotation agroforests for the purpose of producing cellulosic biofuels and enhancing atmospheric CO_2 sequestration (Pacala and Socolow 2004; Canadell and Raupach 2008). Such land-use changes have the potential to not only influence surface albedo and canopy temperatures, but also the emission of BVOCs (Purves et al. 2004). Bioenergy species such as poplars (*Populus* spp.), eucalypts (*Eucalyptus* spp.), reed grass (*Phalaris arundinacea*), pines (*Pinus* spp.) and oil palm (*Elaeis guineensis*) emit large quantities of reactive BVOCs (e.g., Ashworth et al. 2012). Changes in the atmospheric CO_2 concentration will further alter the potential for landscape emissions (e.g., Heald et al. 2009). Many of the changes we might expect on a future Earth trade off against each other in complex ways, making it difficult to predict the ultimate effects of global change on BVOC emissions (Owen et al. 2013). For example, in tropical latitudes, the planting of forests on reclaimed pasturelands, has the potential to: (1) increase CO_2 extraction from the atmosphere (a 'cooling effect'), (2) increase rates of latent heat loss (a 'cooling effect'), (3) increase the formation of secondary organic aerosols and density of clouds (a 'cooling effect'), and (4) increase the production of tropospheric ozone (a 'warming effect'). In the opposite direction, however, forests will cause a lower surface albedo and associated higher flux of sensible heat and long-wave radiation to the atmosphere (a 'warming' effect). The net result of these interactions will require analysis with comprehensive, coupled land-atmosphere climate and chemistry models (Ashworth et al. 2013; Kulmala et al. 2013).

6.5 General Conclusions

The emission of BVOCs from leaves can be understood within the context of a combination of metabolic responses, in which existing metabolic potential responds to changes in substrate availability and enzyme kinetics, and gene-expression responses, in which the regulation of gene transcript number and subsequent translation control the amount of metabolic machinery capable of producing and consuming substrate. In general terms, the metabolic responses tend to reflect the shorter-term (seconds-to-minutes) responses, whereas the gene-expression responses tend to reflect the longer-term (hours-to-days) responses. This control

framework is embedded within two metabolic pathways, the mevalonic acid (MVA) pathway in the cytosol and the methyl-erythritol phosphate (MEP) pathway in plastids. Recent studies have revealed that there is certain cross-talk between these pathways, although not always very strong. The cross-talk is coordinated to control the use of common substrates in the synthesis of BVOCs. The MEP pathway, which produces isoprene and the light-dependent monoterpenes, may be subject to feedback control from its end-product dimethylallyl diphosphate (DMADP), though the exact nature of this control is still uncertain.

The emissions of oxygenated BVOCs show evidence of control by substrate channeling between glycolysis and mitochondrial respiration in the case of emitted bursts of acetaldehyde, whereas the emissions of green leaf volatiles (mostly C5 and C6 aldehydes) are due to oxidation of fatty acid chains in leaf lipids, triggered by mechanical injury. Methanol is emitted in large quantities from expanding leaves, and appears to be the product of pectin demethylation during cell wall expansion. Methanol emissions are largely under stomatal control in the shorter term, but under phenological control over leaf development in the longer term.

Many of the studies conducted to date have concerned controls over one type of BVOC or another, but an integrated perspective as to how cells regulate the biosynthesis and emission of an entire suite of BVOCs, many of which are likely to carry out similar functions, has not been achieved. Interactions among the substrates exchanged between pathways, and coupled responses to cellular cues are likely to create a level of complexity that we have not even begun to appreciate in the ultimate control over BVOC emissions. The emergence of new tools within the realm of genetic manipulation, and the metabolic variants that are produced by such manipulation, are likely to create new opportunities to 'unpeel' the layers of control that regulate this complexity in control dynamics.

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Chapter 7
The Roles of Stomatal Conductance and Compound Volatility in Controlling the Emission of Volatile Organic Compounds from Leaves

Peter C. Harley

Abstract Plants emit more than 30,000 different volatile organic compounds and the extent to which stomata exert control over the emissions of these compounds varies widely. Each of these compounds has unique physico-chemical characteristics, including volatility that characterizes the partitioning between water/air and water/lipid phases. For different volatile compounds, volatility differs by over six orders of magnitude. The volatility of each compound, and to a lesser extent the anatomical characteristics of the leaf, determine for each compound the extent to which the aqueous and lipid phases within the leaf comprise temporary non-specific storage pools between the site of synthesis and the substomatal cavities. This chapter emphasizes that the pool size of each volatile in leaf lipid and water phases is the chief determinant of the strength of stomatal control as well as the responsiveness of emissions to rapid changes in light and temperature.

7.1 Introduction

Plants emit more than 30,000 different volatile organic compounds, each with its own unique physico-chemical characteristics. Biogenic volatile organic compounds (BVOCs) are derived from a variety of biochemical pathways and production occurs in various leaf compartments, including plastids, cytosol, and in cell walls. Some volatiles are emitted more or less immediately upon their production, while others are sequestered in specialized storage structures within, e.g., resin ducts in conifers, or on the surface of leaves, e.g., leaf glandular hairs in the mint (*Mentha*) family

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(Monson 2013 in this volume). With the exception of those terpenoids that are stored in leaf surface structures such as glandular hairs, and presumably escape directly to the atmosphere, BVOCs are assumed to accumulate in the intercellular air space of the leaf and exit almost entirely through the leaf stomata. Though there may be some transfer of BVOCs across the leaf cuticle, cuticular resistance is typically two orders of magnitude higher than stomatal resistance (Nobel 2009). Although BVOCs may diffuse slowly through the cuticle and this flux may comprise a significant percentage of the total flux when stomata are closed, flux across the cuticle will be ignored in the following discussion.

In extant models of BVOC emission from leaves (e.g., Guenther et al. 2006, 2012; Monson et al. 2012; Grote et al. 2013; Guenther 2013 in this volume), short-term control over emissions is largely relegated to leaf temperature and, for those BVOCs whose production has been shown to exhibit a light dependency, also to incident photosynthetic photon flux density (PPFD). In addition, production of at least some BVOC species is directly affected by CO_2 partial pressure (Loreto et al. 2001; Rosenstiel et al. 2003). Although stomata clearly exert control over the rate at which water escapes the leaf, and have considerable influence on CO_2 flux into the leaf, current widely used models of BVOC emissions assume that the stomata have no influence on the flux (e.g., Guenther et al. 2006, 2012). Thus, there is the implicit assumption that the emission rates of those BVOCs which are not stored in specialized structures are equal to the rate of production, while for compounds that are stored, the assumption is that the emission rate equals the rate of release from internal storage structures. This chapter examines the validity of these assumptions, and the implications for modelling BVOC emissions. This chapter emphasizes that for many important plant volatiles, we need to consider not only their rate of synthesis but also their physico-chemical properties in order to predict how the emissions respond to short-term changes in stomatal conductance.

7.2 Theory and Experimental Evidence of Stomatal Control of Trace Gases

7.2.1 The Theory Behind Stomatal Control of BVOC Emissions

Those, who have been involved in attempts to model transpiration and photosynthesis at the scale of the leaf, have long understood the important role of stomatal conductance in controlling the flux of water out of, and CO_2 into, the leaf. Any gas diffusing into or out of a leaf obeys Fick's first law, which states that the flux is proportional to the concentration difference between the leaf intercellular air space and the air outside the leaf boundary layer, and inversely proportional to the sum of the aggregate resistances between them. In the absence of significant

flux across the leaf cuticle, and assuming a low resistance across the leaf boundary layer, a reasonable assumption at moderate wind speeds, this resistance pathway is dominated by the stomata. This relationship has been intensively studied for both CO_2 and H_2O vapour and the reader is referred to the excellent discussion in Nobel (2009). Cowan (1977) introduced the measure of resistance (r) in common usage today, in which r is expressed in molar units (m^2 s mol^{-1}) and the most commonly used formulation for water vapour flux (Cowan 1977; Farquhar et al. 1978) becomes,

$$J_{H_2O} = (w_{l,H_2O} - w_{a,H_2O}) / r_{s,H_2O} = (w_{l,H_2O} - w_{a,H_2O})\, g_{s,H_2O} \tag{7.1}$$

where J_{H_2O} represents the water vapour flux (transpiration rate) in mol m^{-2} s^{-1}, w_{l,H_2O} and w_{a,H_2O} are the mole fractions of water vapour (mol mol^{-1}) in the intercellular air space of the leaf and in the ambient air, respectively, and g_{s,H_2O}, the stomatal conductance to water vapour (mol m^{-2} s^{-1}), is the inverse of r_{s,H_2O}, the stomatal resistance to water vapour.

From Eq. 7.1, it is clear that any increase or decrease in stomatal conductance (g_{s,CO_2}) must lead to a corresponding increase or decrease in the flux (J_{H_2O}) unless the driving force ($w_{l,H_2O} - w_{a,H_2O}$) decreases or increases in order to counteract the change. Whether or not the emission flux of a given trace gas species (J_x) is under stomatal control thus depends largely on whether or not the driving force can change enough to compensate for any change in conductance. Since emissions from the leaf are never sufficiently great to significantly alter the trace gas mole fraction outside the leaf, $w_{a,x}$, the question reduces to whether the intercellular partial pressure of the compound, $w_{l,x}$ can increase sufficiently to balance a decrease in the stomatal conductance to given compound, $g_{s,x}$. Within this context, it becomes clear why transpiration is under tight stomatal control (Sharkey 1991). Air inside the leaf is assumed to be saturated with water; thus, at a given leaf temperature, both w_{l,H_2O} and the driving force ($w_{l,H_2O} - w_{a,H_2O}$) are constant and any change in g_{s,CO_2} must result in a linear change in the flux, J_{H_2O}.

The case for CO_2 is more complex because photosynthetic rate, i.e., the flux, and w_{l,CO_2}, the intercellular CO_2 mole fraction, are not independent, because the rate of CO_2 uptake by the enzyme Rubisco is a function of w_{l,CO_2}. Thus, the driving force, or CO_2 partial pressure difference between the intercellular air space and the ambient air does not remain constant as conductance changes, but decreases somewhat with increasing stomatal conductance to CO_2, g_{s,CO_2} (Sharkey 1991). As a result, photosynthesis is under only partial stomatal control, increasing with increasing g_{s,CO_2}, but in a non-linear fashion with the extent of change depending on w_{l,CO_2}.

For other leaf uptake processes, the extent to which $w_{l,x}$ can change to compensate for changes in $g_{s,x}$ is also limited and some degree of stomatal control is expected. Since w_l can never drop below zero, the driving force ($w_{a,x} - w_{l,x}$) can never exceed the ambient concentration. In the case of O_3, for example, Laisk et al. (1989) reported that intercellular ozone concentration (w_{l,O_3}) was near zero, although this was probably a slight underestimation as a result of a faulty O_3

analyzer (A. Laisk and H. Moldau, personal communication). A small positive value of w_{l,O_3} has been reported by Moldau and Bichele (2002). Nevertheless, w_{l,O_3} has widely been assumed to be close to zero, since O_3 is known to react quickly with cellular components doing considerable damage along the way (Wesely 1989). When internal ozone concentration is essentially zero, the driving force remains constant and O_3 uptake is under total stomatal control. As a result, plants can reduce exposure to O_3 damage by partially closing stomata and thereby limiting uptake. As we will see below, in contrast to the case of deposition, for BVOCs being produced in the leaf and emitted through the stomata, the situation is quite different, and w_l and thus the driving force ($w_l - w_a$) can vary over a very wide range, potentially overcoming stomatal limitations.

7.2.2 *Evidence for the Lack of Stomatal Control over BVOC Emissions*

I think it is fair to say that most plant ecophysiologists, comfortable with the analogy of CO_2 and H_2O vapour exchange, were predisposed to accept a role for stomata in limiting/controlling BVOC emissions. As early as in 1981, Tingey et al. showed that isoprene is emitted through the stomata, by demonstrating that emission from the hypostomatous leaves of live oak (*Quercus virginiana*) occurred overwhelmingly from the adaxial (lower) leaf surface. Paradoxically though, they also found that when stomatal conductance declined by 90 % due to water stress, isoprene emission was unaffected. In an early attempt to understand environmental controls over isoprene emission, Monson and Fall (1989) also clearly demonstrated that allowing g_{s,H_2O} to vary over a wide range, whether by varying humidity or by the addition of abscisic acid (ABA) which leads to stomatal closure, had little or no effect on isoprene emissions. In a follow up study (Fall and Monson 1992), this apparent paradox was resolved in a straightforward application of Fick's law.

In principle, Eq. 7.1 applies to any trace gas. Stomatal conductance for a given gas ($g_{s,x}$) can be directly related to stomatal conductance for water vapour by the ratio of their diffusivities (D), i.e., $g_{s,x} = g_{s,H_2O}\,(D_x / D_{H_2O})$ where the subscript (x) refers to the trace gas of interest. Thus, if one knows its diffusivity, one can estimate the concentration of a trace gas in the intercellular air space necessary to sustain a given measured flux at a certain value of g_{s,H_2O}. Fall and Monson (1992) utilised this concept to explain the apparent lack of sensitivity of isoprene emission to changes in stomatal conductance. Although at that time, the diffusion coefficient of isoprene was unknown, they assumed a value of $1{\cdot}10^{-5}$ m^2 s^{-1}, which is surprisingly close to the current experimental estimate of ca. $0.9{\cdot}10^{-5}$ m^2 s^{-1} (Niinemets and Reichstein 2003a, 2003b). Employing Eq. 7.1 for isoprene, they reasoned that if J_{isop} and $w_{a,isop}$ remain constant, while $g_{s,isop}$ decreases, the only way to balance the equation was for $w_{l,isop}$ to increase in direct proportion to the decrease in $g_{s,isop}$. If isoprene production inside the leaf is unaffected by the concentration of isoprene in the intercellular air space (i.e., there is no feedback inhibition on isoprene production), then $w_{l,isop}$

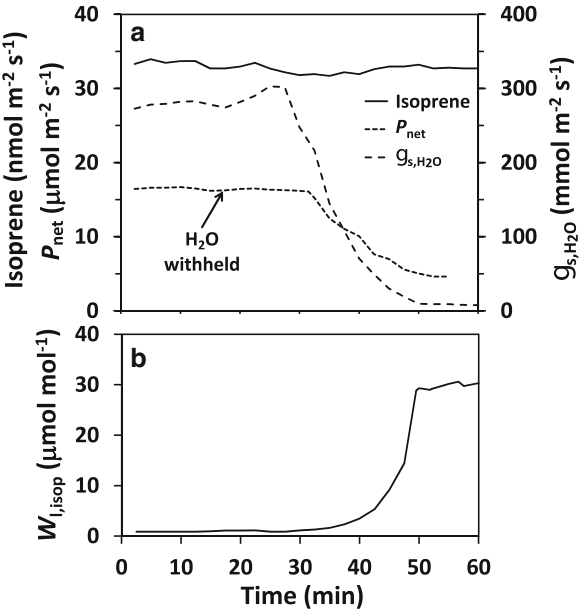

Fig. 7.1 Response of isoprene emission, net photosynthesis (P_{net}) and stomatal conductance (g_{s,H_2O}) to withholding of water from the cut stem of an aspen (*Populus tremuloides*) leaf (**a**). Water was withheld at time $t = 17$ min (*arrow*). Panel (**b**) illustrates the intercellular isoprene partial pressure ($w_{l,isop}$) necessary to sustain the fluxes shown in (**a**), calculated using Eq. 7.1. Leaf temperature was 30 °C (Modified from Fall and Monson 1992)

must rise as $g_{s,isop}$ declines, and a new steady-state condition will arise in which the decline in $g_{s,isop}$ is exactly compensated for by an increase in $w_{l,isop}$. If the rise in $w_{l,isop}$ is sufficiently rapid and exactly balances the decrease in $g_{s,isop}$, the flux will remain unaffected.

Thus, when water was withheld from the cut stem of an aspen leaf (*Populus tremuloides*), g_{s,H_2O} declined from about 300 mmol m^{-2} s^{-1} to about 10, while calculated $w_{l,isop}$ increased from about 1 to over 30 µmol mol^{-1}, but isoprene flux was unchanged (Fig. 7.1). Fall and Monson (1992) were able to verify experimentally that $w_{l,isop}$ did in fact increase dramatically with stomatal closure. They measured the isoprene content of white oak (*Quercus alba*) and aspen leaves by vacuum extraction before and after applying ABA to induce stomatal closure, and found a 25–40-fold increase following stomatal closure in both species.

A similar lack of stomatal control over emissions of α-pinene from *Quercus ilex* was documented by Loreto et al. (1996b) who showed that, by changing ambient CO_2 concentration or air humidity, g_{s,H_2O} could be varied over a fairly wide range with no apparent impact on α-pinene emissions. These isoprene and α-pinene results gave rise to the general notion that, given the absence of feedback inhibition, trace gas emissions ought not to be under stomatal regulation, as any change in conductance for given trace gas will be compensated for by a near simultaneous increase in $w_{l,x}$ (Sharkey 1991; Kesselmeier and Staudt 1999). The implication was that, for those BVOCs not stored in specialized leaf storage structures, the rate of emission reflected only the rate of production. Similarly, for those compounds that were known to be sequestered in specialized internal storage structures such as resin

ducts in conifers, the measured emission rate was assumed to reflect the rate at which they were released from the ducts. With this theoretical underpinning, the early BVOC emission modelling efforts incorporated no direct effects of stomata on emissions, and this has carried over to contemporary models such as MEGAN (Guenther et al. 2006, 2012).

7.2.3 Evidence for Stomatal Control over BVOC Emissions

As an increasing number of BVOC emission patterns were investigated and measurements accumulated, a few reports began to cast doubt on the generalization that stomata exerted no control over emissions. As described below, a correlation was occasionally observed between emissions and stomatal conductance. However, these studies also acknowledged that caution must be exercised before equating correlation and causality. Since g_{s,H_2O} responds strongly to varying PPFD, the emissions of any BVOC whose production is dependent on light may correlate with changes in g_{s,H_2O} without the stomata actually exerting any control. For instance, Kesselmeier et al. (1996) implied a potential role for the stomata in controlling monoterpene emissions from *Q. ilex*, although they suggested that light exerted control over both monoterpene production and stomatal aperture, and assigning control to one or the other was ambiguous. If correlations between the emission flux of a given trace gas species and stomatal conductance are often difficult to interpret, one way to clearly demonstrate a level of stomatal control is to observe emissions following stomatal closure induced by ABA or the withholding of water, where light and temperature are held constant (again assuming that the rate of BVOC synthesis is unaffected).

MacDonald and Fall (1993) reported the first evidence of emissions of methanol from plants, and clearly showed a strong correlation of those emissions with stomatal conductance in leaves of both bean (*Phaseolus vulgaris*) and sweetgum (*Liquidambar styraciflua*). These observations were confirmed and expanded upon by Nemecek-Marshall et al. (1995) who observed large decreases in methanol emissions when stomatal closure was induced, either by withdrawing water from the cut petiole of a bean leaf or by replacing water with an ABA solution (Fig. 7.2a). In addition, following extended periods of darkness, during which the stomata were tightly closed, large transient bursts of methanol emissions were seen upon illumination and stomatal opening (Fig. 7.2b). Although the authors suggested these bursts might be associated with leaf damage or the volatilization of methanol condensed on the leaf surface, the phenomenon was better explained by postulating the accumulation of large pools of methanol somewhere within the leaf when stomata were closed, and their subsequent emptying when stomatal constraints were eliminated (Niinemets and Reichstein 2003a).

In the case of organic acids, Kesselmeier et al. (1997) reported a light dependence for emissions of formic and acetic acids, but were unable to establish whether the

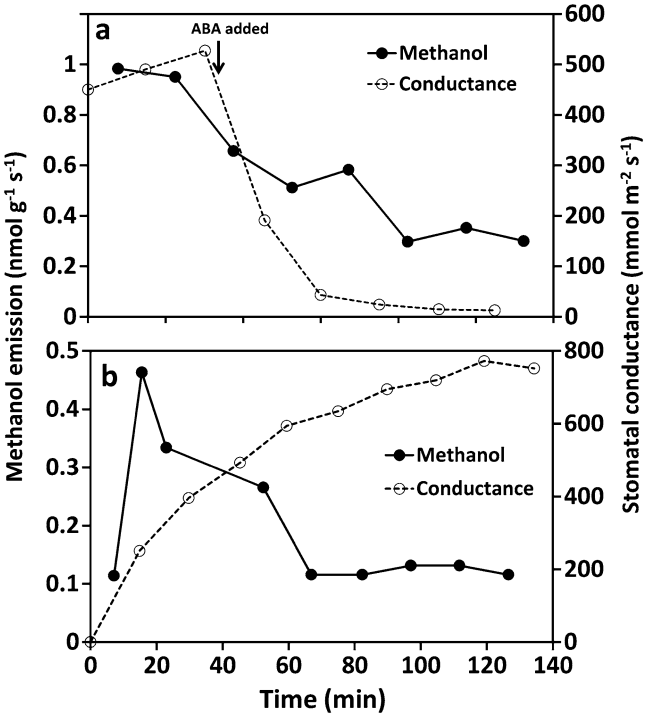

Fig. 7.2 Relationships between rapid changes in stomatal conductance and methanol emission in leaves of a common bean (*Phaseolus vulgaris*). In (**a**), a young stem was cut and immersed in water. After approximately 40 min, 20 μM ABA was added to the transpiration stream (*arrow*). In (**b**), the plant had been in the dark overnight, and light (PPFD = 500 μmol m^{-2} s^{-1}) was turned on at time $t = 0$ min. Leaf temperature was 30 °C (Modified from Nemecek-Marshall et al. 1995)

control was direct, through production, or indirect, through changes in stomatal conductance. Gabriel et al. (1999) also observed a strong correlation between the emissions of formic and acetic acid and g_{s,H_2O}. They established a definitive role for the stomata by demonstrating that when stomatal conductance fell by over 90 % after application of ABA, the emissions of both acids were reduced by over 50 %.

In contrast to the somewhat ambiguous data with regard to stomatal control over monoterpene emissions mentioned above, Niinemets et al. (2002) measured emissions of four different monoterpenes from *Pinus pinea* during the summer drought when stomatal conductance experienced 'midday depression', falling by 75 % between 1000 and 1400 h before recovering slightly in the late afternoon. Two of the monoterpenes, limonene and β-ocimene, were unaffected by the decreased conductance (Fig. 7.3c), while two oxygenated species, linalool and 1,8-cineole, clearly tracked g_{s,H_2O} as it fell and subsequently recovered (Fig. 7.3b).

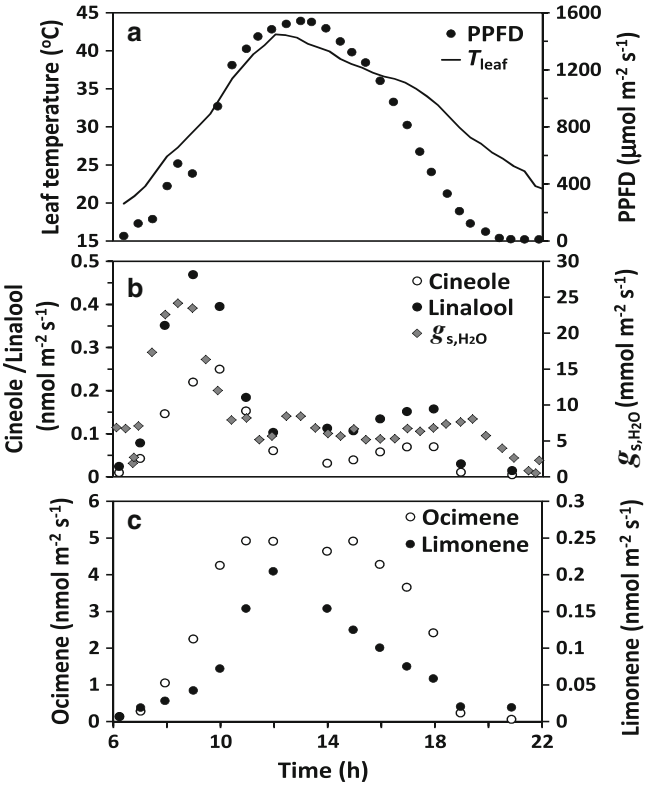

Fig. 7.3 Changes in stomatal conductance (g_{s,H_2O}) and the emissions of four BVOCs from needles of *Pinus pinea* in response to daily variations in leaf temperature and incident photosynthetic photon flux density (PPFD). Panel (**a**) depicts leaf temperature and PPFD, (**b**) stomatal conductance and emissions of oxygenated monoterpenes linalool and 1,8-cineole, and (**c**) the emissions of non-oxygenated monoterpenes trans-β-ocimene and limonene (Modified from Niinemets et al. 2002)

7.3 Dynamic Models Explaining Stomatal Controls of Emissions

7.3.1 Evidence for Temporary Storage Pools for BVOCs within Leaves

As evidence accumulated that BVOC emissions were not always closely coupled to their assumed rate of production, and that for some compounds, changes in stomatal conductance clearly influenced emissions, the notion arose that there must be temporary storage pools within the leaf. Several lines of evidence pointed in this direction (see Niinemets and Reichstein (2002) and Grote and Niinemets (2008) for a more complete discussion). For example, the large bursts of methanol

emission upon re-illumination following extended periods of darkness (Fig. 7.2b) were interpreted to reflect storage within the leaf, the pools emptying rapidly upon stomatal opening (Niinemets and Reichstein 2003a, b; Hüve et al. 2007; Harley et al. 2007). Similarly, although emissions of light-dependent monoterpenes in *Quercus ilex* fall rapidly upon leaf darkening, Loreto et al. (1996a) observed that they did not drop immediately to zero despite the fact that production was assumed to cease. Indeed, after a sharp drop in the first few minutes, emissions continued to fall slowly but were still measurable over an hour later, suggesting the slow emptying of temporary storage pools. Similar results were reported by Pio et al. (2005) and Schuh et al. (1997). In an experiment using a non-emitting chemotype of *Quercus suber*, Delfine et al. (2000) found that fumigating with a mixture of monoterpenes lead to leaf uptake, followed by monoterpene emissions that were measurable over 12 h after fumigation ceased. This clearly indicated that monoterpenes can accumulate in storage pools within the leaf despite the absence of specific storage structures. This was confirmed by direct measurement of monoterpene pools in leaves of *Q. ilex* (Loreto et al. 1998). Although these pools were small relative to the pools in species with specialized storage structures, the pools were sufficiently large to sustain steady-state emissions for approximately 15 min in darkness, a result consistent with the leaf darkening experiment referred to above. Loreto et al. (1998) suggested that these temporary storage pools were related to BVOC solubility and the partitioning of BVOC between the gas and liquid phases.

At physiologically relevant ambient air concentrations, Copolovici et al. (2005) observed significant uptake of the hydrophobic monoterpene α-pinene and hydrophilic monoterpene α-terpineol using a proton-transfer reaction mass spectrometer (PTR MS). Surprisingly, monoterpene uptake did not saturate with time, suggesting that in addition to accumulating in temporary storage pools, monoterpenes might have been metabolized inside the cell. Noe et al. (2008) found that limonene was taken up and re-emitted from a variety of plants and saw a strong correlation between uptake rates and plant lipid content, suggesting that hydrophobic BVOCs might be temporarily stored in the lipid phase of the leaf.

7.3.2 *Role of Temporary Storage in Stomatal Sensitivity of Emissions*

As we have seen, any change in stomatal conductance must lead to a transient change in the intercellular partial pressure of BVOC. A new steady-state will eventually be achieved in which the newly achieved partial pressure exactly compensates for the change in g_s and the flux returns to its value prior to the stomatal adjustment. In the steady state, therefore, stomata can exert no control over BVOC emission. The existing evidence summarized here, however, suggests the existence of non-specific and temporary BVOC storage pools within all leaves. If these storage pools are very small, as for isoprene or α-pinene, the readjustment is very rapid and any change in the emission rate is almost undetectable, implying that the emission

rate closely reflects the rate of production. If, on the other hand, these storage pools are large and slow to re-equilibrate, the time required to return to the steady state may be substantial, and until new steady-state conditions are obtained, emissions will be subject to stomatal control. What then is the nature of these non-specific and temporary storage pools?

These transient storage pools arise because all BVOCs, regardless of where in the leaf they are produced (plastids, cytosol, in association with cell membranes) or stored (resin ducts), must diffuse through aqueous phases in the mesophyll, a complex series of lipid bilayer membranes, and internal air spaces before they are emitted to the atmosphere via the stomata. Along this complex diffusion pathway, BVOCs partition, to a greater or lesser extent, in the liquid and lipid phases, depending on the physico-chemical characteristics of the given BVOC species and on the amount of liquid water and lipids within the leaf (Niinemets and Reichstein 2003a, b).

To explain the various responses of different BVOCs to stomatal conductance (and ignoring for the moment the potential role of the leaf lipid phase), Niinemets et al. (2002) proposed that the aqueous phase of the leaf constituted a temporary storage pool for highly soluble BVOCs, and that the susceptibility of a chemical species to stomatal regulation was directly related to its Henry's law constant (H, Pa m^3 mol^{-1}) which determines the partitioning of a volatile between the liquid and the vapour phases. Table 7.1 lists Henry's law constants for a wide range of BVOCs commonly found in leaf emissions.

For isoprene ($H = 7{,}780$ Pa m^3 mol^{-1} at 25 °C) and α-pinene ($H = 13{,}600$ Pa m^3 mol^{-1}) that are very insoluble in the aqueous phase of the leaf, the aqueous storage pool is very small and the newly produced compounds partition almost entirely to the gas phase. As soon as a new molecule is produced, it can diffuse from the site of production to the intercellular air space in the substomatal cavity. This would also apply to limonene ($H = 2{,}805$ Pa m^3 mol^{-1}) and *trans*-β-ocimene ($H = 3{,}330$ Pa m^3 mol^{-1}) in Fig. 7.3c. For highly soluble compounds (Table 7.1 for Henry's law constants), such as alcohols, e.g., methanol, 2-methyl-3-buten-2-ol, and carbonyls, e.g., carboxylic acids (formic acid and acetic acid), and aldehydes (formaldehyde and acetaldehyde), as well as for oxygenated monoterpenes such as linalool and 1,8-cineole in Fig. 7.3b, newly produced compounds partition strongly to the aqueous phase and large increases in the aqueous phase concentration are necessary to support increases in gas-phase partial pressure sufficient to re-establish steady-state emissions. In effect, dissolution in the aqueous phase constitutes a temporary storage pool. Eventually, continuing BVOC production will lead to aqueous phase concentrations sufficient to support large enough increases in the gas phase concentration to establish a new equilibrium. Steady-state conditions will return and emission rate will once again equal the rate of production. In such circumstances, stomata will exert some level of control over the emissions until a new steady state is reached. Thus, it is the time required to achieve a new steady-state following a perturbation (e.g., stomatal closure, change in production rate) that determines whether or not the emissions of a given BVOC are under stomatal control.

Table 7.1 Henry's law constants (gas/liquid phase partition coefficient) and octanol/water partition coefficients of selected BVOCs at 25 °C

Compound	Henry's law constant (Pa m^3 mol^{-1})	Octanol/water partition coefficient (mol mol^{-1})
Alcohols		
Ethanol	0.507	0.490
Methanol	0.461	0.170
Aldehydes		
Acetaldehyde	7.00	0.457
Formaldehyde	0.0305	0.457
Ketones		
Acetone	3.88	0.575
Organic acids		
Acetic acid	0.0133[a]	1.74[a]
Formic acid	0.0176[a]	0.288[a]
Terpenoids		
Camphene	1,600	28,510
3-Carene	13,640	40,740
p-Cymene	1,100	31,620
Isoprene	7,780	263
Limonene	2,850	30,550
Myrcene	6,300	21,630
cis-β-Ocimene	2,470	23,530
trans-β-Ocimene	3,330	28,200
α-Phellandrene	6,950	38,460
β-Phellandrene	5,670	38,160
α-Pinene	13,600	30,900
β-Pinene	6,830	26,300
Sabinene	6,450	42,660
α-Terpinene	1,960	5,060
γ-Terpinene	3,590	31,620
α-Terpinolene	2,600	29,510
Oxygenated terpenoids		
Bornyl-acetate	44.3	7,244
Camphor	1.22	219
1,8-Cineole	13.6	403
Linalool	2.09	933
Menthol	1.54	2,300
2-Methyl-3-buten-2-ol	1.56	17.8
α-Terpineol	0.239	955
Thymol	0.12	1,995

Adapted from Niinemets and Reichstein (2002) and Niinemets and Reichstein (2003a) where the original references may be found

[a]All physico-chemical properties apply to undissociated form only

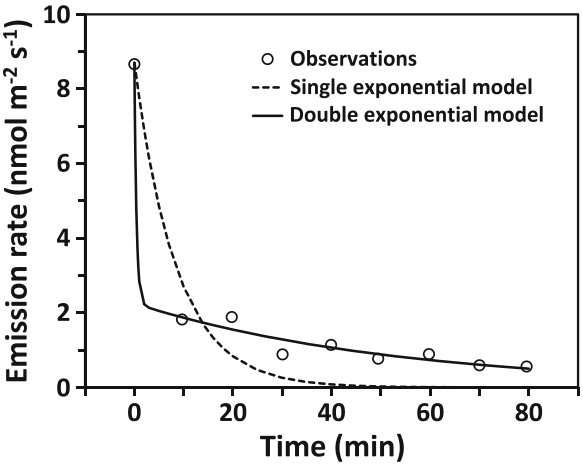

Fig. 7.4 Illustration of the decay in monoterpene emission rate following leaf darkening at time $t = 0$ in holm oak (*Quercus ilex*) (data of Loreto et al. 1996a). The data were fit by both a single-exponential model (the emission flux, $J = J_0 e^{-kt}$) where J_0 is the initial emission rate and k is the decay rate constant, and a double-exponential model ($J = J_0[\eta e^{-k1t} + (1-\eta)e^{-k2t}]$) where k_1 and k_2 are the rate constants of the two pools and η is the initial fraction of monoterpenes emitted from the 'fast' pool (Modified from Niinemets and Reichstein 2002)

The leaf aqueous phase, thus, constitutes one temporary storage pool for water-soluble BVOCs. Niinemets and Reichstein (2002) examined more closely the relatively slow decline in light-dependent monoterpene emissions following darkening in *Q. ilex* (Loreto et al. 1996a) and observed that the decay kinetics were not well described by an exponential decay model, as would be expected if a single pool were being emptied. The data was well described however by a double-exponential model, which implicitly assumes the presence of two independent temporary storage pools, a 'fast' pool and a 'slow' pool, emptying at different rates (Fig. 7.4).

The 'fast' pool was shown to be the aqueous-phase pool described above, which empties in a few seconds, consistent with the discussion above, assuming vales of H greater than 500 Pa m^{-3} mol^{-1}. Consistent with the observations of Noe et al. (2008), the 'slow' pool was assumed to reside in the lipid phase of the leaf in which hydrophobic but lipid-soluble BVOCs might temporarily reside. Thus, in a manner analogous to the aqueous phase, temporary storage in the lipid phase can result in non-steady-state conditions under which the stomata might exert some control over emissions. The extent to which a given BVOC partitions into the lipid phase is related to its octanol/water partition coefficient ($K_{o/w}$, mol mol^{-1}), defined as the ratio of the solubility of a compound in octanol (a non-polar solvent) to its solubility in water (a polar solvent). Values of $K_{o/w}$ for BVOCs of interest are given in Table 7.1. The higher the $K_{o/w}$, the more non-polar the compound and the more likely it is to partition to the non-polar lipid phase. BVOCs with low values of H (highly water-soluble) will thus tend to have low values of $K_{o/w}$

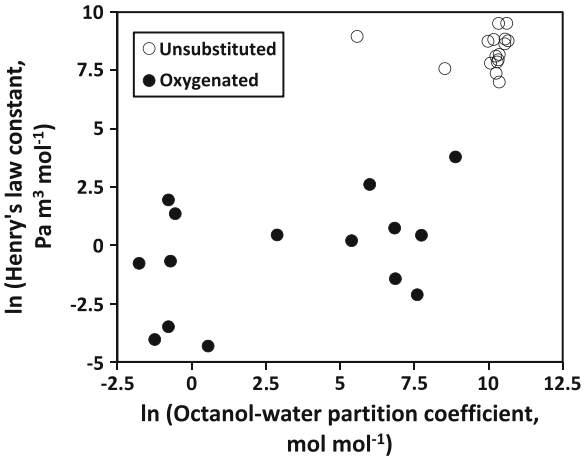

Fig. 7.5 Relationship between Henry's law constant (H) and octanol/water partition coefficient ($K_{o/w}$) for 31 commonly emitted BVOCs (Table 7.1). Unsubstituted hydrocarbons are differentiated from those containing at least one oxygen atom

(low lipid solubility) and *vice versa* (Fig. 7.5). A detailed discussion of both Henry's law constant and octanol/water partition coefficients, including estimates of their temperature dependencies, is found in Copolovici and Niinemets (2005).

7.4 A Dynamic (Non-Steady-State) Model of BVOC Emissions

7.4.1 Description of Tentative BVOC Pools and Their Dynamics

As discussed above, evidence has accumulated that both the aqueous and lipid phases of the leaf can act as non-specific, temporary storage pools. The recognition that the water solubility of a gas, and to a lesser extent the lipid solubility, largely determine whether or not it came under stomatal regulation, lead to the development of a dynamic BVOC emission model that resolved the apparently contradictory reports in the literature regarding the stomatal regulation of trace gas emissions. Building on the concepts outlined above, Niinemets and co-workers (Niinemets et al. 2002; Niinemets and Reichstein 2002, 2003a, b; Noe et al. 2006) developed a dynamic model of BVOC emissions, depicted conceptually in Fig. 7.6, which incorporates these ideas and forms the basis for the remainder of this chapter.

Upon its production (or release from permanent storage structures) at a rate I (mol m^{-2} s^{-1}), each BVOC partitions into the aqueous and lipid phases of the leaf mesophyll, the partitioning ratio being determined by an empirical partitioning coefficient, η. These non-specific, temporary storage pools (mol m^{-2}) are designated S_a (aqueous) and S_l (lipid). The rate of release of each BVOC from the aqueous storage pool (S_a) is described by a compound-specific first-order kinetic constant,

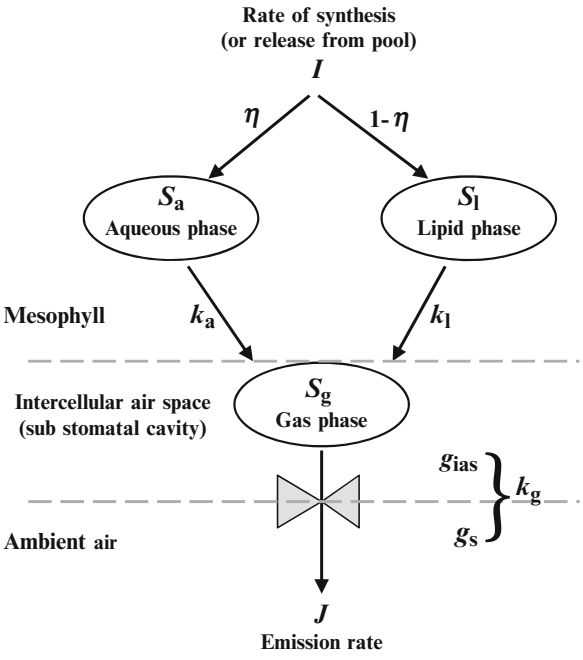

Fig. 7.6 Schematic diagram of the dynamic model of Niinemets and Reichstein (2002, 2003a, b), illustrating the potential role of non-specific storage pools in controlling BVOC emissions. BVOCs are produced (or released from storage structures) at a rate I and eventually diffuse through the stomata to the ambient air at rate J. Along this complex diffusion pathway, BVOCs may be stored temporarily in three non-specific storage pools – an aqueous-phase (S_a) or lipid-phase (S_l) pool in the mesophyll or a gas-phase pool (S_g) in the leaf intercellular air space. Further details in the text (Modified from Noe et al. 2006)

k_a (s^{-1}), that is related both to the complex diffusion pathway within the leaf and to the physico-chemical characteristics of the compound, especially its Henry's law constant and diffusion constant in liquid phase. Analogously, release from the lipid storage pool (S_l) is related to k_l (s^{-1}), dependent again on the diffusion path, as well as on the octanol/water partition coefficient of the compound. These time constants describe the dynamics of BVOC release from S_a and S_l into the leaf gas phase (S_g, mol m^{-2}) in the leaf intercellular air space. The flux out of the leaf gas-phase pool and into the ambient air, i.e., what we typically measure, is described by a third rate constant, k_g (s^{-1}) that is determined by the sum of the gas phase conductance from the outer surface of cell walls to the substomatal cavity (intercellular air space conductance, g_{ias}, mol m^{-2} s^{-1}) and the stomatal conductance between the intercellular air space and the ambient air (g_s, mol m^{-2} s^{-1}).

For a complete derivation of the equations used in the dynamic emissions model, the reader is referred to the original model description describing the role of the aqueous-phase pool (Niinemets et al. 2002; Niinemets and Reichstein 2003a, b) and the expanded version incorporating non-specific lipid phase storage pools as well (Niinemets and Reichstein 2002; Noe et al. 2006). In general, the dynamics of the aqueous-, lipid- and gas-phase pools are described by a system of ordinary differential equations:

$$\frac{dS_a(t)}{dt} = \eta I - k_a S_a(t) \tag{7.2}$$

$$\frac{dS_l(t)}{dt} = (1-\eta)I - k_l S_l(t) \tag{7.3}$$

$$\frac{dS_g(t)}{dt} = k_a S_a(t) + k_l S_l(t) - k_g S_g(t) \tag{7.4}$$

where the measured emission rate of the volatile from the leaf (J) is

$$J = k_g S_g(t) \tag{7.5}$$

The kinetic constants k_a, k_l and k_g are all related to leaf structural properties defining the size of the aqueous-, lipid- and gas-phase pools within the leaf, the relevant transfer conductances between the pools, and, most importantly, the physico-chemical characteristics of the BVOCs (H and $K_{o/w}$). All the kinetic constants require an estimate of the ratio of leaf area (A, m^2) to leaf volume (V, m^3). Additionally, the fractions of the total leaf volume (m^3 m^{-3}) comprising air spaces (f_{ias}), liquid water (f_w) and lipids (f_{lip}) are needed for the determination of k_g, k_a and k_l, respectively. In addition, estimates of the total gas phase, liquid phase and lipid phase diffusive conductances from the site of production (or release from specialized storage pools) are required for each BVOC of interest. These conductances depend on diffusion pathway length, and compound diffusivities in given phases.

7.4.2 *Sensitivity of the Model to Pool Sizes as Determined by Leaf Structure and Compound Solubility*

The rate constant of the gas-phase, k_g, is related to the size of the gas-phase pool and to the total gas phase diffusion conductance (G_G, mol m^{-2} s^{-1}) between the outer surface of the mesophyll cell walls and the ambient air:

$$k_g = \frac{A}{V f_{ias}} \frac{RT}{P} G_G \tag{7.6}$$

where R is the gas constant (8.314 Pa m^3 mol^{-1} K^{-1}) and RT/P converts conductance to units of m s^{-1}. G_G represents the sum of the stomatal conductance for the BVOC in question and the internal conductance from the mesophyll cell walls to the substomatal cavity G_{ias}. Given the serial conductances, $G_G = 1/(1/G_s + 1/G_{ias})$. Using Eq. 7.6, one can calculate a range of values for k_g, which scales linearly with both $A/(V f_{ias})$ and G_G. The half-time (s) for gas pool turnover ($\tau_g = \ln(2)/k_g$) then decreases logarithmically as G_G increases. Plotting τ_g as a function of G_G (Fig. 7.7) for two values of Vf_{ias}/A [the volume of air inside the leaf per unit leaf area (m^3 air m^{-2} leaf)], representing the likely range of values for a variety of leaf morphologies, several important things become apparent.

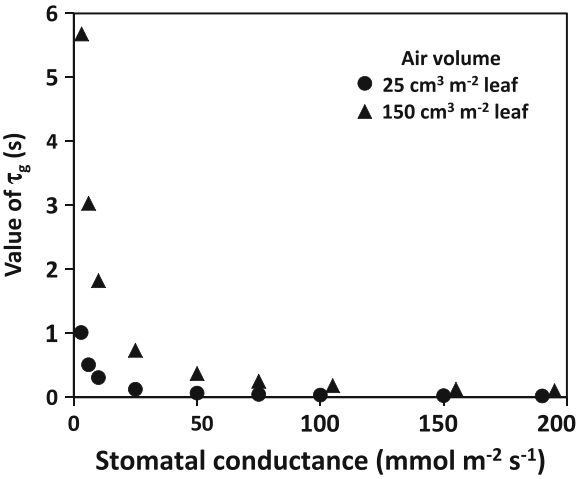

Fig. 7.7 The half-time required for turnover of the gas-phase pool (τ_g) as a function of stomatal conductance, assuming two different values for the amount of air within the leaf ($cm^3\ m^{-2}$). This example assumes a diffusivity similar to that of α-pinene (diffusion coefficient of $6{\cdot}10^{-6}\ m^2\ s^{-1}$ at 25 °C)

The gas pool turnover time increases as stomatal conductance decreases, and as the total volume of air inside the leaf increases. However, the turnover of the gas pool is very rapid, on the order of a few seconds, except at very low values of conductance. Thus, it is assumed that the gas pool reaches a new steady-state value very rapidly following a perturbation, much more rapidly than stomatal movements occur, and cannot therefore contribute significantly to any observed stomatal limitation. Any significant delay in reaching a new steady-state value of w_l must therefore be due to the turnover time of the liquid- and/or lipid-phase pools, determined by the parameters k_a and k_l, respectively.

If we consider the gas-phase to be in the steady state, k_a will depend on the liquid pool size (Vf_w/A) as well as the total liquid phase conductance between the site of synthesis and the outer surface of the cell walls (G_A, $m\ s^{-1}$) and the gas phase conductance, G_G.

$$k_a = \frac{G_A \frac{A}{f_w V}}{1 + \frac{G_A P}{G_G H}} \tag{7.7}$$

where P is the atmospheric pressure. This equation assumes that, following any perturbation, the gas pool re-equilibrates extremely rapidly and may always be considered to be in a steady state. As shown below, this assumption is almost always valid, and contrasts with the situation for the aqueous and lipid pools, which are frequently in a non-steady-state condition. Importantly, the value of k_a is also strongly dependent on the solubility of the BVOC in question, defined by H (see Niinemets and Reichstein 2003a for complete derivation). In contrast to k_g, which is constrained to a relatively narrow range of values, all fairly small, the incorporation of H, which can vary over several orders of magnitude for different BVOCs (Table 7.1), allows k_a to vary over a large range. This translates into a range of liquid

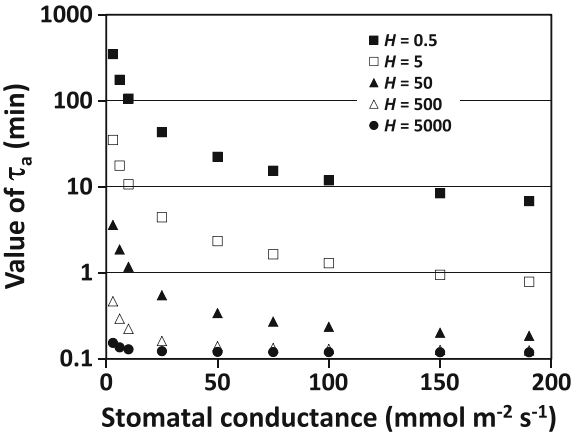

Fig. 7.8 The half-life of the aqueous pool (τ_a) as a function of stomatal conductance for a range of Henry's law constants (Pa m^3 mol^{-1}). The aqueous phase conductance, G_A, is assumed to be 10^{-5} m s^{-1} and the liquid pool size to be 100 cm^3 m^{-2}

pool half-lives, from quite short to very long. As we have seen, the latter correspond to highly soluble BVOCs, with low values of H and long pool turnover times that are often in a non-steady-state condition and thus susceptible to stomatal regulation.

The aqueous phase conductance, G_A, is a complex term, reflecting the diffusion pathway through the plant mesophyll cells and across various membranes. The length of the diffusion path and the resistances encountered differ depening on leaf architecture and the specific site of BVOC synthesis. Estimates of the liquid phase resistances encountered by various BVOCs in leaves of *Pinus sylvestris*, *Phaseolus vulgaris* and *Quercus ilex* (Niinemets and Reichstein 2003a) are available, but estimates of G_A remain somewhat uncertain and G_A is expected to differ across plant species. Fortunately, as demonstrated by Niinemets and Reichstein (2003b), the predicted leaf behavior is relatively insensitive to the value of G_A. For the purpose of illustration, we will use a value of 10^{-5} m s^{-1}, typical of short-chain aliphatic compounds (Niinemets and Reichstein 2003a), in the following analysis.

Figure 7.8 illustrates the importance of H in determining the turnover time of the aqueous pool. As expected, the pool turns over more rapidly as g_{s,H_2O} increases, the half-life of the pool (τ_a) decreasing by over an order of magnitude as g_{s,H_2O} varies from very low to high values. But the effect of H is even more dramatic, with τ_a decreasing by several orders of magnitude as H varies from 0.5 to 5,000, approximately the range observed for plant BVOCs. Note that for those BVOCs with values of H exceeding 50 Pa m^3 mol^{-1}, the half-life of the liquid pool is less than 3 min, even at very low values of g_{s,H_2O}. Stomatal control of emission is unlikely to be of significance for compounds in this range, as aqueous- and gas-phase pools will reach a new steady state following any perturbation in a few seconds to a few minutes, depending on stomatal conductance, and emission will simply equal the rate of production or the rate of release from the internal structural storage pools. As noted above, such lack of stomatal control has been reported for very insoluble compounds isoprene, α-pinene, *trans*-β-ocimene and limonene (Table 7.1), and this will be the case for all non-oxygenated terpenoids ($H > 2{,}000$ Pa m^3 mol^{-1},

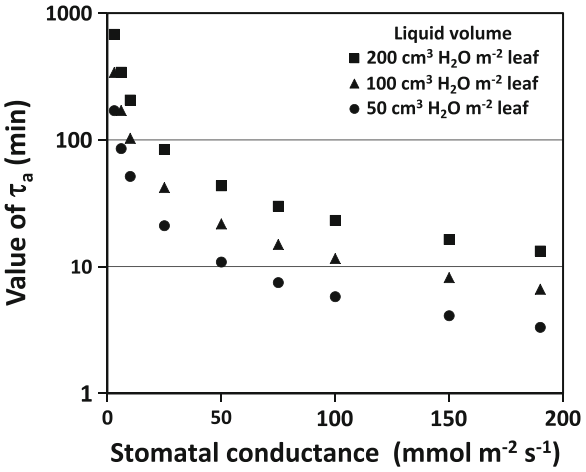

Fig. 7.9 The half-life of the aqueous pool (τ_a) as a function of stomatal conductance for a range of leaf water contents. The liquid phase conductance, G_A, was assumed to be 10^{-5} m s^{-1}, and the assumed Henry's law constant was 0.5 Pa m^3 mol^{-1}

Table 7.1). In contrast, a variety of oxygenated BVOCs, including organic acids, ketones, aldehydes and alcohols (including terpenoid alcohols) have H values less than 5 Pa m^3 mol^{-1} (Table 7.1) and have been shown to be under some level of stomatal control.

A second parameter which strongly affects the turnover time of the aqueous pool, and therefore the extent to which stomata can limit emissions, is the size of the aqueous-phase pool into which a given BVOC can partition. The rate constant, k_a, is inversely proportional to the size of the pool (Eq. 7.7) which depends on both the surface to volume ratio of the leaf and the fraction of the leaf volume occupied by liquid water. Figure 7.9 illustrates the impact of varying the liquid pool size from 50 to 200 cm^3 H_2O per m^2 of leaf on the aqueous-phase pool turnover time (τ_a). For obvious reasons, a leaf containing less liquid water into which a BVOC can partition will reach equilibrium more quickly and stomata will have less opportunity to exert control, even for very soluble compounds.

7.4.3 Dynamic Model Applications in Explaining the Stomatal Controls

As discussed above, among the early evidence of complex stomatal controls on certain trace gas emissions were the observed bursts of methanol emissions when stomata opened following a prolonged period of darkness (Nemecek-Marshall et al. 1995; Hüve et al. 2007; Harley et al. 2007). The dynamic model simulates such behavior well. To demonstrate the stomatal controls during rapid stomatal closure and subsequent re-opening, emissions of three hypothetical BVOC species with values of H ranging from 0.5 to 50 Pa m^3 mol^{-1} were simulated (Fig. 7.10). These H values encompass the range of water solubility over which changes in conductance

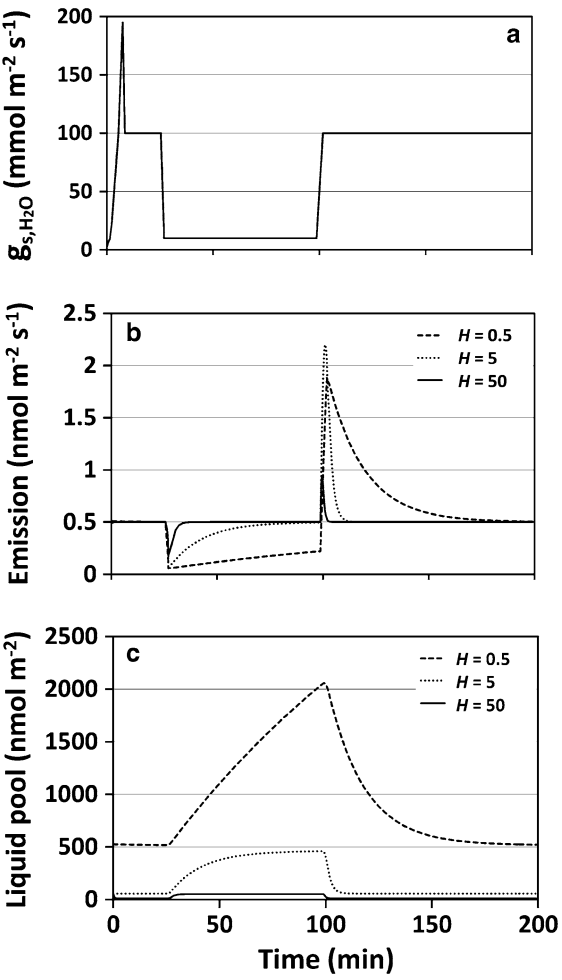

Fig. 7.10 Simulations using the dynamic model of Niinemets and Reichstein (2003a) illustrating the effect of rapid changes in stomatal conductance (**a**) on the emissions of three hypothetical BVOCs with different Henry's law constants (H, Pa m^3 mol^{-1}) (**b**). The production rate of all three is set to 0.5 nmol m^{-2} s^{-1} and G_A to 10^{-5} m s^{-1}. Also shown (**c**) are the simulated changes in the size of the aqueous pool

are expected to have a significant impact on emissions. Note that prior to stomatal closure, when the emissions are in a steady state, the liquid pool size required to sustain emission of 0.5 nmol m^{-2} s^{-1} is much greater for the most soluble BVOCs (Fig. 7.10). This reflects the circumstance that to achieve the gas phase gradient required to sustain the flux, the liquid pool concentration must be greater. Following a rapid change in g_{s,H_2O} from 100 to 10, all three BVOCs experience a transient reduction in emissions, but the pool dynamics are dramatically different. While the least soluble gas ($H = 50$ Pa m^3 mol^{-1}) reaches a new, higher, steady-state liquid pool size within 7 min, the more soluble ($H = 5$ Pa m^3 mol^{-1}) requires about an hour (Fig. 7.10). In both cases, the BVOC emission flux has ultimately returned to its pre-closure value. In the case of the most soluble gas ($H = 0.5$ Pa m^3 mol^{-1},

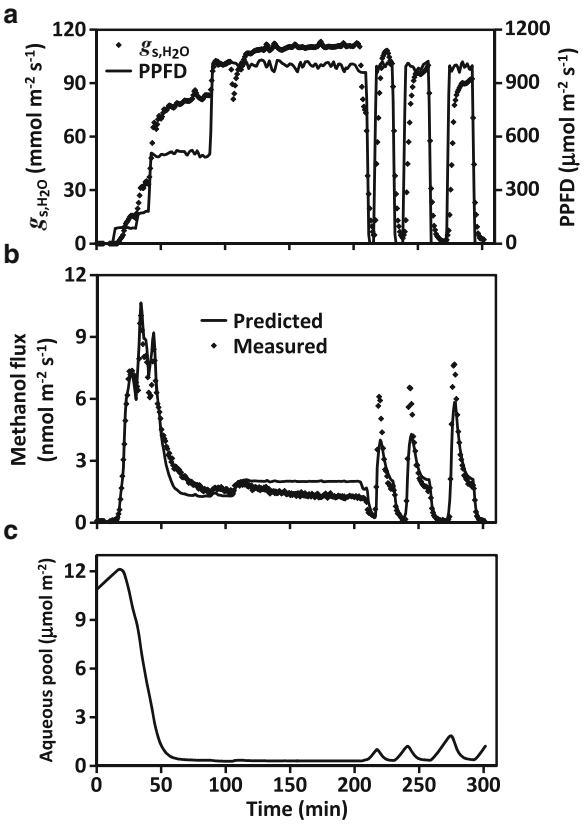

Fig. 7.11 Methanol emissions from a leaf of a sorghum-sudangrass hybrid (*Sorghum bicolor* × *S. bicolor* subsp. *sudanense*). The leaf was kept in darkness for 14 h prior to the experiment, and the light was turned on at time $t = 12$ min. Leaf temperature was 30 °C until time $t = 100$ min, when it was increased to 35 °C for the rest of the experiment. Stomatal conductance (**a**) responded rapidly to abrupt changes in PPFD, accompanied by near simultaneous changes in methanol emissions (**b**, measured). The dynamic model predicted rapid increases in liquid-phase pools of methanol upon stomatal closure and depletions upon stomatal opening (**c**), accompanied by sudden bursts of emission (**b**, predicted) (Modified from Harley et al. 2007)

similar to methanol) the BVOC is still accumulating rapidly in the aqueous phase after 75 min and the emission rate remains less than half of its pre-closure level.

Upon rapid stomatal opening at time $t = 100$ min., equilibration of the liquid pools is greatly accelerated in all three cases, because g_{s,H_2O} is much higher, but the effect of H on the time required to reach the equilibrium remains apparent. When $H \gg 50$ Pa m^3 mol^{-1}, as it is for all non-oxygenated hydrocarbons, equilibration of the liquid pools requires only a few seconds, and stomata exert no control over emissions (not shown).

Niinemets and Reichstein (2003a, Fig. 6) demonstrated that their proposed dynamic model simulated well the emission burst in the data of Nemecek-Marshall et al. (1995). Figure 7.11 provides another example (modified from Harley et al. 2007) illustrating not only a large methanol burst following a prolonged period of darkness, but also that even short periods of stomatal closure (ranging from 4 to 10 min.) are sufficient to allow the accumulation of methanol in the aqueous phase with subsequent release upon rapid stomatal opening. A leaf of sorghum-sudangrass hybrid (*Sorghum bicolor* × *S. bicolor* subsp. *sudanense*) had been in darkness for 14 h and at the end of the dark period, g_{s,H_2O} was extremely low and emissions

were near zero. When the light was turned on at time $t = 12$ min., stomata opened rapidly, accompanied by a large emission burst which lasted over an hour before a new steady state was established with emissions of approximately 1.5 nmol m^{-2} s^{-1}, presumably reflecting the rate of production. Later in the day, the leaf was subjected to three light off-light on cycles, and each time, g_{s,H_2O} fell rapidly, as did methanol emissions, to near zero. Each time the light was turned on, stomata re-opened and short bursts of methanol emissions 4 to 5 times those observed prior to darkening occurred. The size of the burst was proportional to the amount of time spent in the dark, reflecting the expected increase in the size of the aqueous pool (bottom panel) which accumulated under conditions of low stomatal conductance.

The data in Fig. 7.11 clearly demonstrate that stomata can exert some control over emissions of water-soluble BVOCs under the somewhat artificial experimental conditions of sudden and rapid stomatal closure and re-opening. Is the impact of varying stomatal conductance still apparent under more natural experimental conditions in which light and temperature are allowed to fluctuate, leading to both changes in methanol production rates and more gradual changes in g_{s,H_2O}? To address this, rates of methanol emission and g_{s,H_2O} of mature needles of loblolly pine (*Pinus taeda*) were measured during experimental modifications of temperature and light (Fig. 7.12a). Methanol production (Fig. 7.12b) was assumed to increase exponentially with increasing temperature but to be insensitive to incident PPFD. Measured emissions departed significantly from assumed rates of production, sometimes exceeding production, particularly when stomata were opening, and sometimes falling below assumed production rates, especially when g_{s,H_2O} was declining. Emissions predicted using the dynamic model of Niinemets and Reichstein (2003a) closely mimicked much of the observed behaviour, clearly implicating the stomata in short-term control of methanol emissions.

Although it is assumed that methanol production is insensitive to PPFD, g_s and PPFD clearly covary and it is problematic to assign control to one or the other. However, if methanol emissions are plotted against PPFD (Fig. 7.13a) and g_{s,H_2O} (Fig. 7.13b), a stronger relationship with g_{s,H_2O} is clearly indicated. As expected, for a given value of g_{s,H_2O}, emissions are higher when stomata are opening than vice versa. When g_{s,H_2O} is increasing, liquid- and gas-phase pools that had accumulated at lower g_{s,H_2O} are now emptying, resulting in higher emissions that exceed the assumed rate of production (Fig. 7.12c, d).

7.4.4 Dynamic Model Applications: Role of the 'Slow' Pool

To this point, I have focused on the temporary non-specific storage pool represented by the liquid fraction of the leaf, the 'fast' pool depicted in Fig. 7.4, and the consequences of this pool for highly soluble BVOCs ($H < \sim 50$ Pa m^3 mol^{-1}). I now return to the 'slow' pool residing in the lipid phase of the leaf, encountered by BVOCs as they diffuse across lipid bilayer membranes. As depicted above (Fig. 7.6) the flux from this pool is determined by the size of the lipid pool (S_l), the

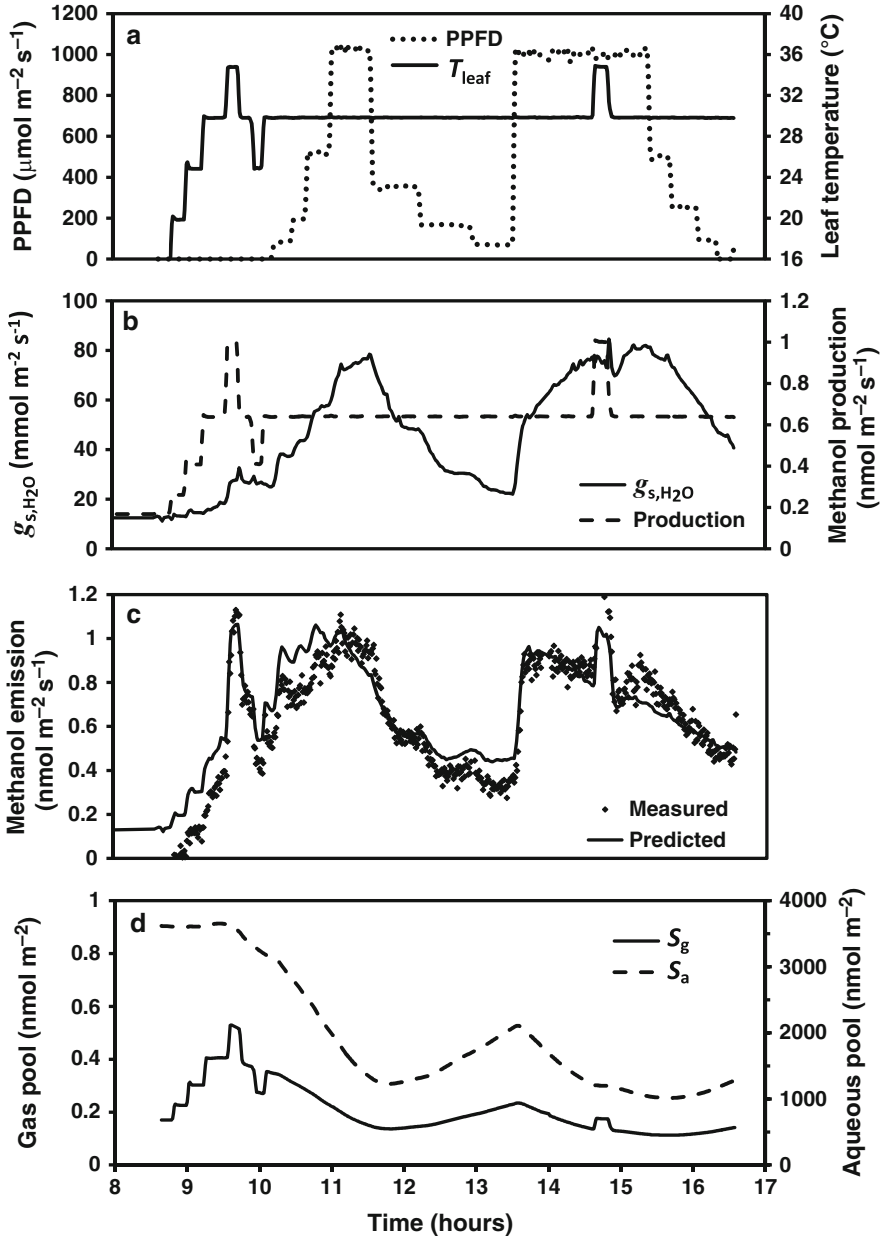

Fig. 7.12 Methanol emissions from needles of loblolly pine (*Pinus taeda*). Incident PPFD and leaf temperature (**a**) were varied and stomatal conductance (**b**) and methanol emissions (**c**) were measured. The fit obtained using the dynamic model of Niinemets and Reichstein (2003a) is shown in (**c**) and modelled rates of methanol production (**b**) and aqueous- and gas-phase pools (**d**) are also shown (Modified from Harley et al. 2007)

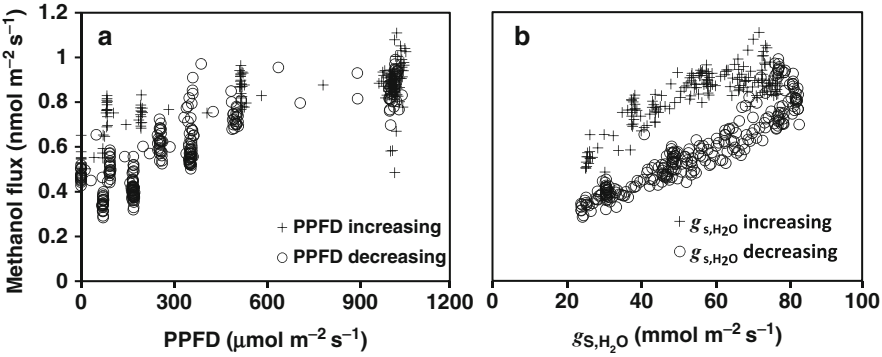

Fig. 7.13 Relationship between methanol emissions and incident PPFD (**a**) and stomatal conductance (**b**) for the data presented in Fig. 7.12. Data were divided into periods of either increasing or decreasing conductance (Modified from Harley et al. 2007)

fraction of produced BVOCs which enters this pool (1−η) and the rate constant, k_l. Analogously to the situation with respect to k_a, k_l is determined by leaf structure and diffusive properties (analysed in detail by Niinemets and Reichstein 2002) and the solubility of a given BVOC in the lipid phase. This latter property has been shown to be well approximated by the octanol/water partition coefficient ($K_{o/w}$, mol mol^{-1}) (Niinemets and Reichstein 2002; Copolovici and Niinemets 2005; Noe et al. 2006). Thus,

$$k_l = G_L \frac{A}{V f_{lip}} \left(1 - \frac{P_i}{C_l K_{A/L}}\right) \tag{7.8}$$

where G_L (m s^{-1}) is the diffusion conductance from the lipid storage pools to the substomatal cavity, C_l (mol m^{-3}) is the lipid phase concentration, P_i (Pa) is the partial pressure of the trace gas in the substomatal cavity, f_{lip} is the lipid volume fraction in the leaf (m^3 m^{-3}) and $K_{A/L}$ (Pa m^3 mol^{-1}; approximated as $H/K_{o/w}$ – see Niinemets and Reichstein 2002 for details) is the air/lipid phase partition coefficient. Thus, in addition to the leaf structural characteristics and lipid pathway diffusion resistances, k_l is related to both H and $K_{o/w}$. As shown above (Fig. 7.5), H and $K_{o/w}$ are strongly correlated. Thus, those water-soluble BVOCs with potentially large liquid phase storage pools are unlikely to have significant transient storage pools in the lipid phase, and vice versa.

The relative importance of temporary storage in the aqueous and lipid phase pools for two contrasting terpenoid species, linalool, a monoterpene alcohol ($H = 2.09$ Pa m^3 mol^{-1}; $K_{o/w} = 933$ mol mol^{-1}) and *trans*-β-ocimene ($H = 3{,}330$; $K_{o/w} = 28{,}200$) has been demonstrated in the Mediterranean conifer *Pinus pinea* (Fig. 7.14). Following the establishment of steady-state emissions, Noe et al. (2006) replaced $^{12}CO_2$ in the air stream with $^{13}CO_2$ at time $t = 10$ min., then measured the incorporation of ^{13}C into the two compounds until labelling was terminated

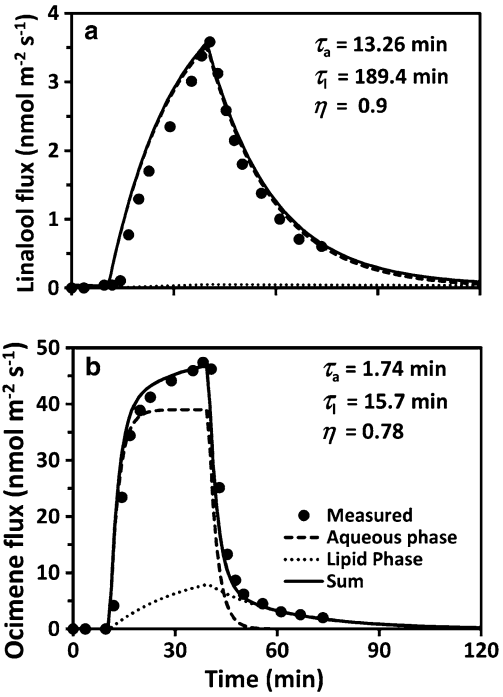

Fig. 7.14 Measured ^{13}C-labelling and de-labelling rates of linalool (**a**) and *trans*-β-ocimene (**b**) in needles of the Mediterranean conifer *Pinus pinea*. Fumigation with $^{13}CO_2$ began at time $t = 10$ min. and ended at time $t = 40$ min. Half-times of the aqueous (τ_a) and lipid-phase (τ_l) pools and the fractional partitioning into the aqueous pool (η) are as shown for both compounds. Also shown are the model simulations of labeled emissions arising from each pool and their sum (Modified from Noe et al. 2006)

at 40 min, after which they monitored the gradual loss of ^{13}C label (Fig. 7.14). Both incorporation and subsequent loss of the label were much more rapid for ocimene than for linalool, consistent with the vastly differing Henry's law constants of these compounds. Simulations obtained using the dynamic model, and assuming two storage pools, are illustrated in the figure using pool half-lives (τ_a and τ_l) and the fractional partitioning coefficients (η) indicated (for additional information on model parameterization, see Noe et al. 2006). For linalool (Fig. 7.14a), labelling and de-labelling dynamics were explained almost entirely by aqueous pool storage and storage in the lipid phase was inconsequential. For the hydrophobic ocimene, however, changes in emission rates were slower than predicted by aqueous phase dynamics alone. As expected, the aqueous phase equilibrated rapidly, and the contribution of the 'slow' lipid-phase pool was necessary to explain the long time-lags in both labelling and de-labelling kinetics (Fig. 7.14b). Thus, this simulation is consistent with the inverse correlation between H and $K_{o/w}$. Temporary storage in the lipid phase is unlikely to make a significant contribution for those water-soluble BVOCs most susceptible to stomatal control (e.g., linalool). For relatively insoluble BVOCs ($H > 50$ Pa m^3 mol^{-1}; e.g., ocimene), however, lipid phase storage explains continuing emissions of light-dependent monoterpenes in the dark in leaves of *Quercus ilex* (Loreto et al. 1996a) and the uptake and slow release of endogenously supplied monoterpenes (Delfine et al. 2000; Noe et al. 2008; Himanen et al. 2010).

7.5 Conclusions

We have seen that, in the steady state, stomatal conductance can exert no control over emissions of a trace gas produced in the leaf. In the absence of feedback inhibition on production, the BVOC gas phase concentration in the substomatal cavity will increase in direct proportion to the decrease in conductance, and the resulting increase in driving force will compensate for the increased resistance. Whether or not stomatal limitations are observed then becomes a function of the time required for a new steady-state condition to be achieved following any perturbation, e.g., a change in stomatal conductance or a change in the rate of trace gas production. The dynamic models developed by Niinemets and colleagues clearly demonstrate that the time required varies widely between different BVOCs, depending largely on their physico-chemical characteristics, as well as on leaf anatomical characteristics which affect the size of the potential aqueous and lipid phase pools and the gas phase and liquid phase resistances encountered by a gas as it diffuses from the site of production to the substomatal cavities.

The potential impact on emissions of water-soluble BVOCs due to changes in stomatal conductance can be dramatic, especially when stomata are induced to open or close rapidly by, for example, suddenly turning off the light or adding ABA to the gas stream. Under natural field conditions, with continuously fluctuating light and temperature conditions, changes in g_s are generally much more gradual, and the impact on emissions more subtle. Nevertheless, if one is interested in understanding the short-term controls over BVOC emissions, a dynamic model such as that described above, is crucial. Integrated over longer time periods, however, the importance of stomatal control becomes less evident. In Fig. 7.12, for example, at any given time the rate of methanol emission may be significantly greater or lower than the assumed rate of production, and can only be understood in the context of temporary pool dynamics. Integrated over several hours or the entire day, however, total emissions will necessarily be similar to total production since storage in aqueous and lipid pools is temporary. An exception may be the large transient increase in emissions observed in the morning when stomata open. For very water-soluble compounds, such transient increases may last several hours.

What emission model to use, and whether or not stomatal effects can be considered significant, clearly depends therefore on the nature of the question being asked. If one is interested in understanding the short-term dynamics of BVOC production and emission, it is critical to include stomatal effects for those BVOCs with Henry's law constants below approximately 50 Pa m^3 mol^{-1} (and perhaps those with high values of $K_{o/w}$, although this is much less studied). If, on the other hand, one is trying to predict average emissions on half-hourly or hourly time scales for incorporation into air quality models or chemistry-transport models, stomatal effects are less likely to strongly influence predictions, and emissions will be influenced almost exclusively by the rate of BVOC production, in which case a static model such as MEGAN (Guenther et al. 2006, 2012) may be appropriate. A clear exception to this generalization is the observed mid-day depression in

emissions of oxygenated monoterpenes in hot, dry situations (e.g., Fig. 7.3). In such a case, traditional emission models such as MEGAN (Guenther et al. 2006, 2012) will greatly overestimate emissions and the dynamic model is more appropriate.

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Chapter 8
The Role of Volatile Organic Compounds in Plant Resistance to Abiotic Stresses: Responses and Mechanisms

Malcolm Possell and Francesco Loreto

Abstract Why plants constitutively emit certain volatile organic compounds is a question that has attracted numerous researchers since the discovery of emissions. A number of hypotheses exist regarding the role of constitutive volatile organic compounds and many of these highlight the role of these compounds in enhancing plant tolerance to certain abiotic stresses. As practically any stress can modify constitutive emissions and also elicit production of novel compounds (induced emissions), this chapter provides a review of the hypotheses with particular foci on the key environmental stresses – heat and drought. Furthermore, we discuss how changes in the atmospheric CO_2 concentration over past and future geologic epochs are likely to affect the role of volatile organic compounds as an adaptation to abiotic stresses.

8.1 Introduction

8.1.1 Volatiles Released by Plants

A multitude of biogenic volatile organic compounds (BVOCs) are emitted by terrestrial and aquatic ecosystems. The emitted compounds can include alkanes, alkenes, alcohols, aldehydes, ethers, esters, carboxylic acids and a variety of isoprenoids (terpenoids). A common feature of the diverse range of compounds emitted is that they have a sufficiently low boiling point such that the vapour

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pressure at physiologically relevant temperatures is high, resulting in significant emissions to the atmosphere. All organisms have the potential to produce BVOCs and since the seminal work of Went (1960), the importance of terrestrial ecosystems, and plants in particular, as sources and sinks of BVOCs has become apparent (Kesselmeier and Staudt 1999; Fuentes et al. 2000). At least 1,700 compounds with many isomers and derivatives are currently known to be emitted by plants (Knudsen and Gershenzon 2006). These can be emitted from all parts of the plants including leaves (Loreto and Schnitzler 2010), roots (e.g., Asensio et al. 2008) and flowers (Knudsen et al. 1993). BVOC production and emission can be constitutive, in which case the emissions can be detected throughout the lifecycle of the plant or at specific developmental stages (e.g., flowering, fruit ripening or/and leaf maturation). Constitutive emissions respond to environmental drivers such as light, temperature, and atmospheric CO_2 concentration (Grote et al. 2013; Li and Sharkey 2013; Monson 2013). In addition, the emission capacity varies depending on ontogeny, nutrient availability and atmospheric CO_2 concentration during plant growth (Niinemets et al. 2010a; Monson 2013). Synthesis of specific compounds can also be induced (induced emissions) in response to mechanical wounding as a consequence of wind or herbivory (e.g., Laothawornkitkul et al. 2008), harvesting (Brilli et al. 2012), or even cellular damage caused by abiotic stresses such as drought (Capitani et al. 2009), air pollution (Pinto et al. 2007; Beauchamp et al. 2005), heat (Copolovici et al. 2012) or flooding stress (Copolovici and Niinemets 2010).

De novo biosynthesis and emission of BVOCs includes products of the lipoxygenase (LOX) pathway, such as oxylipins and green leaf volatiles (GLVs; Feussner and Wasternack 2002), a wide range of terpenoids from the mevalonate and methylerythritol 4-phosphate pathways (homo-, mono, di-, sesquiterpenes; Lichtenthaler 2010), products of the shikimate pathway (e.g., methyl chavicol and methyl salicylate) (Dudareva et al. 2006) and C1 and C2, low molecular weight, compounds such as methanol, acetaldehyde (Kreuzwieser et al. 1999; Fall 2003; Kreuzwieser and Rennenberg 2013; Monson 2013) and ethylene (Lin et al. 2009) which are formed via other mechanisms. Recently, Keppler et al. (2008) identified methane emissions from plants, under aerobic conditions, that were derived from pectin. However, methane is not normally included in BVOC analysis as these emissions likely reflected a "non-biogenic" response due to exposure to ultraviolet radiation (Bruhn et al. 2012). Thus, BVOCs are often also referred to as non-methane hydrocarbons (NMHC). BVOCs are generally classified based upon the biosynthetic pathway they originate from. They could also be classified according to their volatility or atmospheric lifetime to better understand their role in ecosystems (Arneth and Niinemets 2010; Holopainen 2011).

Advances in analytical capabilities during the last 15–20 years (reviewed by Tholl et al. 2006) have led to an increase in our knowledge of the spatial and temporal distribution of BVOC biosynthesis and emission, and the diversity of BVOCs produced. Consequently, for some highly studied compounds, such as isoprene (2-methyl-1,3-butadiene), where knowledge about the sources, biosynthesis and emission is reasonably well constrained, different emission inventories show

good agreement (Arneth et al. 2008; Ashworth et al. 2013; Guenther 2013). For other compounds, such as acetone, where there is limited knowledge of sources, biosynthesis and controls on emission, emission inventories can have large margins of uncertainty (10–148 Tg $year^{-1}$; Singh et al. 2004; Wiedinmyer et al. 2004). Consequently, the global flux of BVOCs from the terrestrial biosphere to the atmosphere is highly uncertain, but is likely in the order of 700–1,000 Tg C $year^{-1}$ (1 Tg = 10^{12} g) (Guenther et al. 1995) which is comparable to the estimated global flux of methane to the atmosphere (500–1,100 Tg $year^{-1}$; Denman et al. 2007; Adushkin and Kudryavtsev 2010). Once emitted, BVOCs play an important role in the oxidative capacity of the atmosphere (e.g., Fehsenfeld et al. 1992) and mediate the formation of secondary organic aerosol (Mentel et al. 2009; Kulmala et al. 2013). BVOC fluxes can account for 5–10 % of total net carbon exchange (Peñuelas and Llusià 2003) or about 1–2 % of the estimated global carbon assimilation by terrestrial ecosystems (Grace and Rayment 2000). The emissions of terpenes from plants are estimated to account for over half the total BVOC burden to the atmosphere (Guenther et al. 1995), but model estimates greatly diverge (Arneth et al. 2008; Ashworth et al. 2013). Obviously, the production of BVOCs by vegetation, and in particular the production of volatile isoprenoids, comes at a cost in terms of carbon and energy and it is reasonable to assume that these costs must be balanced by the benefits of production.

8.1.2 Plants in Natural Stressful Environments

Plants are exposed to a multitude of single and combined stresses at different intensities and durations throughout their lifetime (Fig. 8.1). Every environmental factor deviating from the optimum reduces the rate of primary metabolic processes, such as those associated with reproduction and growth, thereby constituting a stress to the plant (Niinemets 2010a). Plant physiological potentials and stress severity determine whether the plant can acclimate to the given stress and acquire tolerance or exhibit a hypersensitive response ultimately leading to programmed cell death or necrosis. Consideration of the effect of stress beyond the individual plant is also needed as such effects can lead to important short- or long-term shifts in population and species persistence, potentially affecting biological diversity, ecosystem functioning and carbon sequestration (Vickers et al. 2009a; Holopainen et al. 2013).

Considerable research on the response of plants to stress has focussed on the generic production of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), because they are important signalling molecules and serve to initiate defence responses (Apel and Hirt 2004). Similarly, reactive nitrogen species (RNS), especially NO, which are also generically produced under stress, are important signalling molecules, especially activating hypersensitive responses to stress (Lamattina et al. 2003).

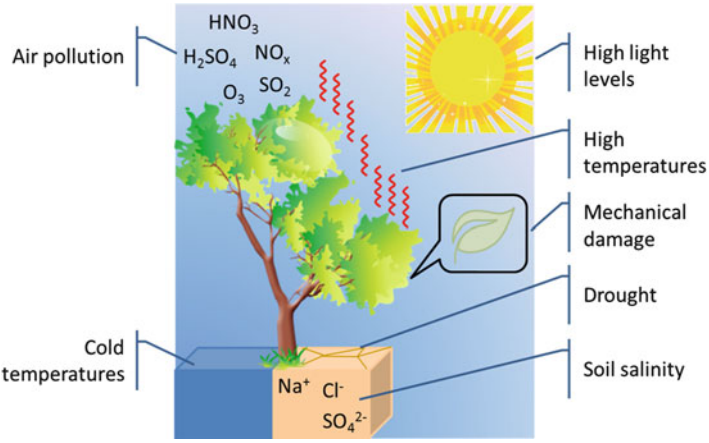

Fig. 8.1 Plants are subjected to a variety of abiotic stresses. Often these stresses coincide with each other, e.g., heat, high light and temperature or high temperatures and drought. The role of constitutive BVOC biosynthesis and emission in maintaining or enhancing plant fitness during stress events by increasing plant tolerance is still a matter of intensive investigations

Although constitutive isoprenoid emissions were initially considered to serve in plant heat stress tolerance only (Singsaas et al. 1997), a general understanding of the role of constitutive BVOC biosynthesis and emission in maintaining plant fitness during stress events by increasing broad-spectrum tolerance to the production of oxidative compounds has started to emerge (e.g., Vickers et al. 2009a; Loreto and Schnitzler 2010). Much attention has been given to heat stress and drought, reflecting the circumstance that both occur alone or in combination in many natural ecosystems (Peñuelas and Llusià 2003). In this chapter, we will focus on these key stress factors. We review the hypotheses of why plants emit certain constitutive BVOCs with an emphasis on their capacity to enhance stress tolerance. This chapter demonstrates the rich spectrum of BVOC emission responses to a variety of stresses and argues that constitutive and induced emissions play key roles in providing plants with broad tolerance to a diversity of stresses.

8.2 Abiotic Stresses: Effect on BVOC Biosynthesis and Emissions

8.2.1 Temperature

Once produced, BVOCs partition between the gas and liquid phases within plants according to their Henry's law constant, but the equilibrium between the two phases is determined by temperature (see Niinemets et al. 2004; Harley 2013).

Consequently, as temperatures increase, more BVOC is expected to enter the gas phase and be emitted from the plant. However, the anatomy of the leaf, especially the presence of specialized storage structures (ducts or glands), and the existence of diffusion resistances, can all modify BVOC emission. Although specialized storage structures can contain large amounts of volatile terpenoids, the diffusion resistances of cell walls of the storage structures are often large, such that plants with extensive storage pools can be only moderate emitters (Kesselmeier and Staudt 1999; Ghirardo et al. 2010), unless the pools are ruptured. Plants that do not store BVOCs have small, temporary pools, and BVOCs diffuse out of the leaf according to their concentration gradients. The limitation of the diffusion process by stomata depends on compound physico-chemical characteristics. Emission of BVOC species with a high Henry's law constant such as isoprene and non-oxygenated monoterpenes are weakly dependent on stomatal conductance (see Niinemets et al. 2004; Harley 2013). For these compounds, stomatal closure leads to a rapid increase in the partial pressure of the BVOC, thereby almost instantly compensating for the increased diffusion resistance. BVOCs that have a lower Henry's law constant, such as methanol or acetone, partition more into the liquid phase so that their emission rates are affected for a longer time-span after reductions in stomatal conductance (see Niinemets et al. 2004; Harley 2013). Changes in stomatal behaviour can occur for a variety of reasons including heat stress or drought.

For de novo synthesized BVOCs in species lacking specialized storage structures, temperature has a strong and immediate effect on emission rate. The response to temperature is an Arrhenius type function with a maximum at temperature optimum, beyond which catalysis and emission rates decline rapidly (see Grote et al. 2013; Niinemets et al. 2010a for a review of these functions). However, there is often a discrepancy between the optimum temperature at which the emissions are the highest compared with the optimum temperatures that give the highest activity of key rate-controlling enzymes such as isoprene synthase and monoterpene synthases. This temperature optimum varies between 40 and 45 °C for enzymes involved in the biosynthesis of chloroplastic isoprenoids and is often a few degrees higher than the emission optimum. This suggests that the temperature response of emission is the combined function of both enzyme activity and substrate supply (Rasulov et al. 2010).

With regard to isoprenoid compounds like isoprene, when temperatures increase beyond the optimum required for peak emission, the production of photosynthetic metabolites and energy needed for their synthesis is inhibited. This leads to a decline in metabolite pool size and hence emission (Singsaas and Sharkey 2000; Magel et al. 2006). If temperatures increase even further, heat-induced enzyme damage can occur which means that the emission is not re-established to the original level when temperatures are reduced (Loreto et al. 2006). However, the shape of the temperature response curve can be modified by acclimation to different environmental conditions. Acclimation of isoprene to high temperature may (Mayrhofer et al. 2005; Wiberley et al. 2005; Vickers et al. 2010) or may not (Centritto et al. 2011) occur. This is currently a controversial issue with important ramifications for accurate prediction of isoprenoid emission responses to temperature.

Leaf temperatures have been observed to fluctuate within seconds and occasionally exceed ambient temperatures by more than 10 °C (Sharkey et al. 1996; Singsaas et al. 1999). For enzymatically produced compounds like isoprene, the emissions do not respond at the same frequency. In a study by Singsaas and Sharkey (1998), the response of isoprene emissions to temperature was measured and two time constants were identified. The first time constant of 8.2 s may reflect the influence of temperature on isoprene synthase reaction kinetics, but it is too slow to fully respond to the very rapid temperature fluctuations. The second time constant of 166 s is likely related to regulation of substrate pool sizes and ultimately depends on changes in the availability of energy, reducing power and carbon.

In addition to short-term extreme temperatures, sustained moderate heat stress from tens of minutes to hours often leads to a reduction in isoprene emissions (e.g., Singsaas and Sharkey 2000) which can be related to reductions in metabolic activity (e.g., Zhang et al. 2009) affecting the production of intermediate compounds for isoprene biosynthesis (Niinemets et al. 2010b). As plants recover from prolonged heat stress, isoprene emission capacities often increase, indicating acclimation to past temperatures (Petron et al. 2001; Sharkey et al. 1999; Fig. 8.2a). Similar responses have been observed in monoterpene emitters (e.g., Loreto et al. 1998; Staudt and Bertin 1998), but the response can be moderated by the combined effect of monoterpene synthase activity and the evaporation of non-specifically stored monoterpenes (Ghirardo et al. 2010). In the case of monoterpenes that can be stored in leaf lipid and liquid phases, non-specific storage may alter the temperature responses (Niinemets and Reichstein 2003; Niinemets et al. 2010b).

Recent studies demonstrate that when the emission of volatile isoprenoids is inhibited by heat stress, the emission of other BVOCs, in particular methanol and GLVs, is enhanced (Loreto et al. 2006; Copolovici et al. 2012; Fig. 8.2b). The emission of these compounds indicates damage of cell walls and membranes, and elicitation of stress signalling pathways. Interestingly, the emission of GLVs was observed to be sustained for the entire heating period and the emissions were maintained long after temperature was returned to optimal levels. Like isoprenoids, long-term changes in temperature can also affect the emission of GLVs. Work on aspen (*Populus tremula*) where nighttime temperatures were elevated by 6–22 °C in 4 °C steps over a 6-week period, showed that GLV emissions increased significantly during the day, peaking when night- and daytime temperatures were equal (Ibrahim et al. 2010). In addition, an increase in the emissions of monoterpenes, sesquiterpenes, and the homoterpene DMNT (4,8-dimethylnona-1,3,7-triene) emission were observed (Ibrahim et al. 2010). In the same study, Ibrahim et al. (2010) discovered that the emissions of DMNT, sesquiterpenes and GLVs from silver birch (*Betula pendula*) were significantly increased by temperature. GLVs have not been the only compound class noticed to have significant fluxes during heat stress. For example, Schade and Goldstein (2001) measured enhanced methylbutenol, ethanol and acetaldehyde fluxes from a ponderosa pine (*Pinus ponderosa*) plantation during high temperature events.

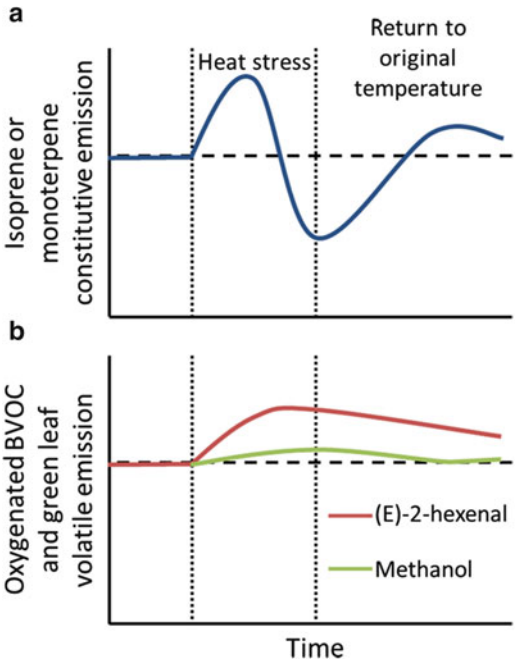

Fig. 8.2 Heat stress dependent changes in (**a**) isoprene and monoterpene constitutive emissions and in (**b**) emissions of (E)-2-hexenal and methanol. Heat stress causes the emissions of isoprene and monoterpenes to rise initially, but as the stress continues, the emissions decline. Upon stress relief, the levels of constitutive isoprene and monoterpene emissions are often higher than before the application of stress, indicating acclimation to stress. The responses of emissions of green leaf volatiles and methanol to heat stress are different from isoprene and monoterpene emissions by being sustained over the stress duration. The generalized responses in (**a**) and (**b**) are based upon Loreto et al. (1998, 2006), Staudt and Bertin (1998), Velikova and Loreto (2005), and Magel et al. (2006)

8.2.2 Drought

Drought is a key stress factor worldwide. During drought, stomatal conductance is directly affected, and combined with enhanced mesophyll diffusion resistance, causes substrate limitations to photosynthesis (Flexas et al. 2004). Intuitively, the reduction of photosynthesis and stomatal conductance would be expected to negatively impact BVOC emission by reducing the supply of carbon and energy to their biosynthesis and/or by increasing the diffusional resistance to their emission. With regard to isoprenoid emissions, drought effects primarily depend on the severity of the drought (Fig. 8.3). Mild drought has been demonstrated to neither affect isoprene (e.g., Pegoraro et al. 2004; Sharkey and Loreto 1993) nor monoterpene (e.g., Staudt et al. 2002; Peñuelas et al. 2009) emissions. Once drought becomes prolonged or heavy, isoprene and monoterpene emissions decline (e.g., Brilli et al. 2007; Peñuelas et al. 2009; Sharkey and Loreto 1993; Staudt et al. 2002).

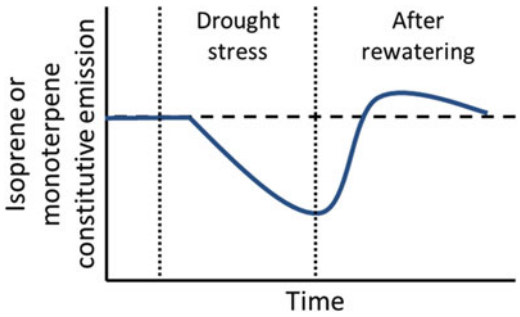

Fig. 8.3 Drought stress dependent changes in constitutive isoprene or monoterpene emissions. Mild drought stress does not affect emissions, but as the stress continues, reductions in emission occur. Upon re-watering, the level of emission is often higher than before the imposition of stress, indicating an acclimation to the stress. The generalized response is based upon Brilli et al. (2007), Peñuelas et al. (2009) and Bertin and Staudt (1996)

8.2.2.1 Isoprene Emissions Under Drought

How can isoprene emissions be sustained under drought stress? Schnitzler et al. (2004) and Loreto et al. (2004a), using ^{13}C-labelling techniques, have demonstrated that while the photosynthetic carbon input is curtailed, alternative carbon sources from starch breakdown or respiration can compensate for reductions in the primary carbon source. Indeed, work by Brilli et al. (2007) has shown that there is a preference for 'old' unlabelled (^{12}C) carbon over recently fixed, ^{13}C-enriched photosynthetic intermediates when photosynthesis and isoprene production become uncoupled during drought stress. The use of this alternative carbon may also explain enhanced isoprene emission rates after re-watering that have been observed in some studies (Sharkey and Loreto 1993; Peñuelas et al. 2009). Once isoprene emission and photosynthesis are re-coupled, the use of alternative carbon sources rapidly ceases (Brilli et al. 2007).

The need to consider the CO_2 response of isoprene (Grote et al. 2013; Monson 2013) is particularly important under plant stress. Niinemets et al. (2010b) have used the shape of the isoprene-CO_2 response as a way to explain isoprene emissions under different drought conditions. In this paper, they argue that the severity of a particular drought event can result in different C_i values and hence different isoprene emission rates. When mild drought conditions occur, they state that C_i will decrease by approximately 50–80 μmol mol^{-1}. A reduction of this magnitude has a small positive effect on isoprene emissions and depending upon the species, may result in no observable changes in emissions or a slight increase. When severe drought occurs, C_i values drop to close to the photosynthetic compensation point, which is where isoprene emissions drop off dramatically. This view may be an oversimplification because (a) when already photosynthetic rate-limiting proteins are downregulated, there might be a reduced sink for CO_2 and CO_2 can accumulate in the intercellular spaces due to respiration. However, this is common only when the

stress is severe and isoprene is probably already limited by substrate availability; (b) low CO_2 acquisition through stomata may be in part compensated by refixation of CO_2 produced by other sources, especially mitochondrial respiration. Standard gas-exchange methodologies allow calculation of the C_i without discerning its carbon sources, but refixed CO_2 does likely contribute to isoprene emission (Loreto et al. 2004a). Furthermore, changes may occur in isoprene synthase activity and source of carbon supply under extreme drought (Brilli et al. 2007; Fortunati et al. 2008). Overall, the existence of a negative correlation between C_i and isoprene emission is now unambiguous, even in un-stressed plants (Guidolotti et al. 2011).

A question remains as to whether the maintenance of isoprene production under drought stress improves plant function and fitness? Studies in transgenic *Arabidopsis* plants containing an isoprene synthase gene concluded that the presence of the trait did not increase drought resistance (Sasaki et al. 2007). Work by Fortunati et al. (2008) showed that during severe drought stress and during the recovery from stress, the temperature dependency of isoprene emission is modified. Although not explicitly demonstrated, Fortunati et al. (2008) postulated that alteration of the temperature response likely resulted from changes in substrate availability and/or isoprene synthase protein concentrations.

8.2.2.2 Drought Effects on Monoterpenes and Oxygenated Volatile Emissions

The effect of drought stress on monoterpene emissions has not been as intensively studied as drought effects on isoprene emissions. Nevertheless, there are field and laboratory observations showing that inhibition of monoterpene emission occurs under severe drought stress, while the emission rates are maintained under less severe stress (e.g., Peñuelas et al. 2009; Bertin and Staudt 1996; Šimpraga et al. 2011). As with isoprene, differential availability of carbon substrates may explain these observations. However, drought may change qualitatively the composition of the monoterpene blend because of differences in the Henry's law constant of monoterpenes. In practice, non-oxygenated terpenes are unaffected by moderate drought, while the emissions of oxygenated volatiles are strongly curbed (Niinemets et al. 2002; Harley 2013).

In terpene-storing species, the effect of drought on monoterpenes might depend upon the season. In a study on Mediterranean woody species, Llusià et al. (2006) showed that leaf concentrations of monoterpenes were greatest in winter and lowest in summer, but responses during drought were highly variable. For example, in Aleppo pine (*Pinus halepensis*), the concentrations decreased in response to drought in winter and increased in summer. In contrast, drought decreased monoterpene concentrations in summer and increased them in winter in *Pistacia lentiscus*. The differences in concentrations were associated with different drought responses of emissions. Emission rates of monoterpenes from Mediterranean shrublands were more strongly affected by drought during spring and summer than during autumn

and winter (Llusià et al. 2008). Even fewer studies have been done on drought effects on sesquiterpenes, but these studies indicate a rapid decline in emissions with increasing drought (Ormeño et al. 2007).

Oxygenated BVOCs, such as methanol or acetone, partition more into the liquid phase than the gas phase (Niinemets et al. 2004). Consequently, stomatal closure in response to drought and salt stress would be expected to reduce the emission of these compounds and affect the gaseous emissions over a much longer time-span than the emissions of isoprene or non-oxygenated volatiles (see Niinemets et al. 2004; Harley 2013). However, even in plants subject to drought stress, morning peaks of methanol and acetone emission, coincident with stomatal opening, have been observed (Fares et al. 2009; Filella et al. 2009) corresponding to "outgassing" of a large liquid-phase pool built up during the night when stomata were closed (Niinemets and Reichstein 2003; Harley et al. 2007; Harley 2013). Green leaf volatiles can be formed also under severe drought if the stress leads to the cell wall and membrane damage (Capitani et al. 2009), although there are much less data for drought effects on GLV than for the effects of other stresses.

8.2.3 Salinity Stress

The effect of salinity on photosynthesis and stomatal conductance is similar to that of drought with significant decreases observed as salinity increases (Loreto and Delfine 2000; Teuber et al. 2008). Therefore, like drought, the reduction of photosynthesis and stomatal conductance would be expected to negatively impact BVOC emission by either reducing the supply of carbon and energy to their biosynthesis or by increasing the diffusional resistance to their emission. However, isoprene emission rates measured under conditions of transient or continual salinity stress were found to be unaffected relative to controls (Loreto and Delfine 2000; Teuber et al. 2008). In contrast to the isoprene emission rates, Teuber et al. (2008) showed that in grey poplar (*Populus* x *canescens*), continuous salinity stress led to a decrease of the leaf concentrations of the immediate substrate for isoprene synthesis, dimethylallyl diphosphate (DMADP). They argued that this decline is probably due to the limited availability of photosynthates for isoprene biosynthesis (Teuber et al. 2008). Thus, the high demand of isoprene synthase for its substrate in salt-stressed plants results in an increased turnover rate of DMADP in the salt-stressed plants. Analogous to isoprene, acetaldehyde, formaldehyde and acetone emission rates were not influenced by salinity in this study (Teuber et al. 2008).

Very few studies have looked at the effect of salinity on monoterpenes. In perennial evergreen sage (*Salvia officinalis*), leaf monoterpene contents were found to increase with increasing salinity, but once the salinity reached a threshold level, monoterpene content began to decline (Ben Taarit et al. 2009). The same study also found that the composition of the monoterpenes changed depending upon the level of salinity. Similar results have been found in marjoram (*Origanum majorana*) (Jelali et al. 2011).

8.2.4 Combined Stresses

In addition to heat, drought and salinity, plants can be subjected to a number of other environmental stresses. The effects of flooding on BVOC emissions are reported in the chapter of Kreuzwieser and Rennenberg (2013), and the effects of air pollution in chapters of Calfapietra et al. (2013) and Holopainen et al. (2013). However, stresses often occur in combination, for example, drought may occur with increased salinity and/or heat or biotic stress can also occur with abiotic stress (Loreto and Schnitzler 2010; Niinemets 2010a). Although we could predict this behaviour based upon the known responses for each factor, the response by a plant can be unique and not directly extrapolated from the response of plants to each of the stresses applied individually (Mittler 2006; Niinemets 2010b). For instance, important modifications in the temperature response of isoprene emission rates under drought have been observed. Individually, an increase in drought and temperature would normally lead to an increase in isoprene emission, but Fortunati et al. (2008) discovered that, after drought, the plants in higher temperature treatment had suppressed emissions for at least for 2 weeks (i.e., leaves from 25 and 35 °C treatments showed the same isoprene emission rates), despite photosynthesis quickly returned to pre-stress levels. Work by Centritto et al. (2011) not only supported this finding but also demonstrated that increases in temperature cannot offset the inhibition of isoprene under water-stress. This highlights the need for more multi-factorial studies focusing on BVOC biosynthesis and emission and leads us to question how the biosynthesis and emission of constitutive BVOCs can enhance stress tolerance, especially in multi-stress situations?

8.3 How Do BVOCs Enhance Stress Tolerance?

Obviously, the constitutive biosynthesis and emission of BVOCs must benefit the plant in some way. As highlighted above, the biosynthesis and emission of BVOCs, especially isoprene, appears to be tuned to changes in the level of abiotic stress that a plant is exposed to. If the plant is benefiting from volatile emissions in some way, how is it benefiting and through what mechanism (Fineschi et al. 2013)? Studies of transgenics either engineered to emit isoprene (Sasaki et al. 2007; Velikova et al. 2011; Vickers et al. 2011) or to knock out isoprene (Behnke et al. 2007; Rosenkranz and Schnitzler 2013) have conclusively indicated improved abiotic stress tolerance in isoprene emitting genotypes. These studies are reviewed in detail in the chapter of Rosenkranz and Schnitzler (2013). Here we analyse the evidence from both wild, and to some extent transgenic plants that highlights the specific mechanisms via which isoprene enhances abiotic stress resistance.

As a consequence of the dominance of isoprenoids, and in particular isoprene, in BVOC emissions (e.g., Guenther et al. 1995) the majority of studies of different stress factors have also concentrated on isoprene. This was best illustrated by Peñuelas and Staudt (2010) were they showed that research into isoprene dominated

all the potential environmental stress factors with respect to global and climate change drivers. Given the wealth of the volatiles released by plants, we echo the plea for more detailed studies of other key BVOCs.

8.3.1 Membranes

One of the oldest hypotheses as to why plants produce isoprene is that it enhances the thermotolerance of the photosynthetic apparatus by stabilising the chloroplastic (thylakoid) membranes under transient heat shocks (Sharkey and Singsaas 1995). This was based upon two main observations. First, higher rates of photosynthesis and electron transport were measured from plants fumigated with isoprene when they were exposed to rapidly increasing temperatures. Second, previous reports indicated that other isoprenoid compounds are important in membrane physiology (Ourisson and Nakatani 1994). Subsequent experiments have confirmed that isoprene does confer thermotolerance to photosynthesis (e.g., Sharkey et al. 2001; Velikova et al. 2006; Behnke et al. 2007, 2010; Way et al. 2011) and this has also been demonstrated with light-dependent monoterpene emissions (Loreto et al. 1998; Delfine et al. 2000) but only for certain monoterpenes (Copolovici et al. 2005). In all of these studies, photosynthetic rates measured after stress were significantly higher than in plants with suppressed isoprene or monoterpene emission rates.

In silico studies by Siwko et al. (2007) indicated that isoprene partitions into the centre of the phospholipid membranes and maintains their order without significantly changing the dynamic properties of the membrane (Fig. 8.4a). Isoprene occupies the volume between the lipid tails thereby increasing the adhesive forces and acting as molecular glue. As temperatures decrease, an exponential decline in isoprene biosynthesis would concurrently occur preventing solidification of the membrane. Conversely, an increase in temperature would lead to more isoprene and greater membrane stability. Consequently, Siwko et al. (2007) concluded that isoprene-producing plants could maintain membrane in functional liquid-crystalline phase and photosynthetic activity over a wider temperature range. However, Logan et al. (1999) failed to demonstrate isoprene protection on fluidity or functions of reconstituted membranes. Indirect evidence that isoprene stabilises thylakoid membranes comes from the use of inhibitors of isoprene production even in the absence of heat stress. However, Possell et al. (2010) showed that the application of fosmidomycin to inhibit isoprenoid production led to significant reduction in the operating efficiency of photosystem II. Recently, laboratory studies by Velikova et al. (2011), using three different techniques, showed that isoprene improves the integrity of the photochemistry of photosynthesis under heat-stress conditions by affecting large-scale thylakoid membrane organisation. The authors also demonstrated that a continuous biosynthesis of isoprene was necessary for this to occur, which would be consistent with the need for a volatile substance that could rapidly escape after transient heat shocks to prevent transition of membranes from liquid-crystalline phase to solid-gel phase (Siwko et al. 2007).

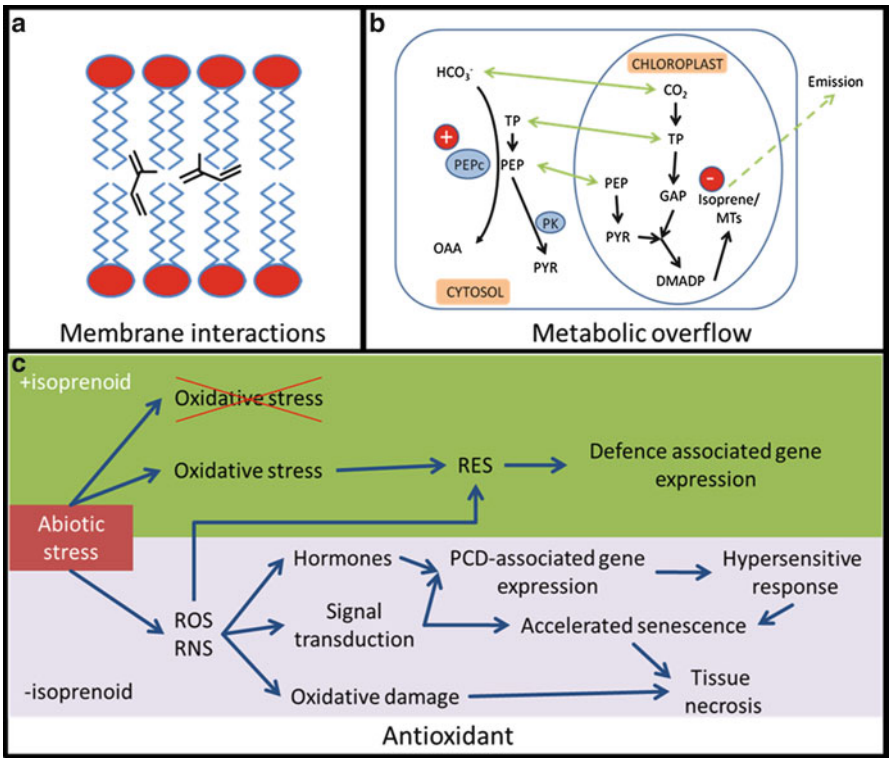

Fig. 8.4 Schematic overview of the proposed metabolic functions of volatile terpenes: (**a**) stabilisation of phospholipid membranes under heat stress by isoprene as indicated by *in silico* modelling (Siwko et al. 2007); (**b**) 'metabolic overflow hypothesis' as proposed by Rosenstiel et al. (2004) and (**c**) the 'single biochemical mechanism for multiple physiological stressors' model proposed by Vickers et al. (2009a). The model shows how volatile isoprenoids may exert protective effects through antioxidant activity. In (**b**), the *negative sign* indicates the impact of an increase (*positive sign*) in phosphoenol pyruvate carboxylase (PEPc) activity on isoprenoid biosynthesis and emission. DMADP – dimethylallyl diphosphate, GAP – glyceraldehyde 3-phosphate, MTs – monoterpenes, OAA – oxaloacetate, PEP – phosphoenol pyruvate, PEPc – phosphoenol pyruvate carboxylase, PK – pyruvate kinase, Pyr – pyruvate, TP – triose phosphate. In (**c**), PCD – programmed cell death, RES – reactive electrophilic species, RNS – reactive nucleophilic species, ROS – reactive oxygen species

8.3.2 Role as Antioxidants

Under stress conditions, a variety of ROS are produced within plant cells, which once left detoxified, can cause significant damage. Plants have a well-developed antioxidant system consisting of liphophilic (e.g., tocopherols and carotenoids), hydrophilic (e.g., ascorbate and glutathione) and enzyme components which together mediate the effect that ROS have upon the plant cell. The production of ROS even under non-stressed conditions is an integral part of photosynthetic apparatus. Hence,

ROS concentrations are tightly regulated by scavenging excess ROS to prevent cytotoxic effects (Apel and Hirt 2004). Experiments with inhibitors of isoprene biosynthesis and those using transgenic plants have demonstrated that the presence of isoprene keeps ROS and the levels of lipid peroxidation to a level much lower than isoprene-inhibited or non-emitting plants (e.g., Loreto and Velikova 2001; Velikova et al. 2006, 2012; Vickers et al. 2009b). These findings indicate that volatile isoprenoids have a role in moderating the oxidative load under stress conditions.

How volatile isoprenoids moderate oxidative load is still a matter of debate, but the current evidence points to two main avenues: either direct reactions between volatile isoprenoids and oxidizing species or mediation of the signalling responses (Fig. 8.4c). Many BVOCs, especially isoprenoids, contain double bonds which make them susceptible to oxidation via reactions with ROS. When an oxidizing agent, such as ozone, enters a leaf, it reacts with reactive hydrocarbons, but it can also elicit a cascade of reactions potentially resulting in formation of other damaging ROS. Thus, a capacity to rapidly regulate plant oxidative status is essential. In this regard, it is relevant that hydroxyl radicals react with isoprene in the aqueous phase to form 2-methyltetrols (Santos et al. 2006). Isoprene is also known to protect against singlet oxygen (Affek and Yakir 2002; Velikova et al. 2004) which is produced at the thylakoid membranes when absorbed energy is in excess of that used in photosynthesis (Asada 2006). This may occur because excess light is present at high light intensities and/or because the use of excitation energy is retarded by various abiotic stresses (Vickers et al. 2009a).

Mediation of signalling responses is the other possible mechanism by which BVOCs such as isoprenoids can contribute to protective effects. Isoprene is oxidized to methyl vinyl ketone (MVK) and methacrolein (MAC) in the atmosphere (Fuentes et al. 2000), and the same compounds have been recently demonstrated to appear when isoprene is oxidized within leaves (Jardine et al. 2011). Both MVK and MAC are reactive electrophilic species (RES) and, along with unsaturated carbonyl compounds such as GLVs like (E)-2-hexenal, are known to stimulate the expression of defence genes (Almeras et al. 2003) (Fig. 8.2c). Similarly to isoprene, nitric oxide (NO) is involved in a number of stress-related physiological processes including the direct scavenging of ROS (Beligni and Lamattina 2002) and the plant hypersensitive response (Gould et al. 2003; Wilson et al. 2008). Interestingly, isoprene-emitting leaves produce less NO compared to isoprene-inhibited leaves when subjected to oxidative stress (Velikova and Loreto 2005; Velikova et al. 2008). Although the exact mechanism for these observations is unknown, any change in NO concentration can potentially mediate the hypersensitive response and the onset of programmed cellular death.

Unlike exposure to ozone or heat stress, there does not appear to be any evidence that isoprene or monoterpenes protect against the consequences of flooding. ROS formation in leaves with flooded root systems has been shown to play a role in flooding-driven decline in plant physiological activity (Visser et al. 2003; Yordanova et al. 2004; Kreuzwieser and Rennenberg 2013). Thus, isoprenoids could play an important role in quenching ROS in flooded plants. However, Copolovici and

Niinemets (2010) demonstrate that the capacity for isoprene emission was not linked to flood tolerance in three species studied. Isoprene emission was lacking in the most flood-tolerant species black alder (*Alnus glutinosa*), while the species with highest isoprene emission rate red oak (*Quercus rubra*), was most intolerant of flooding and could not sustain high isoprene emission rates under flooding.

8.3.3 Safety Valve

A number of studies have observed that the emission of isoprene is strongly correlated with photosynthetic electron transport (e.g., Possell et al. 2004) and hence leaf ATP content (Loreto and Sharkey 1993; Rasulov et al. 2009b). On a molar basis, a considerable amount of photosynthetic energy is utilised in the biosynthesis of isoprene (20 ATP, 14 NADPH; Sharkey and Yeh 2001). In reality, the consumption of energy for isoprene production under non-stressed conditions is relatively small. The emission of isoprene is measured in nmol m^{-2} s^{-1}, and the emissions are typically an order of magnitude smaller than the rate of photosynthesis. Sharkey et al. (2008) estimated that for an isoprene emission rate set at 2 % of photosynthesis only 2.7 % of ATP and 3.4 % of NADPH is required for isoprene biosynthesis compared to the 20–40 % used in photorespiration. Based upon these calculations Sharkey et al. (2008) argued that the postulation by Magel et al. (2006) that the function of isoprene emission was to dissipate unused energy does not hold up to quantitative scrutiny. However, if under high temperature and high light, up to 50 % of the isoprene produced in the chloroplast is actually oxidised to MVK and MCR prior to leaving the leaf, then the sink for electrons would be much higher (Jardine et al. 2011).

Rosenstiel et al. (2004) hypothesised that isoprene synthase, and its associated isoprene emission, is a mechanism for balancing the demand for DMADP (by higher isoprenoid biosynthesis) against the supply of DMADP (Fig. 8.4b). This would ensure that chloroplastic DMADP will be available for the crucial synthesis of carotenoids and chlorophyll when it is needed, and isoprene synthase prevents DMADP from accumulating to levels that unnecessarily sequester phosphate. According to this hypothesis, cytosolic phosphoenol pyruvate carboxylase plays a pivotal role in dividing the phosphoenol pyruvate pool between isoprene biosynthesis and other forms of carbon metabolism (Fig. 8.2b). Sharkey et al. (2008) argued that because of the sensitivity of the first enzyme in the MEP pathway to feedback from other metabolites of the MEP pathway, there is no need for this type of regulatory mechanism. Although methods to investigate MEP pathway substrates exist (e.g., Rasulov et al. 2009a) and information on how the MEP pathway is regulated is being ascertained (e.g., Rivasseau et al. 2009; Mongelard et al. 2011; Li and Sharkey 2013 a, b), it is still not entirely clear how feedback modulation of the MEP pathway operates and by how much chloroplastic DMADP fluctuates.

8.3.4 Indirect Consequences on Ecosystem Engineering

Hastings et al. (2007) defined ecosystem engineering as a concept that focuses on how organisms physically change the abiotic environment and how this feeds back to the biota. The concept applies well to the feedbacks between BVOCs produced by the ecosystems and the atmosphere. A consequence of the emission of BVOCs into the atmosphere is that their oxidation leads to the formation of secondary organic aerosols (SOA). The formation of SOA has been demonstrated in both the laboratory (O'Dowd et al. 2002; Joutsensaari et al. 2005; Hao et al. 2009; Kiendler-Scharr et al. 2009; Mentel et al. 2009; Virtanen et al. 2010) and forests for isoprene (Claeys et al. 2004), monoterpenes (Tunved et al. 2006; Kiendler-Scharr et al. 2009; Virtanen et al. 2010), sesquiterpenes (Boy et al. 2008), oxygenated VOCs (Mentel et al. 2009) and GLVs (Hamilton et al. 2009). Aerosols modify the Earth's radiative balance though direct scattering of radiation, the formation of cloud condensation nuclei that lead to enhanced cloud albedo, or even absorbing energy and promoting warming (Ramanathan et al. 2007; Spracklen et al. 2008; Kulmala et al. 2013).

Vegetation is sensitive to changes in radiation, in particular to the diffuse and direct fractions of sunlight. Roderick et al. (2001) showed that increases in particulate matter increased the amount of diffuse radiation and this penetrated inside tree canopies enhancing the overall photosynthetic rate. Therefore, any stress that leads to changes in the emission of BVOCs has the potential to affect SOA formation, radiative forcing and photosynthetic rates (Kulmala et al. 2013). As woodland landscapes are estimated to contribute to 75 % of the total annual BVOC emissions (Guenther et al. 1995) any rapid change in BVOC emission has the massive potential to rapidly influence, or engineer, their surrounding environment. However, the potential benefits of SOA formation from BVOCs for the ecosystem have to be balanced against the potential negative effects of BVOC oxidation, such as ozone formation, and the consequences these effects have upon the ecosystems (Lerdau 2007).

8.3.5 Multifunctional Compounds?

Vickers et al. (2009a) argued that volatile isoprenoids played an important role in the protection against abiotic stresses by helping plants improve their ability to deal with oxidative changes regardless of the nature of the stressor. The authors suggest that the activity of ROS is mediated through the combined action of direct reactions with ROS species and the indirect consequences this has for ROS signalling. The authors also state that membrane stabilisation decreases lipid peroxidation, thus directly impacting the oxidative status of the cell. Indeed, Velikova et al. (2012) argue that the stabilisation of the thylakoid membranes by isoprenoids may be the reason for reduced ROS levels observed in heat-stressed plants. Until these questions are resolved, there will be a continued debate within the BVOC community as to the primary function of isoprene. However, the biosynthesis of isoprene may actually

be multifunctional. A compound that can stabilise membranes against transient heat stress and minimise ROS production, remove ROS through direct reactions and through its reaction products, generate reactive electrophilic species (RES) that initiate the expression of defence genes, while at the same time modifying the atmosphere to enhance photosynthetic light use efficiency, is certainly worth the substantial investment in carbon and energy.

8.4 Evolution of the Stress Tolerance Function

Plants produce a wide range of BVOCs but not all plants produce the same BVOCs and it remains to be seen if plants have the capacity to produce a wider range of BVOCs than those measured so far. In the sections above, we saw evidence that isoprene biosynthesis and emission play a significant role in tolerating heat, drought and oxidative stress induced by pollutants. A puzzling issue of isoprene in stress tolerance is that given the many benefits isoprene confers for stress tolerance, why not all plants have evolved the capacity to produce it? Isoprene emitters can be found among mosses (Hanson et al. 1999), ferns (Tingey et al. 1987) and in gymnosperms and angiosperms (see http://bai.acd.ucar.edu/Data/BVOC/for a comprehensive list and (Fineschi et al. 2013) for a detailed discussion), but not all species of a particular genus make isoprene. For example, species in the sub-genus *Sclerophyllodrys* from the genus *Quercus* are light-dependent monoterpene emitters, while the remaining sub-genera (except for species from sub-genus *Cerris*, which are predominantly non-emitters) are isoprene emitters (Loreto 2002).

Evolutionary aspects of BVOC emission have been discussed in some detail in several other chapters in this book (Fineschi et al. 2013; Li and Sharkey 2013; Rajabi Memari et al. 2013). Monson et al. (2013) hypothesise that there is a narrow range of conditions in which isoprene is advantageous as protection against stress. Dealing with the stress tolerance function of isoprene, we only surmise here that changes in atmospheric CO_2 concentrations might have been a selective filter, modifying selection pressures for or against the evolution of isoprene biosynthesis. Recent studies by Darbah et al. (2010) and Way et al. (2011) into thermotolerance under different [CO_2] regimes suggested greater tolerance under low [CO_2] conditions than elevated [CO_2] conditions. Thus, Way et al. (2011) argued that the evolutionary pressure that led to the independent evolution of isoprene emission was a low [CO_2] atmosphere. This supports the views of Sharkey and Yeh (2001) that the evolution of isoprene emission was to cope with environmental conditions not conducive to photosynthesis. Indeed, there has been a number of epochs in Earth's history where CO_2 concentrations have been lower than the current concentration and such conditions can make photosynthesis more susceptible to heat and light damage (Cowling and Sage 1998).

We must also consider that selective pressures could lead to the loss of isoprene emission in different genera and epochs. For example, if a low CO_2 concentration could lead to the evolution of isoprene biosynthesis, then an elevated

[CO_2] atmosphere could lead to its loss. Evidence from long-term [CO_2]-isoprene studies does show a suppression of isoprene emissions by reducing both substrate availability and enzyme activity (Scholefield et al. 2004; Possell and Hewitt 2011; Calfapietra et al. 2013) (but see Sun et al. 2012). However, explanations of the patterns of why plant lineages do or do not emit isoprene also have to consider complex interactions with other environmental factors, plant phenotypes and patterns of geographic migration (Monson et al. 2013).

The mass production of a single, volatile compound, particularly over a long period of time would be an inefficient way to sustain protection. Indeed, there is no evidence to date that isoprenoid emitting species cope better in the long-term in the presence of environmental stresses. However, the production of reactive electrophilic species (RES) from the oxidation of volatile isoprenoids could lead to the biosynthesis of efficient mechanisms for protection or defence (e.g., Almeras et al. 2003) which can result in acclimation and tolerance to a particular stress. For example, other known thermotolerance mechanisms, such as changes in xanthophyll epoxidation state, synthesis of heat shock proteins, and changes in membrane lipid composition, can take many minutes, hours, or days to respond to a temperature episode (Singsaas and Sharkey 1998). Sustained stress results in the reduction of constitutive BVOCs in most cases (see above) and only the prolonged fumigation with ozone can lead to a sustained increase in emissions (Velikova et al. 2005a). Once the stress has been relieved, however, increases in emissions have been observed. This has been observed after heat stress (Velikova et al. 2005b), drought (Sharkey and Loreto 1993; Pegoraro et al. 2004; Peñuelas et al. 2009), salinity (Loreto and Delfine 2000; Teuber et al. 2008), and ozone (Loreto et al. 2004b). Niinemets (2010a) suggested that the previous condition now constitutes a new mild stress for the leaves acclimated to the stressed environment, explaining maintenance of the higher emissions, or it may prime the defensive apparatus to maintain an active antioxidant system in order to rapidly react to other forthcoming stresses.

8.5 Summary and Conclusions

A large number of stress factors affect the emission of BVOCs and the existing tolerance, timing, duration and severity of the stresses is known to mediate the magnitude of the emissions of constitutive BVOCs or induce *de novo* synthesis of new induced BVOCs (e.g., Loreto et al. 2006). In this chapter we have seen that this is especially true for the biosynthesis and emission of isoprenoids, oxygenated BVOCs and GLVs in response to heat and drought stress. Research in the role of BVOCs in stress tolerance has predominantly concentrated on isoprene, and to a lesser extent monoterpenes, but substantially more research is required with regard to other BVOCs that may be more difficult to detect and to better understand the role other isoprenoids and non-isoprenoid compounds undertake in stress tolerance and acclimation. The development of transgenic plants that have had isoprene synthase genes added or silenced is helping us understand the role that isoprene plays in

stress tolerance and the mechanism by which it performs this function (Rosenkranz and Schnitzler 2013). Similar developments in identifying genes responsible for other BVOCs would be a necessary next step in assessing their potential in plants acquiring stress tolerance to abiotic stress, biotic and multi-factorial stresses, and in maintaining fitness and survival. Such research may allow us to identify common mechanisms, such as the one suggested by Vickers et al. (2009a) for isoprenoids, which will enable us to improve and simplify our understanding of plant responses to stress. These developments could aid in the improvement of models of BVOC emissions under transient and complex environmental conditions, allowing for better assessment of the impact BVOCs have upon atmospheric chemistry, climate and their associated feedbacks.

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Chapter 9
Flooding-Driven Emissions from Trees

Jürgen Kreuzwieser and Heinz Rennenberg

Abstract Anoxia in the root system leads to the formation of ethanol in roots, the transport of ethanol to the leaves and strong foliar emissions of ethanol and acetaldehyde. In addition, emissions of typical stress-related volatiles are elicited. This chapter reviews the environmental, biochemical and physiological controls on flooding-driven products of anoxic metabolism and stress signalling compounds. It demonstrates that the various controls operate at different timescales and, furthermore, that these emissions are characterized by strong differences between species.

9.1 Introduction

Higher plants are aerobic organisms depending on a continuous supply of oxygen which is required to maintain crucial physiological processes such as mitochondrial respiration (Drew 1997). Nevertheless, if adverse environmental stress factors lead to diminished or even completely reduced oxygen availability, plants can survive for a limited period of time. The fate of plants stressed by anoxia (complete lack of oxygen) or hypoxia (a limited amount of oxygen is still available) strongly depends on plant species and age, season, duration, and severity of the stress event, and other environmental conditions during stress (Drew 1997). Depending on these factors, plants can survive stress periods for up to several months or are severely injured after only some hours under stress (Drew 1997; Vartapetian and Jackson 1997). Stress tolerance can be a consequence of (i) morphological adaptations helping to avoid or delay the occurrence of oxygen deprivation and/or (ii) changes at the metabolic level required to endure the period of reduced oxygen supply (Bailey-Serres and

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Voesenek 2008). Several omics approaches with anoxia-tolerant and -sensitive herbaceous and tree species have indicated that metabolic adaptations to anoxia or hypoxia need well-orchestrated changes in numerous physiological processes, including the onset of fermentative processes, a stimulated flux through glycolysis, and the down-regulation of energy demanding processes (Klok et al. 2002; Liu et al. 2005; Loreti et al. 2005; Lasanthi-Kudahettige et al. 2007; Kreuzwieser et al. 2009; Licausi et al. 2010).

Because the lack of oxygen dramatically affects mitochondrial respiration, the plants' main process of ATP production, energy metabolism is strongly impaired by anoxia or hypoxia. The ability to induce alternative ATP generating pathways in stress-exposed tissues is therefore essential for the plants' survival. In particular, fermentative pathways play a crucial role in the acclimation to flooding (Crawford and Finegan 1989; Drew 1997; Bailey-Serres and Voesenek 2008). As a first response to reduced oxygen availability, several plant species induce lactic acid fermentation. Because accumulation of lactic acid considerably reduces the cytoplasmic pH, this pathway can only be maintained transiently and rapidly has to be replaced by alcoholic fermentation (Davies 1980; Roberts et al. 1984). The energy (ATP) yield of fermentative pathways is only 2 mol ATP per mol glucose consumed. This is very inefficient compared to the energy yield of mitochondrial respiration of up to 38 mol ATP per mol glucose consumed. Many changes observed at the metabolome and transcriptome level induced by flooding can be explained by impaired energy metabolism and the need to maintain energy supply (Bailey-Serres and Voesenek 2008). Consequently, several energy-consuming processes such as transport of nutrients, biosyntheses of cellulose, lignin and other macromolecules, and growth are strongly inhibited under anoxia (Loreti et al. 2005; Lasanthi-Kudahettige et al. 2007; Kreuzwieser et al. 2009). Surprisingly, clear differences between anoxia-tolerant and -sensitive plant species at the metabolic level have not been identified so far (Kreuzwieser et al. 2009). Independent of the species' flood tolerance, energy-consuming pathways are slowed down, while the glycolytic flux – leading to ATP production during fermentation – is enhanced (Klok et al. 2002; Liu et al. 2005; Loreti et al. 2005; Branco-Price et al. 2005; van Dongen et al. 2008; Kreuzwieser et al. 2009). Major differences between flood-tolerant and -sensitive species include a much higher number of differentially expressed genes in flood-tolerant compared to -sensitive species and modified temporal patterns of gene expression (Kreuzwieser et al. 2009). For example, in poplar, a flood-tolerant species, a maximum of 5,000 genes with altered expression was observed after 14 days of flooding (Kreuzwieser et al. 2009) whereas in flood-sensitive *Arabidopsis*, a maximum of only ca. 1,500 genes with altered expression was found after 2–4 h of anoxia; afterwards the number of differentially expressed genes even declined in *Arabidopsis* (Liu et al. 2005).

The present chapter summarizes recently gained knowledge of flooding-induced volatile emissions. It examines the physiological determinants of flood tolerance with main emphasis on flooding-induced volatile metabolic intermediates and stress marker compounds. Although flooding stress is a key limiting factor in a number of ecosystems, the reasons for interspecific variations in flood tolerance and also

in flooding-driven volatile release are still poorly understood. Here we argue that a plant internal cycling of ethanol might contribute to flood tolerance of trees. Ethanol is produced in anoxic roots of trees. It is then transported with the transpiration stream to the leaves and oxidized in the leaves to acetaldehyde and acetic acid as volatile intermediates. The main portion of the ethanol is, however, used in the leaves' primary carbon metabolism thereby allowing the use of the energy-rich reduced carbon of this fermentation product.

9.2 Products and Intermediates of Alcoholic Fermentation

9.2.1 Key Fermentation Products Emitted from Flooded Plants

The emission of volatile compounds from leaves of flooded plants is closely connected to the altered metabolism in anoxia exposed roots. Several studies have demonstrated that the end-product of alcoholic fermentation, ethanol, is loaded into the xylem and transported with the transpiration stream to the leaves of the plants (Crawford and Finegan 1989; MacDonald and Kimmerer 1991; Kreuzwieser et al. 1999). In the leaves, only a small part, a few percent of the ethanol delivered is emitted into the atmosphere. The main part, however, is oxidized in the leaves (MacDonald and Kimmerer 1993; Ferner et al. 2012), and the oxidation products are also either emitted or metabolized by the plant. In particular, the first intermediate of ethanol oxidation, the relatively volatile compound acetaldehyde, is also partially emitted into the atmosphere (Kreuzwieser et al. 1999; Ferner et al. 2012). The majority of acetaldehyde is further oxidized to yield acetic acid, which is much less volatile than acetaldehyde (the Henry's law constant for acetic acid is 0.0133 Pa m^3 mol^{-1} compared to 7 Pa m^3 mol^{-1} for acetaldehyde, Niinemets and Reichstein 2003), although its emission has also been reported from flooded trees (Rottenberger et al. 2008).

In general, the emission rates for ethanol are around one order of magnitude higher compared to acetaldehyde emissions. In 24 h flooded *Quercus robur* trees, for example, ethanol emission rates peaked at about 4.2 nmol m^{-2} s^{-1}, whereas acetaldehyde emission amounted to only 0.3 nmol m^{-2} s^{-1} (Kreuzwieser 2002). Similarly, in *Alnus glutinosa*, *Q. rubra*, *Populus tremula* and *Q. ilex*, ethanol emission rates exceeded acetaldehyde release by factors of 5–10 (Holzinger et al. 2000; Copolovici and Niinemets 2010). Also, in non-woody species such as rice, similar emission patterns have been observed (Boamfa et al. 2005; Mustroph et al. 2006). However, some tree species from the Amazonian floodplain exhibit somewhat different ethanol to acetaldehyde emission ratios (Parolin et al. 2004; Rottenberger et al. 2008; Bracho-Nunez et al. 2012), with occasionally greater acetaldehyde than ethanol emissions at some periods of flooding (e.g., *Laetia corymbulosa* in Parolin et al. 2004). Unfortunately, there is not much data available on the emissions of acetic acid as a consequence of flooding. In the work of

Rottenberger et al. (2008), flooded trees of *L. corymbulosa* emitted this organic acid at slightly higher rates than non-flooded control trees. Compared to ethanol (up to 8.3 nmol m^{-2} s^{-1}) or acetaldehyde (5 nmol m^{-2} s^{-1}) release, acetic acid emission rates were very low amounting to ca. 0.05 nmol m^{-2} s^{-1} in this species. In other species studied, no such flooding effect was observed (Rottenberger et al. 2008).

The flux of a volatile compound from the leaf is determined by stomatal conductance and the concentration gradient of the compound between substomatal cavities and the atmosphere (Niinemets and Reichstein 2003; Harley 2013 in this book). As stomatal conductances for ethanol, acetaldehyde and acetic acid do not differ strongly (Niinemets and Reichstein 2003), differences in emission rates must be mainly based on the concentration gradient which is determined by the compound concentration in the liquid phase of the apoplast and its volatility, reflected by the Henry's law constant. Of the three C2 compounds, acetaldehyde has the highest Henry's law constant and therefore the highest volatility. Ethanol is ca. 14-times less volatile than acetaldehyde and acetic acid even 3–4 orders of magnitude less volatile than ethanol (depending on pH). Much higher ethanol than acetaldehyde emission rates observed in many species therefore must be caused by high ethanol concentrations in the apoplast as well as rapid acetaldehyde consumption (Monson 2013 in this book). As ethanol in flooded trees is produced in the roots, its leaf concentrations depend on the transport rate in the xylem. On the other hand, the concentrations of acetaldehyde in the leaves of flooded trees depend on (i) leaf ethanol abundance (Kreuzwieser et al. 2001), (ii) activity of alcohol dehydrogenase which converts ethanol to acetaldehyde (Parolin et al. 2004; Ferner et al. 2012), and (iii) activity of aldehyde dehydrogenase, the enzyme degrading acetaldehyde thereby producing acetic acid. Consequently, acetic acid concentrations are determined by acetaldehyde abundance, activity of aldehyde dehydrogenase (production) and the activity of acetic acid consuming enzymes. Unfortunately, information on leaf apoplast concentrations of these volatiles is scarce (ethanol) (Jaeger et al. 2009; Ferner et al. 2012) or even completely missing (acetaldehyde, acetic acid). In leaves of non-flooded *Fraxinus excelsior*, *Fagus sylvatica* and *Q. robur*, the ethanol concentrations were in a range of 50 $\mu g\ g^{-1}$ FW to 200 $\mu g\ g^{-1}$ FW and, thus, significantly higher than in the roots (Jaeger et al. 2009; Ferner et al. 2012). The effects of flooding on leaf ethanol concentrations seem to be inconsistent. Whereas leaf ethanol increased in *F. sylvatica* and *Q. robur*, it was unaffected in *F. excelsior* and *Fraxinus angustifolia* (Jaeger et al. 2009; Ferner et al. 2012).

Only a limited number of studies has investigated alcohol dehydrogenase activity in leaves of flooded trees. Recently, it was demonstrated that there are no strong species-specific differences in enzyme activity (Jaeger et al. 2009; Ferner et al. 2012). Nevertheless, similar to ethanol concentrations, in some species, alcohol dehydrogenase activities in the leaves increased in response to flooding, but remained constant in other species (Jaeger et al. 2009; Ferner et al. 2012). In addition to the very similar alcohol dehydrogenase activity in different species, there was a lack of any species-specific differences in the use of ^{14}C-labelled ethanol in the foliar C metabolism; therefore, it was concluded, that acetaldehyde emission is

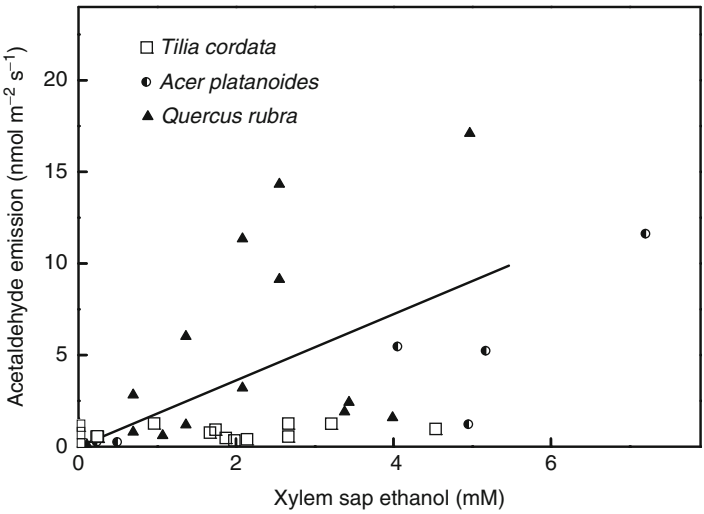

Fig. 9.1 Correlation between xylem sap ethanol concentration and acetaldehyde emission in three temperate deciduous species, *Quercus rubra*, *Acer platanoides* and *Tilia cordata*. Trees were flooded for 0, 1, 2, 10 and 15 days (unpublished data, Kreuzwieser). At the end of each treatment acetaldehyde emission rates were determined and xylem sap was extracted using the pressure chamber technique (Scholander et al. 1965)

controlled by the amount of ethanol transported to the leaves (Ferner et al. 2012) (Fig. 9.1). The good correlation of acetaldehyde emission rates with xylem sap ethanol concentrations and ethanol abundance in leaves (Fig. 9.1) (Kreuzwieser et al. 2001; Ferner et al. 2012) indicates that ethanol availability is the main parameter determining the emission rates. This assumption is supported by the close correlation of ethanol and acetaldehyde emissions from leaves of trees (Copolovici and Niinemets 2010; Bracho-Nunez et al. 2012).

9.2.2 Relationships of Emissions of Fermentation Volatiles with Species Flood Tolerance

Kreuzwieser et al. (1999) proposed that the production of ethanol in anoxia exposed roots, its transport via the transpiration stream to the leaves and its oxidation and re-use in the oxygen-exposed leaves of trees might be a physiological mechanism of flood tolerance at the whole-plant level. It was further hypothesized that acetaldehyde emission rates correlate with flood tolerance of trees (Kreuzwieser et al. 2004). More recent results with moderately flood-tolerant *Q. robur* and flood-sensitive *F. sylvatica* suggest that the metabolism in the leaves of at least these trees species is less decisive for acetaldehyde emission and re-use of ethanol in oxidative metabolism in the leaves (Ferner et al. 2012), and that rather the root processes

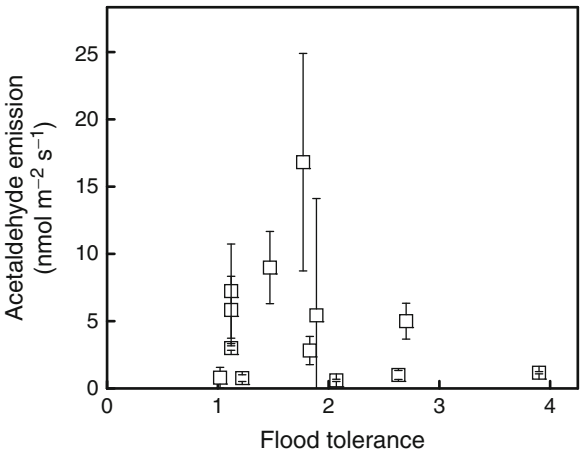

Fig. 9.2 Correlation of flood tolerance with acetaldehyde emission by flooded trees. Data are mean ± SD values for *Pinus sylvestris*, *Picea abies*, *Fagus sylvatica*, *Quercus robur*, *Q. rubra*, *A. platanoides*, *Populus x canescens*, *P. tremula*, *Tilia cordata* and *Alnus glutinosa*. Data are from Copolovici and Niinemets (2010), Kreuzwieser et al. (1999) and Kreuzwieser and Rennenberg (unpublished). Scoring of flood tolerance is according to Niinemets and Valladares (2006). Higher values of the flood tolerance score indicate greater flood tolerance

determine the trees' flood tolerance. The study of Copolovici and Niinemets (2010) suggests that ethanol and acetaldehyde emission are inversely related to the species' flood tolerance; for example, flood-sensitive *Q. rubra* emitted much more of these volatiles than flood-tolerant *P. tremula* or *A. glutinosa*. Assuming that the ethanol emitted by leaves was synthesized in the roots of the trees, it can be concluded that trees' flood tolerance is negatively associated with the amount of ethanol produced in the roots, i.e., with root oxygen availability. However, flood tolerance and ethanol production do not necessarily correlate (Raymond et al. 1985). In fact, there is no general correlation between foliar acetaldehyde emissions the species flood tolerance (Fig. 9.2).

Of course, the production of ethanol in the roots and its re-use in aerobic leaves is only one physiological aspect of trees' flood tolerance. This might be particularly important for tree species not capable of extensive morphological acclimation to flooding such as formation of adventitious roots and aerenchyma tissue. The capacity for such morphological modifications that allow the plants to increase the oxygen availability in the root zone, can be more strongly linked to species flood tolerance (Drew 1997; Vartapetian and Jackson 1997). Such an increase of root zone oxygen availability is expected to reduce the rate of fermentative processes, thereby reducing ethanol and acetaldehyde emissions. This idea is supported by the occurrence of aerenchyma rich adventitious roots in some Amazonian tree species and subsequent reduced acetaldehyde and ethanol emission rates (De Simone et al. 2002a, b; Parolin et al. 2004).

9.3 Physiology of Ethanol and Acetaldehyde Emission

9.3.1 Diurnal Variation Patterns: Influence of Stomata

Typically, acetaldehyde and ethanol are emitted from leaves of flooded trees at high rates during the day and at low rates during night (Fig. 9.3). There is often a peak of highest emissions in the early morning hours which decreases to lower levels during the rest of the light-period. Such patterns are assumed to result from daily patterns of stomatal conductance and consequently of transpiration rates (Kreuzwieser et al. 2000; 2001). Especially the morning peak emission of ethanol which has a low Henry's law constant of 0.51 Pa m^3 mol^{-1} (Niinemets and Reichstein 2003) can reflect stomatal control on emissions because a large pool of ethanol in the liquid phase is rapidly released upon stomatal opening (Harley 2013 in this book; Niinemets and Reichstein 2003). In the case of acetaldehyde with a somewhat higher Henry's law constant of 7 Pa m^3 mol^{-1} (Niinemets and Reichstein 2003), the stomatal constraints are expected to pose a less strong effect on the emissions. Application of abscisic acid to grey poplar (*Populus* x *canescens*) leaves has clearly demonstrated that – similarly to isoprene (Fall and Monson 1992) – stomatal opening does not play a dominant role in controlling acetaldehyde emission rates (Kreuzwieser et al. 2001), although moderate temporal control by stomata has been suggested by simulation analyses (Harley 2013 in this book; Niinemets and Reichstein 2003). More importantly, the correlation between stomatal opening and acetaldehyde emission suggests mainly an indirect control over acetaldehyde emissions by determining the transpiration rates and thereby ethanol transport into and abundance inside the leaf tissue. In fact, a significant linear correlation of

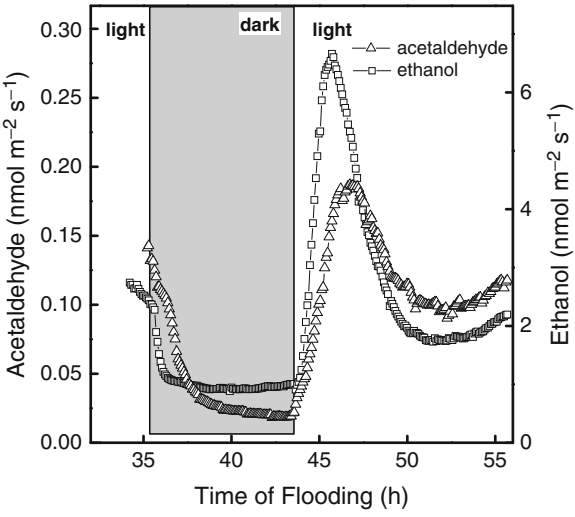

Fig. 9.3 Daily pattern of ethanol (*squares*) and acetaldehyde (*triangles*) emissions from flooded *Quercus robur* trees (Modified from Kreuzwieser 2002)

acetaldehyde emission rates with xylem sap ethanol concentrations (Kreuzwieser et al. 2000; Ferner et al. 2012) suggests that acetaldehyde emission is strongly controlled by the abundance of ethanol in the leaf tissue. A steady transport of ethanol from roots to the leaves is only ensured when transpiration takes place, i.e., during the light period of a day. As discussed above, because acetaldehyde is produced from the oxidation of ethanol by alcohol dehydrogenase, the activity of this enzyme is also involved in determining the acetaldehyde emission rate. Daily patterns of the alcohol dehydrogenase activity have, however, not been demonstrated so far, although induction by ethanol has been demonstrated (Good and Crosby 1989).

9.3.2 Effects of Flooding Duration

Besides the emission strength, the temporal pattern of elicitation of trace gas emissions is strongly species-dependent. Some species, e.g., flood-tolerant *Populus x canescens* or flood-sensitive *Quercus rubra*, react quickly with significantly enhanced ethanol and acetaldehyde emission rates after some hours of oxygen shortage in the roots (Kreuzwieser et al. 2004; Copolovici and Niinemets 2010). This response can occur in a transient manner. In both species, the emission rates drop after some days, reaching a steady emission level that is moderately higher than in non-flooded controls. Other species react more slowly and show steadily enhanced emission levels after some days of flooding (Kreuzwieser et al. 2004; Parolin et al. 2004; Copolovici and Niinemets 2010) (Fig. 9.4). The driving forces for such emission patterns remain unclear.

Apart from intrinsic differences among species, differences in soil conditions in species habitat such as soil redox status development under anoxia (rate of reduction of oxygen content of soil micropores and soil water by plant roots and aerobic bacteria) may contribute to the observed emission patterns and apparent differences between species. Soils with a lower portion of air-filled pores will be faster oxygen depleted than soils entrapping more air. In natural ecosystems, the specific flooding conditions are also of importance. It has been shown that stagnant flood water promotes oxygen deficiency much stronger than a flowing water body (Kreuzwieser et al. 2004). Since in none of the above mentioned studies soil characteristics were investigated in detail, it cannot be excluded that differences in the establishment of hypoxia/anoxia have contributed to the different emission patterns. Nevertheless, in studies simultaneously investigating multiple species, same soils and same treatment conditions have often been used, e.g., in the study of Copolovici and Niinemets (2010), suggesting that detected patterns reflect intrinsic species responses.

As discussed in Sect. 9.2.2, differences between species in the rate of development and level of expression of morphological adaptations such as aerenchyma and adventitious root development may be involved in the drop of emissions after prolonged flooding. Moreover, physiological features, such as a depletion of

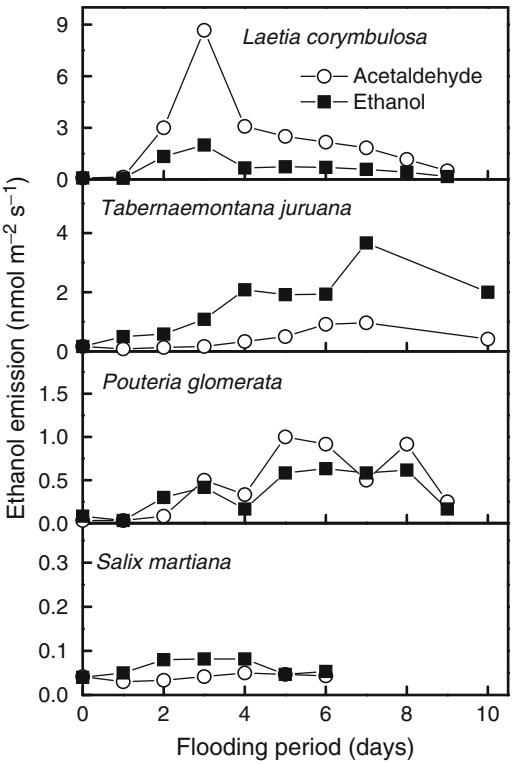

Fig. 9.4 Different temporal patterns of acetaldehyde and ethanol emissions through an extended flooding period in Amazonian trees (Modified from Parolin et al. 2004)

carbohydrates, the substrate for alcoholic fermentation, may at least partially be responsible. A reduction in fermentation-driven volatile emissions occurred at the same time when a drop in plant sugar status was observed in flooded *Fagus sylvatica* trees (Ferner et al. 2012).

9.4 Other Volatiles Released During Flooding

9.4.1 Emissions of Stress Volatiles

Besides the emission of fermentation-related volatiles ethanol, acetaldehyde and acetic acid, the release of some other BVOCs is affected by flooding. A series of publications have reported emissions of stress marker compounds in response to soil flooding. This includes the emission of ethylene, wound BVOCs and the nitrogen-containing volatile nitric oxide (Holzinger et al. 2000; Grichko and Glick 2001; Agarwal and Grover 2006; Bracho Nunez et al. 2009; Copolovici and Niinemets 2010) (Fig. 9.5).

The release of some of these trace gases is clearly stimulated under flooding. Such gases are either related to stress-induced injury of the leaves or might be a

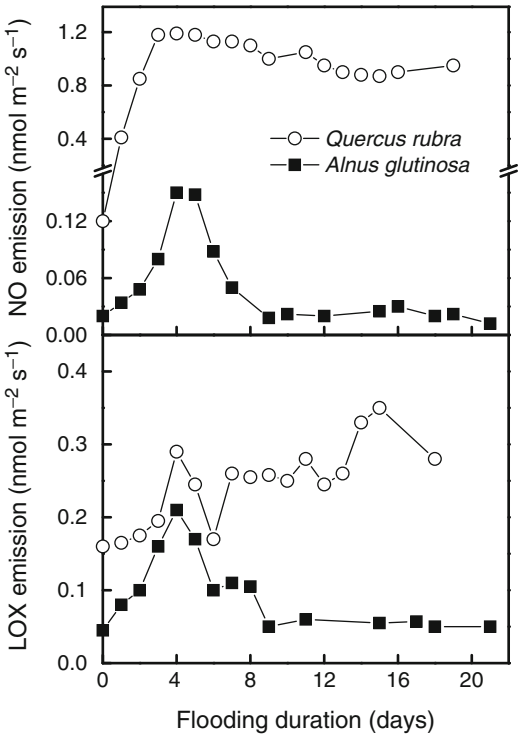

Fig. 9.5 Temporal changes in the emissions of NO and volatile products of lipoxygenase (LOX) reaction from flooded *Quercus rubra* and *Alnus glutinosa* trees (Modified from Copolovici and Niinemets 2010). The plant root systems were completely submerged at day 1 and maintained in waterlogged conditions through the experiment

consequence of acclimation of the trees to flooding. Emission of volatile products of the lipoxygenase pathway (Feussner and Wasternack 2002) (LOX products, mainly C6 aldehydes) from leaves of flooded trees is certainly a stress indicator. Consequently, emissions are higher from flood-sensitive plants than from more tolerant species (Fig. 9.5; Copolovici and Niinemets 2010). LOX products are derived from linoleic and linolenic acids released from cell membranes by the action of phospholipases as a response to oxidative stress (Feussner and Wasternack 2002). The strength of LOX product emission is a marker of the severity of a given stress factor (Beauchamp et al. 2005) and, in addition, of the sensitivity of a species towards the particular stress (Copolovici and Niinemets 2010). The leaf NO emission of flooded trees as observed by Copolovici and Niinemets (2010) might also be an indicator of oxidative stress; similar to LOX product emissions it was highest in flood-sensitive *Q. rubra* and lowest in flood-tolerant *A. glutinosa*.

In general, stress-induced NO emissions from leaves of plants are well documented; NO is involved in signal transduction pathways of biotic and abiotic stress resistance (Delledonne et al. 1998; Mata and Lamattina 2001; Zhao et al. 2007, 2009; Xuan et al. 2010). The main pathway for NO production is most probably the enzyme nitrate reductase, although there is also evidence of synthesis of NO by a L-arginine-dependent nitric oxide synthase (NOS) (Besson-Bard et al. 2008; Corpas et al. 2011).

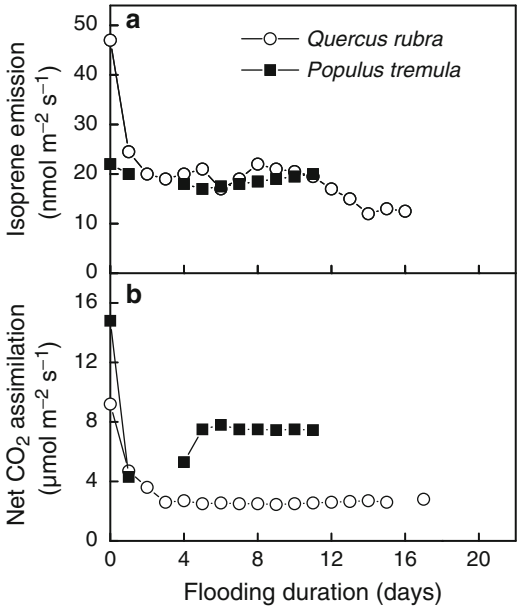

Fig. 9.6 Changes in isoprene emission (**a**) and net CO_2 assimilation (**b**) rates in *Quercus rubra* and *Populus tremula* through the flooding treatment (Modified from Copolovici and Niinemets 2010, the same experiment as in Fig. 9.5)

Stimulation of ethylene emission is a classic flooding response (Holzinger et al. 2000; Grichko and Glick 2001). Ethylene production and emission is closely connected with the development of aerenchyma (for review see Evans 2003). In aerenchyma formation, ethylene acts as a signal, inducing cell apoptosis and the formation of air filled pores.

Release of methanol is also enhanced due to flooding (Copolovici and Niinemets 2010). Methanol formation in plant tissues is closely related to cell wall modifications. It is released as the result of the activity of cell wall associated pectin methylesterases, which catalyze the demethylation of cell wall pectins during relaxation and rigidification cycles during leaf expansion (Fall and Benson 1996; Hüve et al. 2007; Ricard and Noat 1986), but also during degradation of cell walls in maturing fruits and senescing leaves (Frenkel et al. 1998; Sun et al. 2012). Thus, the formation of aerenchyma during flooding is closely connected to cell wall degradation and the formation of intercellular air spaces, processes known to stimulate methanol production (MacCann and Roberts 1991; Levy and Staehelin 1992; Nemecek-Marshall et al. 1995; Fall and Benson 1996).

9.4.2 *Effects of Flooding on the Release of Constitutively Emitted Volatiles*

The emission of isoprene was reduced by waterlogging in the flood-tolerant *Populus tremula* and flood-sensitive *Quercus rubra*, but this effect was much more pronounced in the latter species (Copolovici and Niinemets 2010) (Fig. 9.6a). Reduced

isoprene emission was also found in flooded *Garcinia macrophylla*, an Amazonian tree species (Bracho-Nunez et al. 2012). However, in the same study, other species did not show any flood-induced changes in isoprene emission. Similarly, flooding effects on monoterpene emission were inconsistent. The provenances of *Hevea spruceana* derived from an igapó-type forest characterized by brownish, nutrient- and sediment-poor flood water with low pH responded with a strong increase in monoterpene emission (Bracho-Nunez et al. 2012). In contrast, the provenances from a várzea-type forest characterized by nutrient and sediment rich "whitewater" showed the opposite pattern (Bracho-Nunez et al. 2012). Similar to the igapó type trees, flooded lemon (*Citrus limon*) trees also showed higher monoterpene emissions than non-flooded control trees (Velikova et al. 2012). A function of isoprene in quenching ROS in flooded plants has been discussed (Copolovici and Niinemets 2010), although there was no correlation of the capacity for constitutive isoprene release and flood tolerance of a given species. The sharp decrease in isoprene emissions from *Q. rubra* leaves due to flooding might be related to a general disturbance of leaf metabolism, as can be concluded from strong reductions in assimilation rates in this flood-sensitive species (Fig. 9.6b).

9.5 Conclusions

Flooding considerably affects the profile of volatile compounds emitted from leaves of trees. While the emission of isoprene drops, reflecting disturbance of photosynthetic processes, the release of products of glucose fermentation and stress volatiles is stimulated. Ethanol is the key BVOC formed in the roots due to fermentative processes in flooded trees and transported to the leaves via the transpiration stream. In the leaves, ethanol is converted to acetaldehyde and acetic acid, both of which can be emitted into the atmosphere in addition to ethanol.

The significance of these processes for ecosystem-scale acetaldehyde and ethanol fluxes is still unclear. Most of the present data and conclusions are based on experiments with tree seedlings investigated under more or less controlled conditions in the greenhouse. The mechanisms of flooding-induced BVOC emission might be of importance for ecosystems such as the Amazon forest where flooding regularly occurs (Rottenberger et al. 2008). However, the fact that flooding-induced BVOC emission rates usually drop during prolonged flooding, likely reflecting acclimation suggests that the emissions are of limited significance for atmospheric processes during the rest of the wet season. Other sources for these oxidized volatiles, ethanol, acetaldehyde and acetic acid, such as e.g., photochemical production due to oxidation or more reactive BVOCs, are assumed to be dominant compared to direct emission by vegetation (Millet et al. 2010).

The typical stress volatiles induced in response to flooding are volatile products of lipoxygenase (LOX) pathway (also called green leaf volatiles) and NO. There

is evidence that flood-sensitive trees emit these compounds at higher rates than tolerant species. In addition, ethylene and methanol can also be released in response to flooding. The site of release of the volatiles is currently unclear, but it is likely that ethylene and methanol are produced both in the roots and leaves. Ethylene stimulates the formation of aerenchyma, a morphological adaptation of trees to flooding that improves the supply of the root system with atmospheric oxygen during the flooding period, while methanol is released from cell wall pectins during aerenchyma formation.

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Chapter 10
Modification of BVOC Emissions by Changes in Atmospheric [CO_2] and Air Pollution

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Abstract Biogenic volatile organic compounds (BVOCs) produced by trees participate in the formation of air pollutants such as ozone and particulate matter. At the same time, the metabolic processes responsible for these emissions are sensitive to ozone and other air pollutants, as well as the solar radiation flux, which is affected by atmospheric particulate concentration. Recent anthropogenic increases in the atmospheric carbon dioxide concentration are also capable of affecting BVOC emissions, although the mechanisms behind these responses can produce variable effects depending on the plant species. Mechanisms of air pollutant effects on BVOC emissions are reviewed and dose-response relationships across a variety of trees with differing pollutant tolerance and emission capacity are compared. From this broad analysis, generalized response patterns have been developed. This chapter emphasizes the need to consider the interactions between BVOC emissions and ozone to understand plant behaviour in future climates.

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10.1 Introduction

10.1.1 Global Change Projections

It is widely acknowledged that the atmosphere has been affected by human activities, beginning from the Industrial Revolution and continuing to the present. Human-induced increases in the emissions of greenhouse gases are likely responsible for an increase in global average temperature, altering the hydrological cycle, with more intense droughts and floods (IPCC 2007). Anthropogenic carbon dioxide (CO_2) emission is the most important forcing variable affecting changes in climate over the past century. CO_2 concentration in the atmosphere remained relatively constant in the range of 260–280 μmol mol^{-1} over long periods before the Industrial Revolution, when it has continuously increased from approximately 270 μmol mol^{-1} in the late nineteenth century to a current atmospheric average of approximately 400 μmol mol^{-1} (Keeling et al. 2009). Projections from global carbon cycle models show that concentrations of atmospheric [CO_2] could increase to 500–1,000 μmol mol^{-1} by the year 2100 (IPCC 2007). The main cause of rising CO_2 emissions is burning of fossil fuels (coal, oil and natural gas), but emissions are also high due to deforestation and subsequent biomass burning and due to cement manufacturing.

Other greenhouse gases produced by human activities contribute to radiative forcing. Production of tropospheric ozone (O_3) is the third most important contributor to anthropogenic radiative forcing after emissions of carbon dioxide and methane (CH_4). Ozone is produced in the troposphere by nitrogen oxides (NO_x) interacting with non-methane volatile hydrocarbons (NMVOCs) in the presence of blue light in the visible spectrum. Background tropospheric O_3 concentrations continue to rise due to human activities (IPCC 2007), increasing from pre-industrial concentrations of less than 10 nmol mol^{-1} (ppb) (Volz and Kley 1988) to average summertime concentrations of 40 nmol mol^{-1} (Fowler et al. 1999). Ozone concentrations in the troposphere have been monitored by the environmental agencies of several countries because it, as a highly reactive oxidizing agent, is a principal component of urban and suburban air pollution.

10.1.2 Role of BVOC Emissions in Changing the Atmosphere

Biogenic volatile organic compounds (BVOCs) are released by vegetation to the atmosphere in significant quantities accounting for up to 5–10 % and more of total net carbon exchange into the atmosphere (Peñuelas and Llusià 2003). They can strongly contribute to the global change and play a crucial role in atmospheric composition because of their reactivity. For isoprene, this amount is on the order of ~400–500 Tg C year^{-1} (Archibald et al. 2011). Many studies have demonstrated that biogenic volatile organic compounds (BVOCs) contribute to the formation of tropospheric ozone (Chameides et al. 1988; Tao et al. 2003; Bell and Ellis 2004;

Hogrefe et al. 2011) in the presence of NO_x. The release of BVOCs also constitutes a significant input of precursors for photochemical oxidants (Simpson et al. 1995; Trainer et al. 1993). Most recently, evidence has been provided that the emission of BVOCs from tropical forests, particularly isoprene, plays an important role in recycling hydroxyl radicals, implying that BVOC emissions occupy a central role in buffering the oxidative capacity of the atmosphere (Lelieveld et al. 2008; Taraborrelli et al. 2012). Thus, studies of the relation of BVOC emissions to increases in atmospheric O_3 concentrations are likely to reveal key processes that control the oxidative capacity of the troposphere and control important surface-atmosphere feedback loops with implications for climate change (Ashworth et al. 2013; Kulmala et al. 2013 in this volume). Changing forest management practices lead to further important implications. Millions of hectares of fast-growing plantations are being planted across the world for biomass production and with the aim to sequester large amount of atmospheric CO_2. Most of the species used are poplars (*Populus* spp.), willows (*Salix* sp.) and eucalypts (*Eucalyptus* spp.) that are strong BVOC emitters (Owen et al. 2013). This means a large BVOC load into the atmosphere, especially in developing areas of the planet where growing levels of NO_x are being emitted, thus exacerbating the pollution potential in these areas (Hewitt et al. 2009). These aspects recall the need to reconsider the bio-engineering for the future, considering that we are now able to clone the isoprene/monoterpene synthase gene from many species and to create transgenic lines of several species which do not produce and emit BVOCs (Miller et al. 2001; Behnke et al. 2011).

In this chapter, we will focus on the effects of atmospheric [CO_2] and [O_3] on BVOC emission at the leaf scale and these effects will be considered within the broader context of climate change (Fig. 10.1). We first analyse the experimental setups and methodologies in studies of [CO_2] and [O_3] effects. Then we analyse the leaf-level responses to [CO_2] and [O_3] and combinations of both, and finally investigate the opportunities of how the reported effects can be best included in models. We separate between instantaneous effects that result from biochemical responses (Li and Sharkey 2013 for detailed description of overall emission mechanisms) and acclimation effects that reflect modifications in enzymatic capacities for BVOC synthesis (Monson 2013 for the overall philosophy of separating the BVOC flux controls).

10.2 Rationale and Methodological Issues of BVOC Studies Under Elevated [CO_2] and [O_3]

10.2.1 Experiments in Controlled Conditions

The responses of BVOC emissions to changes in atmospheric composition have been investigated with different methods both in the laboratory controlled conditions and in the field, manipulating or focusing on natural gradients of one or more factors. However, the response of plant emissions can vary considerably depending on the

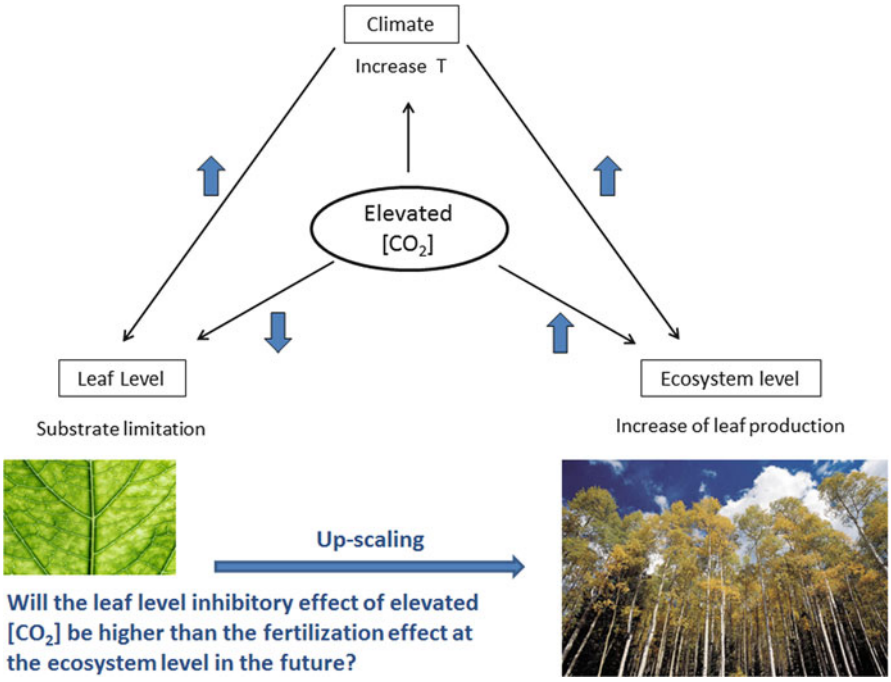

Fig. 10.1 Direct and indirect effects of increased CO_2 concentration at leaf and ecosystem levels on BVOC emissions. [CO_2] may directly stimulate (*upwards arrows*) or inhibit (*downwards arrows*) the emissions, and the [CO_2] effects may further indirectly be altered by [CO_2] effects on temperature

specific BVOC emitted, the plant species involved, the scale of interest (single leaf vs. whole plant) and the time of interest (short-term vs. long-term responses). Several laboratory studies have been conducted on the effects of high concentrations of CO_2 and/or O_3, where concentrations could be controlled artificially and changed quickly according to experimental design. Experiments with rapid changes in CO_2 concentrations have been originally coupled with studies of photosynthesis (Monson and Fall 1989) and provided important insights regarding the regulatory processes behind the formation and emission of BVOCs. These experiments are usually carried out at the leaf level and are often limited to a temporal scale that is in a range of few hours or some days. These instantaneous responses are different from those observed in leaves grown for a long period under elevated [CO_2] or elevated [O_3] because of acclimation mechanisms which can occur over time (Fig. 10.1, Monson 2013 in this volume). Both relatively rapid and longer-term experimental manipulations can be conducted by exposing entire plant or entire ecosystems to elevated concentrations of CO_2 and/or O_3, because in these cases, the regulations can occur at different temporal and spatial scales (Niinemets 2012 for a review). This is due mainly to canopy effects on microclimatic conditions, which, in turn, can influence BVOC emission directly and indirectly.

10.2.2 Localized Fumigation (LOF) System Experiments

The single leaf laboratory fumigation experiments provide insight into the physiological and biochemical changes induced by acute and short-term exposure to elevated CO_2 and/or O_3 concentrations. However, the results observed with these laboratory fumigation systems are not necessarily transferable to field conditions. The localized CO_2 and O_3 fumigation (LOF) system is a promising compromise between highly controlled laboratory experiments and experiments under field conditions (Pinelli and Tricoli 2004; Brilli et al. 2007; Loreto et al. 2007). LOF systems provide short-term fumigation of individual leaves on field-grown or potted plants, which have developed under the normal background atmospheric CO_2 or O_3 concentration. The LOF system enables investigation of physiological responses of leaves with different age on the same plant and exposed to a broad range of CO_2 or O_3 concentrations. This enables researchers to continue to control for other factors determining plant response to fumigation, such as genetic variability, meteorological dynamics and soil factors (Pinelli and Tricoli 2004).

10.2.3 Field Experiments

Studies focusing on BVOC emissions under different atmospheric compositions in the field are often biased by the diverse experimental conditions used in the different studies. Plants growing at different CO_2 and/or O_3 concentrations can exhibit different within-canopy light and/or temperature gradients due to the effects of seasonal changes in leaf area profiles and changes in soil moisture, which can affect canopy latent heat exchange (Gielen et al. 2003; Liberloo et al. 2007). Thus, in field studies, it is particularly important to measure BVOC emission rates for individual leaves under standard light and temperature (30 °C and 1,000 $\mu mol\ m^{-2}\ s^{-1}$) conditions, and study within-canopy variation in foliage BVOC emission capacity (Centritto et al. 2004; Niinemets et al. 2010b).

When experiments are performed in the field, generally only one or two factors can be manipulated, while laboratory conditions allow researchers to impose multiple factors and their combinations when needed and produce observations under comparable conditions. Experimental manipulation of pollutant concentration at the scale of several hectares or an entire region is impossible with current technology and resources. It is possible, however, to perform experiments on the effects of changed atmospheric composition on isolated trees or stands of trees in natural environments. In addition, studies of O_3 pollution in natural environments can be carried out using both natural (Winner et al. 1989) and regional gradients (Karnosky et al. 1999; Arbaugh et al. 2003). Unfortunately, the concentration of O_3 across the gradient is not constant, and the presence of gradients in other environmental factors could confound the O_3 effects (Paoletti et al. 2005).

In the last 20 years, the free-air CO_2 enrichment (FACE) technique has allowed researchers to fumigate portions of ecosystems in field condition, thus maintaining

control over other environmental variables and avoid the limitations of the open-top chambers (OTC) where alteration of microclimatic characteristics often occurred. This technique has been applied also to [O_3] (Karnosky et al. 1999; Dickson et al. 2000).

An important factor when working with air pollutants in free-air exchange experiments, and particularly when working with O_3, is the high reactivity between many BVOCs and O_3 causing the reduction of BVOC concentrations within the treatment rings. This effect may be especially important in the most polluted treatments due to BVOCs and O_3 and BVOCs and other, secondary oxidants (Fares et al. 2010; Jardine et al. 2012). Thus, when leaves are isolated for study in these experiments, and leaf-level measurements are performed, leaves should be measured in the presence of clean air flows to gain insight into BVOC emission potentials (Loreto and Velikova 2001; Brilli et al. 2011; de Gouw and Warneke 2007).

10.2.4 Natural CO_2 Spring Experiments

Natural CO_2 springs provide an opportunity to study the effects of elevated CO_2 concentrations on BVOC emissions, together with other related parameters, in trees or stands of trees growing near the CO_2 source. A limitation of these studies can be the frequent fluctuation of [CO_2] that can compromise the establishment of a "target" concentration at any particular distance from the CO_2 source, and the lack of control plots at ambient [CO_2] with similar characteristics of soil, water, and nutrient availability and with similar plant species, and often also with similar light level. In addition to difficulties with appropriate control treatment, another key shortcoming of CO_2 spring experiments is the lack of replication. Moreover, the presence of pollutants in several of these CO_2 springs, mostly sulphur-containing compounds such as H_2S and SO_2, can make it difficult to isolate the CO_2 effect on BVOC emission and can compromise the measurement by increasing background contamination and the reactivity with BVOCs (Paoletti et al. 2005).

10.3 Effects of Elevated Atmospheric [CO_2] on Plant BVOC Emissions

The effect of the progressive rise of atmospheric CO_2 concentration on the emission rate of BVOCs has been investigated in a number of studies, and different responses have often been observed (Table 10.1, Fig. 10.2). The responses of plant emissions to rising [CO_2] can differ among species and among plants of different ages. The responses can also vary with different experimental lay-outs, times of exposure, and with water and nutrient supply, thus complicating the comparison of the results

Table 10.1 Variability in BVOC emission under changing CO_2 concentrations in different plant species and different fumigation experiments

Species	Experimental system	Effect	Reference
Isoprene emission			
Short-term experiments			
Populus tremuloides	Leaf cuvette	−	Monson and Fall (1989)
Quercus rubra	Leaf cuvette	−	Loreto and Sharkey (1990)
Quercus pubescens	Natural CO_2 springs	−	Rapparini et al. (2004)
Long-term experiments			
Populus deltoides	Biosphere 2 facility	−	Rosenstiel et al. (2003)
Populus x euramericana	Free-air CO_2 enrichment (FACE)	−[a]	Centritto et al. (2004)
Populus deltoides	Biosphere 2 facility	=[b]	Pegoraro et al. (2004)
Quercus robur	Well-ventilated greenhouse	−	Possell et al. (2004)
Quercus pubescens	Natural CO_2 springs	=	Rapparini et al. (2004)
Phragmites australis	Natural CO_2 springs	−	Scholefield et al. (2004)
Mucuna pruriens	Whole-plant chambers	(+)[c]	Possell et al. (2005)
Populus tremuloides	Free-air CO_2 enrichment (FACE)	=	Calfapietra et al. (2007)
Populus alba	Free-air CO_2 enrichment (FACE)/Laboratory experiment	=	Loreto et al. (2007)
Liquidambar styraciflua, *Populus tremuloides*	Free-air CO_2 enrichment	−	Monson et al. (2007)
Populus deltoides	Biosphere 2 facility	=[d]	Pegoraro et al. (2007)
Populus tremuloides	Free-air CO_2 enrichment (FACE)	−[e]	Calfapietra et al. (2008)
Eucalyptus globulus, *Liquidambar styraciflua*, *Populus deltoides*, *Populus tremuloides*	Controlled-environment growth chambers	−	Wilkinson et al. (2009)
Ginkgo biloba	Open-top chambers	+	Li et al. (2009)
Acacia nigrescens	Controlled-environment growth chambers	−	Possell and Hewitt (2011)
Populus tremula x P. tremuloides	Open-top chambers	=	Sun et al. (2012)
Monoterpene emission			
Short-term experiments			
Quercus ilex	Natural CO_2 springs	−	Rapparini et al. (2004)
Long-term experiments			
Pinus ponderosa	Open/top chambers and controlled-environment Terracosm	=	Constable et al. (1999)

(continued)

Table 10.1 (continued)

Species	Experiment	Effect	Reference
Pseudotsuga menziesii			
Quercus ilex	Open-top chambers	−, +[f]	Loreto et al. (2001)
Quercus ilex	Controlled-environment greenhouse	+	Staudt et al. (2001)
Adenostoma fasciculatum	Biological field station	=	Baraldi et al. (2004)
Ceanothus greggii			
Quercus ilex	Natural CO_2 springs	=	Rapparini et al. (2004)
Betula pendula	Open-top chambers	=	Vuorinen et al. (2005)
Pinus sylvestris	Closed-top environmental chambers	+	Räsänen et al. (2008)
Ginkgo biloba, *Taxodium distichum*	Growth chambers	+,−, =[g]	Llorens et al. (2009)
Metasequoia glyptostroboides			
Sequoia sempervirens,			
Nothofagus cunninghamii			

The treatment time varied between 3 h and 10 days for short-term and from 30 days to 8 years for long-term treatments
[a]Decrease age-dependent
[b]Drought and high vapour pressure deficit (VPD) offset the inhibitory effect of elevated CO_2
[c]Increase in sub-ambient atmospheric [CO_2]
[d]Plants grown at elevated [CO_2] were less sensitive to drought stress
[e]Decrease mainly in the O_3-sensitive clone
[f]Only limonene emission increased
[g]Species-specific response

reported in the literature. Here we analyse the patterns for key BVOCs, isoprene and monoterpenes, and address the possible sources of variation in experimental observations.

10.3.1 Effects on Isoprene Emissions

10.3.1.1 General Patterns

There is a high degree of species-specific variability in the response of isoprene emission to elevated [CO_2], although generally a decreased emission is observed both in the short-term and in the long-term experiments (Fig. 10.2). Here we summarize a few responses in several case studies to emphasize the general trends, but also highlight exceptions.

Different species of *Populus* have exhibited a certain variability in responses to increasing [CO_2], although an inhibition effect prevailed in most of the studies. Decreased emissions were observed in the hybrid poplar *Populus x*

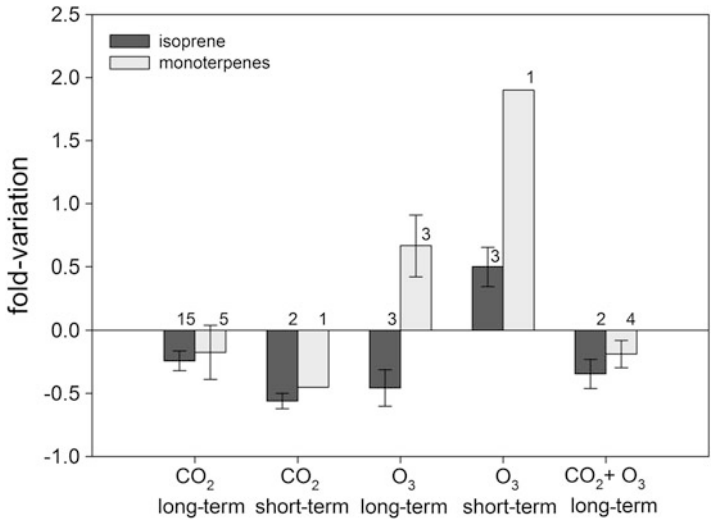

Fig. 10.2 Effects of elevated CO_2, O_3 and $CO_2 + O_3$ concentrations on isoprene and monoterpene emissions. *Bars* represent mean (±SE) values derived from studies in Tables 10.1 and 10.2. Included are only studies where enough quantitative information was available to derive estimates for different treatments. In studies with multiple additional treatments such as drought or warming, only the control treatments were used. In studies with multiple CO_2 and O_3 treatments or species, a mean value for each study was used. Across the included treatments, the ranges of [CO_2] were 550–1,200 μmol mol^{-1} (ppmv) for elevated [CO_2], and the applied [O_3] concentrations were 75–300 nmol mol^{-1} (ppbv) for elevated [O_3]. The treatment time varied between 3 h and 10 days for short-term and from 30 days to 8 years for long-term treatments. The *numbers* next to the *bars* denote the number of studies

euramericana (Centritto et al. 2004) and in *P. deltoides* (Rosenstiel et al. 2003; Wilkinson et al. 2009). The decreased emissions in *P. deltoides* were not observed when the plants were exposed to drought stress (Pegoraro et al. 2004), possibly due to decreased intercellular CO_2 concentrations, which likely counteracted the influence of elevated atmospheric [CO_2] due to reductions in stomatal conductance (Sects. 10.3.1.2 and 10.3.2). Significant decreases in isoprene emission in quaking aspen (*Populus tremuloides*) have also been observed (Wilkinson et al. 2009), especially in one clone (Calfapietra et al. 2008). Monson et al. (2007) demonstrated evidence of an active down-regulation of isoprene emissions during long-term growth in FACE experiments with sweetgum (*Liquidambar styraciflua*) forest in Tennessee, USA and *P. tremuloides* stands in Wisconsin, USA. However, no significant effect of elevated [CO_2] on isoprene emission was observed in an experiment with white poplar (*Populus alba*) (Loreto et al. 2007), and in hybrid aspen (*Populus tremula* x *P. tremuloides*) (Sun et al. 2012). Furthermore, in the study of Sharkey et al. (1991), elevated [CO_2] resulted in increased isoprene emissions in white oak (*Quercus alba*) and inhibited emissions in *P. tremuloides*. In gymnosperm *Gingko biloba*, Li et al. (2009) showed an increase of isoprene emission under elevated [CO_2].

10.3.1.2 Drought, N Availability, Temperature and Ontogeny Effects

Of particular interest for the responses of isoprene emissions to changes in [CO_2] is the effect of drought, which can offset the negative effect of elevated [CO_2] (Loreto et al. 2001; Rapparini et al. 2004; Pegoraro et al. 2004, 2007). Results from *Populus deltoides* in Biosphere 2 facility clearly showed this effect (Pegoraro et al. 2007). Emissions from poplar trees grown under elevated [CO_2] showed a significant stimulation under drought as compared to those grown under ambient [CO_2]. Only when drought stress became severe, the emission decreased under elevated [CO_2]. Increases in isoprene emissions under elevated [CO_2] and drought are likely due to decreases in leaf stomatal conductance (g_s) and decreases in intercellular CO_2 concentration (C_i) that alleviated the inhibitory effect of elevated [CO_2] (Sect. 10.3.2). These results were consistent with studies in open-top chambers by Loreto et al. (2001).

It is not yet conclusively clear how nitrogen availability alters isoprene emissions under ambient [CO_2], but nitrogen availability can modify the CO_2-response of isoprene emission. Possell et al. (2004) showed that the addition of nutrients reduced, but did not completely cancel, the negative impact of elevated [CO_2] on isoprene emission in *Quercus robur*. Overall, study-to-study variations in elevated [CO_2] effects on isoprene emission (Sect. 10.3.1.1) have been attributed to variations in plant nutrition among the treatments, with studies demonstrating inhibition being possibly characterized by more sever nutrient limitations under elevated [CO_2] (Sun et al. 2012).

Generally, elevated temperatures stimulate isoprenoid emission (Monson and Fall 1989; Monson et al. 1994; Sharkey et al. 1999; Petron et al. 2001) so it is likely that the inhibitory effect of elevated [CO_2] can be counteracted by the higher temperatures that is predicted in future high [CO_2] environments (Rapparini et al. 2004). In fact, at high temperature, the rate of isoprene emission has been shown to became less sensitive to [CO_2] (Loreto and Sharkey 1990; Rasulov et al. 2010). Observations on the effects of the stress factors on isoprene emission support the hypothesis that other environmental stresses, such as drought and high temperature, particularly important in the Mediterranean area, can increase the isoprene emissions and offset the negative effect of elevated [CO_2] (Rapparini et al. 2004).

Centritto et al. (2004) studying *P. x euramericana* saplings growing in a FACE experiment showed that isoprene emission was age-dependent and decreased under elevated [CO_2]. In fact, only fully-mature leaves emitted isoprene, while young developing leaves did not emit isoprene. Leaf ontogeny was accelerated by growth at elevated [CO_2], although the leaf plastochron index for reaching the maximum isoprene emission rate and its decline in aging leaves remained unaltered under elevated and ambient [CO_2]. There is evidence of enhanced leaf area formation under elevated [CO_2], but the stimulation of total leaf area by elevated [CO_2] is often a transient effect and can disappear after the first years of growth (Saxe et al. 1998; Norby et al. 1999; Gielen and Ceulemans 2001; Nowak et al. 2004). Thus, provided total leaf area remains similar, the integrated whole-plant isoprene

emission is expected to be significantly lower under elevated [CO_2] as has been observed by Loreto et al. (2007). However, there is evidence that leaf area can increase under elevated [CO_2], resulting in enhanced whole plant isoprene emissions (Sun et al. 2013).

10.3.1.3 Sub-ambient [CO_2] Responses

Isoprene emissions have been shown to be enhanced in many species under sub-ambient CO_2 concentrations similar to those during the Last Glacial Maximum (about 180 μmol mol^{-1}). Such an increase has been reported in *Eucalyptus globulus* (Wilkinson et al. 2009), *Mucuna pruriens* and *Arundo donax* (Possell et al. 2005). Possell et al. (2005) suggested that during the glacial period the emissions were still lower due to cooler temperatures, and possibly lower leaf area index (LAI). In fact, Way et al. (2011) using transgenic poplar (*P. x canescens*) with suppressed isoprene emissions suggested that isoprene biosynthesis may have evolved under low atmospheric CO_2 concentrations. They grow emitting and non-emitting trees under low (190 μmol mol^{-1}) and high (590 μmol mol^{-1}) CO_2 concentrations and tested the effects of short, transient periods of high light and temperature. Isoprene-emitting plants grown under low [CO_2] emitted twice as much isoprene as those grown under high [CO_2] and had significantly greater tolerance of sunflecks than non-emitting trees (Way et al. 2011). Thus, this evidence suggests that rising [CO_2] might reduce the functional benefits of isoprene in the future (see also the chapter of Fineschi et al. 2013).

10.3.2 Mechanisms of [CO_2] Responses of Isoprene Emission

There is still a debate about the mechanism behind the decrease of isoprene emissions under elevated [CO_2]. Rosenstiel et al. (2003) provided evidence of a clear reduction of isoprene emission with the rise of CO_2 concentration, at the protoplast, leaf and ecosystem levels, despite the increase of biomass and photosynthetic rates under elevated [CO_2]. There was a positive correlation between the rate of isoprene production and the cellular concentration of dimethylallyl diphosphate (DMADP), the immediate precursor of isoprene biosynthesis. Such a reduction of DMADP pool size by high [CO_2] has been further shown in several other studies (Rasulov et al. 2009; Possell and Hewitt 2011; Sun et al. 2012). Thus, the reduction of DMADP production can constitute the main reason for suppression of isoprene emission under elevated [CO_2]. However, in the reported patterns, it is important to distinguish between instantaneous and acclimation responses. Isoprene emission rate essentially instantaneously decreases after rising the [CO_2] above ambient level (Rapparini et al. 2004; Scholefield et al. 2004) (Loreto and Sharkey 1990; Rasulov et al. 2009; Sun et al. 2012). Thus, when measured under given growth [CO_2] environment, i.e., ambient-[CO_2]-grown plants are measured under

ambient [CO_2], and elevated-[CO_2]-grown plants are measured under elevated [CO_2], the response to elevated [CO_2] consists of two processes, immediate, short-term response to differences in CO_2 concentration and longer-term acclimation effect to growth [CO_2] (Sun et al. 2012; Monson 2013), and here we separately discuss both processes.

10.3.2.1 Instantaneous [CO_2] Response

DMADP formation via chloroplastic 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway) starts with condensation of glyceraldehyde 3-phosphate and pyruvate. The origin of chloroplastic pyruvate is not fully clear, but it has been postulated that a significant fraction of carbon entering the chloroplastic DMADP pool might originate from cytosolic phosphoenol pyruvate (PEP) (Monson et al. 2009; Wilkinson et al. 2009). PEP, derived from cytosolic glycolysis, is the substrate for a number of cellular metabolic pathways and for mitochondrial respiration. PEP enters into the Krebs cycle after conversion to oxaloacetic acid by the cytosolic enzyme PEP carboxylase (PEPc). Rosenstiel et al. (2003) hypothesized that the formation of isoprene depends on the transport of PEP into the chloroplast from the cytosol. As inhibition of cytosolic PEPc seemingly caused an increase in isoprene emissions under elevated [CO_2], they postulated a metabolic competition for PEP between PEP carboxylase and its transport into the chloroplast (Rosenstiel et al. 2003). These experiments were used to hypothesize that reduction of isoprene emission under elevated [CO_2] is due to reduced DMADP concentrations in the chloroplast due to competition between PEP carboxylase and chloroplast uptake.

Additional pieces of evidence have provided support to the postulated mechanism. Loreto et al. (2007) observed a positive, although weak, correlation between dark respiration and isoprene emission rates in leaves exposed to or grown under different [CO_2] both in the field and laboratory experiments. At the same time, a negative correlation was observed between the activity of PEPc and the isoprene emission rate (Loreto et al. 2007). Thus, they provided further evidence for the role of competition for PEP on isoprene emissions, but ruled out a role of mitochondrial respiration as part of this competition. Using $^{13}CO_2$-labelling, Trowbridge et al. (2012) observed in *P. x canescens* plants grown under sub-ambient [CO_2] a greater incorporation of "older" non-labeled carbon in isoprene than under high [CO_2], and demonstrated that the older carbon comes from pyruvate substrate. They suggested that this older carbon in pyruvate is most likely of cytosolic origin (Trowbridge et al. 2012).

An alternative hypothesis to explain the inhibitory effect of high CO_2 concentration on isoprene emission is the regulation of isoprene synthesis pathway by the availability of ATP and NADPH (Rasulov et al. 2009). There is a positive correlation between ATP level and isoprene emission (Loreto and Sharkey 1993), and the pool size of ATP is lower under higher than under ambient [CO_2] (Cardon and Berry 1992; Delwiche and Sharkey 1993). Furthermore, Rasulov et al. (2009)

demonstrated that isoprene emission was inhibited by both high and very low CO_2 concentration (below ca. 100–180 μmol mol^{-1}). Both are conditions under which ATP formation is inhibited (Kiirats et al. 2009). This reduction of isoprene emission by low CO_2 concentration cannot be explained by PEP transport. We note that both PEPc and ATP hypotheses of CO_2 effects on isoprene emission agree in that the inhibition results from reduced DMADP pool size, but the debate is over what causes DMADP pool size to decrease under high [CO_2].

10.3.2.2 Acclimation Response

Nowadays, acclimation of isoprene emission capacity to increased CO_2 concentrations is considered as a key gap in understanding biochemistry of isoprene formation (Sharkey 2009) and identification of possible adaptive modifications is difficult. Available data on these adaptive changes are contrasting. Some studies have been carried out in the vicinity of natural CO_2 springs which provided access to the long-term exposure to elevated [CO_2] and the onset of possible adaptation mechanisms (Rapparini et al. 2004; Scholefield et al. 2004). Scholefield et al. (2004) studied *Phragmites australis* grown in the vicinity of natural CO_2 springs along three streams of freshwater at the bottom of the crater. The basal isoprene emission rate was higher in plants grown at ambient [CO_2] (control site) than in those grown under elevated [CO_2], but the inhibition under elevated [CO_2] was attenuated when expressed on a leaf nitrogen content basis. It was also evident that the inhibition of isoprene emission due to high CO_2 concentration followed the gradient of CO_2 concentration from the bottom to the edge of the CO_2 vent. In particular, isoprene synthase activity was strongly reduced at the bottom of the springs (where CO_2 concentration is around 900–1,100 μmol mol^{-1}) than in the peripheral stand (CO_2 concentration around 700 μmol mol^{-1}). However, no inhibition at the branch-level isoprene emissions was observed in *Q. pubescens* grown in natural CO_2 springs (Rapparini et al. 2004). The higher leaf temperature at elevated [CO_2] in natural springs and the interaction with multiple stresses as recurrent drought may compensate the negative effect of long-term exposure to high concentrations of CO_2. The same findings have been obtained in a recent study of Sun et al. (2012) which showed no significant differences in isoprene emission rate among ambient- and elevated-[CO_2] grown plants, and in fact enhanced emissions from elevated-[CO_2] grown plants when studied at the same ambient CO_2 concentration. In this study, isoprene emission rate was enhanced at higher [CO_2] under saturating light conditions.

Changes in isoprene emission capacity in plants developed under different [CO_2] may reflect changes in isoprene synthase activity, and/or changes in the precursor DMADP pool size. Calfapietra et al. (2007) studying the response of field-grown quaking aspen (*P. tremuloides*) trees exposed to elevated [CO_2] observed a non-significant decrease of isoprene synthase (IspS) protein levels under elevated [CO_2]. However a significant inhibitory effect of elevated [CO_2] on isoprene emission rates was observed as a result of decreased DMADP levels (Calfapietra et al. 2008).

Analogous patterns were reported for hybrid aspen (*P. tremula* x *P. tremuloides*) by Sun et al. (2012). In Possell and Hewitt (2011), elevated [CO_2]-driven inhibition of isoprene emissions in the tropical African tree *Acacia nigrescens* was associated with both reduced DMADP pool size and isoprene synthase activity. Clearly, there are study-to-study differences in changes in isoprene synthase activity and DMADP pool size in plants acclimated to elevated [CO_2], and future work should focus on resolving this variability.

10.3.3 Influence of Elevated [CO_2] on Monoterpene Emissions

In understanding monoterpene emission responses to elevated [CO_2], it is critical to distinguish among storage emissions that are prevailing in species with specialized storage structures and emissions from immediate synthesis that are dominating the emissions in species lacking specialized storage (Grote et al. 2013 for a discussion). In the case of storage emissions, elevated [CO_2] is expected to alter the size of storage pool and accordingly, the effects can be explained on the basis of possible alterations of sink-source balance (Constable et al. 1999; Litvak et al. 2002). In contrast, the response of immediate emissions in species lacking storage tissues may approximate the elevated [CO_2] effects on isoprene emissions (Niinemets et al. 2010a). Thus, differences among species in growth [CO_2] effects on monoterpene emissions can be accounted for by differences in the emission mechanisms, tissue storage vs. immediate emission.

Monoterpene-storing conifer species such as *Pinus ponderosa* and *Pseudotsuga menziesii* showed no differences in tissue monoterpene concentrations and emission rates in response to growth under increased [CO_2] (Constable et al. 1999). This lack of response was also observed in *Adenostoma fasciculatum* and *Ceanothus greggii* (Baraldi et al. 2004) and *Betula pendula* (Vuorinen et al. 2005). Llorens et al. (2009) investigated four monoterpene emitting "living fossil" gymnosperm and one angiosperm tree species growing in a simulated Cretaceous polar environment with doubled atmospheric CO_2 concentration of 800 μmol mol^{-1}, and observed that only the deciduous gymnosperm species were affected by high [CO_2]. However, there were interspecific differences with enhanced emissions in *Metasequoia glyptostroboides* and inhibited emissions in *Taxodium distichum*, and variable responses in *Ginkgo biloba* depending on the sampling date (Llorens et al. 2009). However, it is not fully clear to which extend the monoterpene emissions in these deciduous conifers came from storage or reflected de novo monoterpene synthesis.

The "immediate" emitter *Quercus ilex* exhibited an increase in monoterpene emissions when grown under elevated [CO_2] (Staudt et al. 2001). Results from the study of Staudt et al. (2001) showed that leaves of *Q. ilex* in elevated [CO_2] treatment did not allocate more carbon to monoterpene production, but the emission capacity of leaves increased together with the capacity of photosynthesis. In another studies in *Q. ilex*, Loreto et al. (2001) found a slight inhibition of α-pinene, β-pinene and

sabinene emission, but an increase of limonene emission. These differences were correlated with changes in corresponding monoterpene synthase activities (Loreto et al. 2001). Further studies are needed to gain insight into differential regulation of different monoterpene synthases by elevated [CO_2].

10.4 Effects of Elevated [O_3] on Plant BVOC Emissions

BVOCs not only contribute to the formation of O_3 and other oxidants (Sect. 10.1), but O_3 itself can influence the rate of BVOC emission from plants. There is a large study-to-study variability in BVOC responses to [O_3], and thus, no general response direction of the effect of [O_3] on isoprenoid emissions (Fig. 10.2). The evidence has demonstrated that exposure of leaves to elevated [O_3] can increase (Velikova et al. 2005a, b; Fares et al. 2006) or decrease (Fares et al. 2006; Calfapietra et al. 2007, 2008) isoprene emission rates (Table 10.2). Here this controversial evidence is analysed and possible explanations for the variability in the reported patterns are given.

10.4.1 Influence of Acute Ozone Episodes

In laboratory experiments acute and short-term (300 nmol mol^{-1} for 3 h) exposure to O_3 significantly increased isoprene emission by reed (*Phragmites australis*) leaves (Velikova et al. 2008). Analogously, acute, high O_3 concentrations have been observed to stimulate monoterpene emissions from *Quercus ilex* leaves in laboratory experiments, and this effect was apparent even for leaves that had developed in an otherwise low [O_3] atmosphere (Loreto et al. 2004).

In the case of stress-signalling volatiles and sesquiterpenes, fumigation with [O_3] also stimulated the emissions in Scots pine (*Pinus sylvestris*) and in tobacco (*Nicotiana tabacum*) ozone-sensitive (Bel W3) and ozone–resistant (Bel B) cultivars (Heiden et al. 1999). An ozone treatment of 120–170 nmol mol^{-1} for 5 h induced visible damage in the ozone-sensitive tobacco cultivar, while the more tolerant cultivar was not affected (Heiden et al. 1999). Both cultivars emitted methyl salicylate and a range of sesquiterpenes after the [O_3] treatment, but the emission of several C6 compounds of the lipoxygenase pathway (LOX, also called green leaf volatiles; *cis*-3-hexen-1-ol as the most prominent one, *trans*-2-hexen-1-al, 1-hexanol and *cis*-3-hexenyl acetate) was only observed in the ozone-sensitive cultivar (Heiden et al. 1999). High O_3 concentration of 120–200 nmol mol^{-1} also induced emissions of homo- and sesquiterpenes in lima bean (*Phaseolus lunatus*), a non-isoprene emitter, when grown in the stirred tank reactors (Vuorinen et al. 2004). Following exposures to different ozone concentrations from 80 to 1,700 nmol mol^{-1} and timing (1.0–8.8 h), typical LOX volatile products were emitted from ozone-sensitive

Table 10.2 Variability in BVOC emission responses to elevated ozone exposure in different plant species and different fumigation experiments

Species	Experimental system	Effect	Reference
Isoprene emission			
Short-term experiments			
Phragmites australis	Enclosed chamber	+	Velikova et al. (2005a, 2008)
Quercus pubescens	LOF system	−, +, =[a]	Velikova et al. (2005b)
Long-term experiments			
Populus alba	Enclosed chamber	+,−[b]	Fares et al. (2006)
Populus tremuloides	Aspen FACE	−, =[c]	Calfapietra et al. (2007, 2008)
Ginkgo biloba	Open-top chamber	+	Li et al. (2009)
Monoterpene emission			
Short-term experiments			
Quercus ilex	Enclosed chamber	+	Loreto et al. (2004)
Long-term experiments			
Pinus sylvestris	Continuously stirred tank reactors	+	Heiden et al. (1999)
Ceratonia siliqua *Olea europaea*, *Quercus ilex ssp. ilex* *Quercus ilex ssp. ballota*	Open-top chamber	+,−, =[d]	Llusià et al. (2002)
Ginkgo biloba	Open-top chamber	+	Li et al. (2009)
Other BVOC emission			
Short-term experiments			
Phaseolus lunatus	Growth chamber	+[e]	Vuorinen et al. (2004)
Nicotiana tabacum	Continuously stirred tank reactors	+[f]	Beauchamp et al. (2005)
Long-term experiments			
Nicotiana tabacum	Continuously stirred tank reactors	+[g]	Heiden et al. (1999)
Pinus halepensis *Solanum lycopersicum*	Open-top chamber	+, =[h]	Peñuelas et al. (1999)
Peatland microcosms	Growth chamber	+[i]	Rinnan et al. (2005)

The treatment time varied between 3 h and 10 days for short-term and from 30 days to 8 years for long-term treatments
[a]Dose effect, emission burst after recovery
[b]Age-dependent effect
[c]Decrease in the ozone-sensitive clone
[d]Seasonality and species dependence
[e]Homo- and sesquiterpenes
[f]C_6-volatiles (LOX products)
[g]Sesquiterpenes
[h]Seasonal dependence
[i]Non-methane BVOC

tobacco cultivar Bel W3 (Beauchamp et al. 2005). These LOX products are derived from lipoxygenase activity after release of polyunsaturated free fatty acids from membranes (Feussner and Wasternack 2002; Liavonchanka and Feussner 2006). They are considered as products of membrane lipid peroxidation related to leaf

damage (Loreto et al. 2006). Thus, emissions of LOX products from plants exposed to high [O_3] during acute exposure episodes provide evidence that these acute episodes result in physiological damage.

10.4.2 Localized [O_3] Fumigation (LOF) System Experiments

Using a LOF system (Sect. 10.2.2), it was demonstrated that exposure to low (60 nmol mol^{-1}) and intermediate (190 nmol mol^{-1}) O_3 concentrations for three consecutive days (9 h/day) did not affect isoprene emissions from pubescent oak (*Quercus pubescens*) leaves (Velikova et al. 2005b). High O_3 concentration (300 nmol mol^{-1}) significantly reduced isoprene emission by leaves sampled immediately after the treatment as compared with control leaves, but isoprene emission rates were increased significantly in 288 h after termination of the high [O_3] treatment (Velikova et al. 2005b), suggesting that isoprene stimulation is delayed relative to the onset of O_3 stress, probably reflecting the activation of a whole class of constitutive and induced genes (Sharkey et al. 2005). It is likely that the high isoprene emissions help to quench reactive oxygen species (ROS) and to enhance the membrane stability in leaves recovering from O_3 stress (Possell and Loreto 2013).

10.4.3 Long-Term [O_3] Fumigation Effects

Using open-top chamber experiments, Peñuelas et al. (1999) observed a significant positive relationship between tropospheric O_3 exposure and BVOC concentrations in the atmosphere, which followed seasonal patterns with a maximum in early summer. However, there were species-specific responses with no significant effect of O_3 on BVOC emissions in Aleppo pine (*Pinus halepensis*), but a highly significant O_3 effect in tomato (*Solanum lycopersicum*) (Peñuelas et al. 1999). In open top chamber experiments with different Mediterranean woody plant species, an overall enhancement of BVOC emissions under high O_3 concentrations was shown, indicating that a positive feedback on tropospheric O_3 formation can be expected (Llusiá et al. 2002). When considering specific BVOCs, there were variable results among species and time of the year. Emission of α-pinene decreased with [O_3] fumigation in *Olea europaea*, while α-pinene and limonene emissions increased in *Quercus ilex* (Llusiá et al. 2002).

Stimulation of isoprene emission after ozonation has been also observed in white poplar (*Populus alba*) leaves (Fares et al. 2006). However, this increase of isoprene emission was restricted to leaves that developed inside the cuvette during the ozone treatment; when leaves that had developed outside the cuvette, in an atmosphere with low [O_3], were exposed to the higher treatment O_3 concentration, isoprene emission rate was reduced (Fares et al. 2006).

Long-term adaptation (4 months) to an O_3 concentration of 80 nmol mol^{-1} (9 h/day) increased the emissions of isoprene and monoterpenes from *Ginkgo biloba*

(Li et al. 2009). In *Pinus sylvestris*, exposure to elevated [O_3] concentrations (daily mean concentration of 50 nmol mol^{-1}, 8 h/day) in open-top chambers for a period of 2 years also led to a significant increase of monoterpene emissions, while no visible damage to the needles was observed (Heiden et al. 1999). Analogously, in a growth chamber experiment, high O_3 concentration (150 nmol mol^{-1} for 36 days with 9 h/day) increased the emissions of all BVOCs that were emitted from a peatland (Rinnan et al. 2005). Emissions of aromatics, terpenoids and N-containing compounds were doubled at 150 nmol mol^{-1} O_3 levels (Rinnan et al. 2005).

In contrast to these observations, long-term field experiments showed a significant decrease of isoprene emission in an O_3-sensitive aspen (*Populus tremuloides*) clone, whereas smaller decreases in isoprene emissions were observed in the O_3-tolerant clone (Calfapietra et al. 2008).

10.4.4 Mechanisms of Long-Term [O_3] Influences

When considered across the entire range of experiments and species, these past findings on the effect of short- and long-term [O_3] exposures suggest that BVOC emissions might be stimulated by acute O_3 doses, although with species-dependent and microenvironment-dependent variation. Chronic O_3 exposure may inhibit emissions, but the responses seem to be different for isoprene and monoterpenes (Calfapietra et al. 2009, Fig. 10.2). The differences observed in acute and chronic exposure experiments likely reflect important differences in the capacity for the photosynthetic systems of plants to tolerate oxidative stress, and also the biochemical acclimation responses associated with alterations in gene expression. In the study of Calfapietra et al. (2007), lower isoprene emission rate in the sensitive aspen clone correlated with reduced isoprene synthase (IspS) mRNA level and reduced concentrations of IspS protein in the leaves. Although the drop in IspS protein level induced a drop in the isoprene emission rate under elevated [O_3], other mechanisms could also have contributed to the observed inhibition, such as the reduction in the pool size of DMADP, the main substrate for the formation of isoprene (Calfapietra et al. 2008). This may have occurred because of [O_3]-induced damage to the photosynthetic apparatus, including the electron transport system and trans-thylakoid proton gradient, both of which control the production of NADPH and ATP, which are required for DMADP synthesis. Clearly, there is a need for more quantitative studies looking into the biochemical limitations under different ozone exposures.

10.4.5 Mechanistic Approaches to Understand [O_3] Response

Variation in ozone responses suggests that there is a need for experiments that explicitly compare acute and chronic responses with the aim of identifying the limits of ozone tolerance as driven by ozone dose, and short- and long-term emission

responses. In order to improve O_3 dose/effect predictions, a mechanistic approach has been developed, which is based on analyses that take into account the O_3 flux through stomata (Emberson et al. 2000). The leaf O_3 uptake rate has been shown to depend on species-specific or genotype-specific maximum and minimum stomatal conductances (Fares et al. 2008). It also depends on environmental variables such as light, temperature and water availability in the plant-soil system, all of which modify leaf stomatal conductance (Löw et al. 2006). It has been demonstrated that O_3 uptake by strong isoprene emitter *Populus nigra* is limited by stomatal aperture, and it was suggested that O_3 removal due to reactions inside the leaves occurs much faster than delivery of O_3 through the stomata (Fares et al. 2008). Relatively low reactivity of O_3 with isoprene likely limits significant O_3 losses in reactions in the leaf-atmosphere boundary layer in isoprene-emitting species. On the other hand, compared to isoprene, monoterpenes have greater potential to react with O_3 within or just above leaves (Atkinson 1997). The results obtained with *Quercus ilex* show that gas-phase reactions may contribute substantially to O_3 losses within the leaves of monoterpene-emitting species (Fares et al. 2008). It was also shown that O_3 flux into the leaf may be driven by the reaction with monoterpenes (Loreto and Fares 2007) and other antioxidant molecules (Burkey and Eason 2002). In fact, O_3 uptake in the leaves of isoprenoid emitting species, such as *Populus alba*, *Phragmites australis* and *Quercus ilex*, was higher than in the leaves of the non-emitting species *Nicotiana tabacum* and *Betula pendula* (Loreto and Fares 2007). More studies are needed to understand the biochemistry of O_3 reactions inside the leaf to exploit the capacity of vegetation to naturally scavenge O_3. At this point, it is interesting that Jardine et al. (2012) showed significant emissions of oxygenated compounds presumably coming from within-leaf gas-phase reactions between isoprene and OH. It would be interesting to determine if similar emissions of reaction products between O_3 and monoterpenes come from the leaves of monoterpene-emitting species such as *Quercus ilex*.

10.5 Inclusion of [CO_2] and [O_3] Effects in Models

Accurate prediction of BVOC changes to future air concentrations of CO_2 and air pollutants is necessary for estimating consequences and possible feedbacks to the chemistry of the atmosphere (Guenther et al. 1995; Wang and Shallcross 2000; Karl et al. 2004; Yokouchi and Ambe 2007; Lerdau 2007; Sitch et al. 2007). Improved knowledge of the environmental and physiological factors that regulate BVOC synthesis and emission has made it possible to develop different models that take into account the response of isoprene emissions to changing CO_2 concentrations including both the direct effect on the emission rate and indirect effects on photosynthesis and photosynthetic products required for isoprenoid biosynthesis (Monson et al. 2012; Grote et al. 2013).

There is little information available on the combined effects of elevated CO_2 and elevated O_3 concentrations on BVOC emission and the combination of these factors

with warming. These interactions are potentially relevant as there may be secondary effects of [O_3] and [CO_2] on the temperature or light algorithms as opposed to direct effects of these parameters on isoprene emission. For example, both elevated [CO_2] and [O_3] can lead to reduced stomatal conductance. Low stomatal conductance can increase leaf temperature by reducing latent heat loss. The [CO_2]-dependent higher leaf temperature (Zavala et al. 2013) might increase isoprene synthesis while [CO_2]-dependent reduced stomatal conductance allows isoprene to build up in the leaf (Sharkey 1991; Fall and Monson 1992) thereby enhancing leaf thermotolerance (see the chapter of Possell and Loreto 2013).

10.5.1 Simulating Atmospheric [CO_2] Effects on Emissions

The effect of CO_2 concentration on terpenoid emissions has been included in leaf-level algorithms through relations to physiological processes and in particular to photosynthetic and biochemical processes (Niinemets et al. 1999; Martin et al. 2000; Zimmer et al. 2000; Bäck et al. 2005; Grote and Niinemets 2008; Wilkinson et al. 2009; Rasulov et al. 2009; Possell and Hewitt 2011). In most of the cases, increases in CO_2 concentration are associated with linear or non-linear decreases in isoprene emissions (Possell et al. 2005; Wilkinson et al. 2009, Sect. 10.3). The shape of this response can have profound influences on predictions of future isoprene emission in elevated [CO_2] atmospheres (Arneth et al. 2007; Heald et al. 2009). A few available studies of CO_2 effects on monoterpenes emissions show a more variable picture, partly due to different responses of storage- and "non-storage"-emitters (Constable et al. 1999; Staudt et al. 2001; Loreto et al. 2001; Snow et al. 2003; Baraldi et al. 2004; Vuorinen et al. 2005; Llorens et al. 2009).

Two factors that have been conflated in past models of isoprene emission analysing its impact on air quality in future atmospheres of higher [CO_2] are the effects of CO_2 on net primary production (NPP) (a positive effect, Norby et al. 2005), which can increase global emissions due to more emitting leaf biomass, and the effect of [CO_2] on isoprene emissions per unit of leaf area (a negative effect). This conflation was noted by Monson et al. (2007), and as a result, many past predictions of atmospheric chemistry in future atmospheres with elevated [CO_2] are highly uncertain. The contribution of elevated [CO_2] to both NPP and isoprene emissions must be considered in models aiming to project future trajectories of atmospheric chemistry.

More recently, some global models have incorporated both the direct and the indirect effects of elevated [CO_2] on isoprenoid emissions using different models and approaches. Arneth et al. (2008) have shown that in Europe we should expect in most of the areas an increase in isoprene emission due to a stimulation of leaf area index (LAI) and due to land-use change, mainly because of the changes in the coverage of forested areas. They also showed that including the direct CO_2 inhibitory effect will completely counteract the effect of a warmer climate, with an overall decrease in the isoprene emission rate in most regions of Europe.

Heald et al. (2009) showed similar results at the global scale separating the short-term effect of elevated [CO_2] on isoprene emission and the long-term effect of elevated [CO_2] on plants and highlighting in particular that when changes in vegetation distribution and leaf area density are included in models, future isoprene emissions could increase by more than a factor of two.

It is clear that in assessing the sensitivity of isoprene emissions to future increases in atmospheric CO_2 concentration, we cannot ignore the parallel increases in temperature that are likely to occur. The positive effect of global warming, in terms of temperature and its effect on terrestrial productivity, tends to be of the same magnitude and sometimes even higher than the CO_2 inhibition of isoprene production (Arneth et al. 2007, 2008; Heald et al. 2009; Young et al. 2009). For instance large increases in the emission by the end of this century (27–70 % relative to present-day emissions) are expected due to the effect of global warming and more productive vegetation (Sanderson et al. 2003; Lathière et al. 2005; Wiedinmyer et al. 2006; Arneth et al. 2008; Ashworth et al. 2013). Moreover the increase in the frequency of extreme high temperature events will likely produce episodic peaks of isoprene emission.

Several models have been built not only to predict future CO_2 scenarios but also to investigate past CO_2 concentration effects on and temporal changes in BVOC emissions. Recent studies suggest that isoprene emissions were generally enhanced by low [CO_2], but only at the leaf level, as the overall effect on canopy isoprene emission was counterbalanced by reduced leaf area (Possell et al. 2005). Simulations of BVOCs emissions since the Last Glacial Maximum (LGM) showed that total emissions of isoprene and monoterpenes over Europe increased due to higher temperatures and an increase of vegetation abundance; despite the counteracting effect of CO_2 inhibition, the relative increase in isoprene and monoterpene emissions was larger than the increase in gross primary production (GPP), due to the stronger temperature sensitivity of terpenoid production compared to photosynthesis (Schurgers et al. 2009).

We note that it is important to couple the models predicting the effects of increased CO_2 concentrations on BVOC emissions with models predicting the ground ozone levels, integrating the future scenarios of biogenic emissions with air quality models and with regional climate data (Wiedinmyer et al. 2006; Young et al. 2009; Pacifico et al. 2009).

10.5.2 Towards Models of [O_3] Responses

Incorporation of the effects of air pollutants on isoprenoid emissions is more complicated and requires new models that include a stress signalling component (Niinemets et al. 2010c; Grote et al. 2013). Although such models are currently under development (Grote et al. 2013), air pollutant effects cannot currently be effectively simulated by process-based approaches. Nevertheless, at leaf level, an hypothesis based on the short- and long-term studies of [O_3] fumigation has been

formulated by Calfapietra et al. (2009) linking BVOC emission to the O_3 dose according to a hormetic-dose response characterized by a stimulation of BVOC emission at low O_3 doses and an inhibition at high O_3 doses. Modelling the O_3 effects over the long-term would result in an inhibition due to both decreased leaf area and decreased emission at leaf level, which is exacerbated when it occurs in combination with elevated [CO_2] (Calfapietra et al. 2008). Nevertheless, more experimental work is needed to test the generality of this hypothesis (Fig. 10.2).

10.5.3 Modelling Changes in Competitive Relations as the Result of [O_3] Elevation

Although simulation of [O_3] responses is currently difficult, [O_3] elevation is involved in altering numerous biological interactions, including alteration of terrestrial carbon sink (Ashworth et al. 2013) and multitrophic interactions (Holopainen et al. 2013). Here we address the important feedback loop between ozone tolerance and changes in community composition.

It is likely that rising [O_3] will provide a competitive advantage for isoprene-emitting species over non-emitting species because of the protection that isoprene synthesis provides against O_3 stress (Lerdau 2007). This could lead to a decrease in forest biodiversity, with isoprene-emitting species becoming more abundant and thus causing an increase of isoprene emissions at the global scale, with a strong positive feedback on tropospheric O_3 concentration (Lerdau 2007; Laothawornkitkul et al. 2009). On the other hand, higher O_3 concentrations could shorten the growing season and decrease standing leaf biomass (Karnosky et al. 2003). These secondary effects could negatively affect isoprene emissions and counteract any positive feedback generated by climate warming (Arneth et al. 2008). In summary, it is evident that future efforts of modelers should include O_3 effects and feedbacks with BVOCs for a better understanding of BVOCs-O_3 interactions in the future.

10.6 BVOCs-Air Pollution Interactions and Implications in a Changing Atmosphere

The interactions between BVOCs and O_3 pollution are complicated, especially if we consider the long-term responses. On one hand, BVOC emissions contribute to the formation of O_3 and other photochemical pollutants through reactions involving NO_x and OH radicals (Fuentes et al. 1999). In addition to being a greenhouse gas, O_3 is also a toxic pollutant that significantly reduces crop and forest yields worldwide and is responsible for human health problems during pollution episodes. Ozone phytotoxicity may significantly accelerate future climate warming due to reductions in the terrestrial carbon sink. On the other hand, BVOCs protect plants

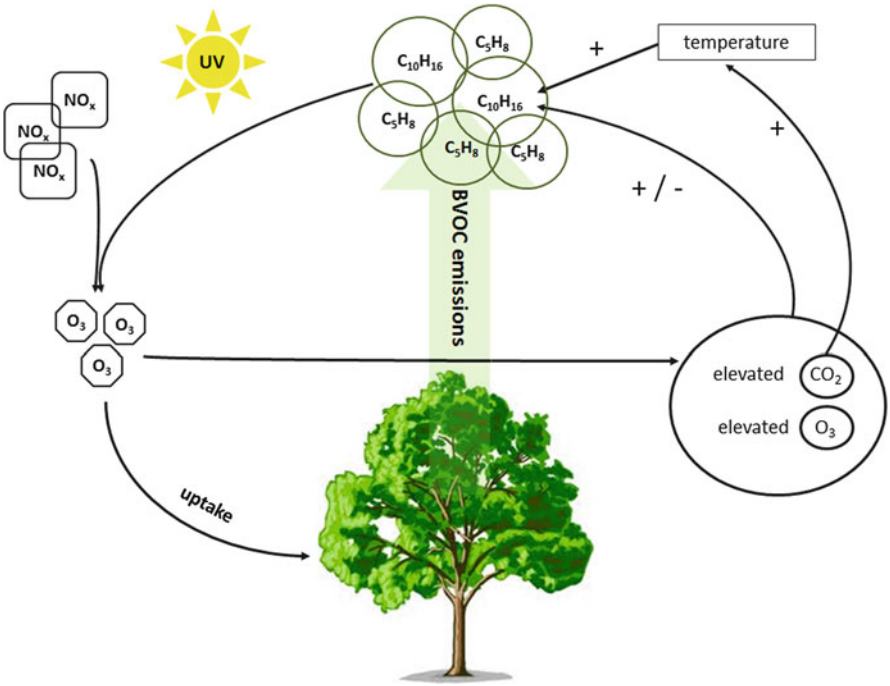

Fig. 10.3 Effects of BVOC emissions (including mainly isoprene and monoterpenes) on the O_3 formation and direct and indirect effects of increased CO_2 and O_3 concentrations on BVOC emissions. The indirect effect of [CO_2] and [O_3] results from the influence of these greenhouse gases on temperature. The figure demonstrates a positive loop resulting from the rise in temperature and O_3 on BVOC emissions. The overall positive effect is only partially alleviated by the O_3 uptake by plants, potentially negatively affecting emissions

against oxidative stress such as O_3 (Vickers et al. 2009; Possell and Loreto 2013), and it has been hypothesized that increasing levels of O_3 might stimulate BVOC synthesis and emission (Loreto et al. 2004). This aspect has raised the hypothesis of a positive loop considering that increasing levels of O_3 in the future will favor BVOC emitter species because of the protective role of these compounds and this will result in higher BVOC load into the atmosphere and higher O_3 production (Lerdau 2007, Sect. 10.5.3).

A positive dangerous loop could also occur in the future if there is a long-term O_3 stimulation of BVOC emissions as has been consistently observed in the short term (Fig. 10.3). Such a long-term enhancement as observed in some studies (Fig. 10.2) is, however, not general. For example, measurements carried out in plants exposed for several years to elevated [O_3] at the AspenFACE site demonstrated inhibition of isoprene emissions by [O_3] (Calfapietra et al. 2008).

The interactions between BVOC emission and atmospheric composition are complicated also by the recent findings related to the O_3 uptake capacity by plants

(Fig. 10.3). Ozone uptake was higher in BVOC-emitting plants than in non-emitters (Loreto and Fares 2007; Fares et al. 2010). However, what are the stomatal and non-stomatal contributions of this uptake and how relevant this process is for plant physiological activity and ambient ozone levels is still uncertain. It has been confirmed, however, that reactions between BVOCs and reactive oxygen species (ROS) occur already inside the leaves and that reaction products such as methyl vinyl ketone and methacrolein are emitted by most plants (Jardine et al. 2012), suggesting that a similar reactivity might also occur between BVOCs and O_3. This topic is central to understanding the vegetation capacity for uptake of air pollutants, particularly in urban environments (Nowak et al. 2006) where we might currently significantly underestimate the abundance of BVOC emitters (Niinemets and Peñuelas 2008; Owen et al. 2013). To conclusively assess the importance of vegetation in oxidant uptake, an important task for future research is to determine ozone uptake characteristics in relation to BVOC emissions for the main plant species. This can be done by laboratory measurements controlling the physiological status of the plant and the microclimatic conditions around it. Another key question, considering all these interactions between BVOCs and O_3, is which effect is stronger: the contribution of BVOCs to the O_3 formation or the contribution of plants (and even of BVOCs) on O_3 removal. So far, the variabilities and uncertainties in and understanding of the biological and chemical processes do not allow to draw clear conclusions on the relevance of different processes and connecting feedback loops.

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Chapter 11
Multitrophic Signalling in Polluted Atmospheres

Jarmo K. Holopainen, Anne-Marja Nerg, and James D. Blande

Abstract Volatile compounds emitted by plants in response to herbivory serve as important cues within and between trophic levels, and as cues over more than two trophic levels, such as in the attraction of enemies of herbivores. However, many of the volatiles elicited by herbivory are highly reactive with key atmospheric pollutants, implying that the signal is communicated over increasingly shorter distances with increasing pollutant concentrations in the atmosphere. Thus, polluted atmospheres can importantly alter the multitrophic interactions between trees, herbivores and herbivore enemies. This chapter highlights the alterations in multitrophic interactions and resulting modifications in plant fitness in polluted atmospheres.

11.1 Introduction

Atmospheric pollution is a major environmental factor and long known to have an impact on plants (Bell and Treshow 2002), herbivores, especially insects (Docherty et al. 1997), and the natural enemies of herbivores (Butler et al. 2009). Foresters have seen these impacts in practice as trees languish and frequent insect outbreaks occur in the surroundings of urban and industrial areas polluted by sulphur dioxide (SO_2) and oxides of nitrogen (NO_X). Heavy metal pollution distributed among other air pollutants has often resulted in death of surrounding vegetation and subsequent decline of communities of herbivores (Zvereva and Kozlov 2010) and their natural enemies (Butler et al. 2009). Recent environmental and ecological research has indicated that more ubiquitous air pollutants such as ozone (O_3) will affect vegetation over much longer distances from the urban and industrial point sources (Sitch et al. 2007; Calfapietra et al. 2013 in this volume). This means that in

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principle, all terrestrial ecosystems may suffer to a certain extent from air pollution and subsequently air pollutants may globally disturb the biogenic volatile organic compound (BVOC) signals evolved as facilitators of communication between plants and plants and animals across multiple trophic levels.

This chapter is aimed at highlighting how polluted atmospheres can be involved in altering multitrophic interactions communicated and mediated by BVOC signals and how this may result in modifications in plant fitness. First we review the current knowledge of the effects of air pollution on arthropod communities that live on woody plants. Knowledge of multitrophic interactions in arboreal communities under stress is needed for better understanding of BVOC signalling in forest ecosystems and how much these signals are dependent on the health of trees.

The mechanisms of multitrophic signalling between plants, between plants and herbivores and between plants and carnivores, which act as natural enemies of herbivores, have been subjected to extensive research in recent years. However, most of the studies have been conducted in controlled laboratory conditions and less is known about how variability in air quality affects the volatile signals. We will survey recent studies where the role of the atmospheric pollution load is considered as an additional factor adding "noise" to BVOC signals. Then we take a closer look at the impacts of different atmospheric pollutants, which have distinctly different impacts on the lifetimes of the volatile signalling compounds in the atmosphere. Three specific oxidants: ozone (O_3) and OH and NO_3 radicals react with volatile signalling compounds produced by plants. These oxidants also occur in less polluted environments, but at much lower concentrations than in areas currently suffering from direct impacts of air pollution. Finally, we discuss future directions for research on multitrophic signalling and how forecasted changes in atmospheric conditions due to global change could impact the multitrophic signalling patterns. In the coming decades, human population growth, increasing urbanization and global climate change will extensively influence the environmental conditions for both cultivated and wild plants. Therefore, a better understanding of the function and pollutant sensitivity of BVOC-dependent multitrophic chemical signalling networks is essential. In this chapter we primarily focus on stress-induced volatiles. For [O_3] effects on constitutive emissions, we refer to the Calfapietra et al. (2013) chapter in this volume.

11.2 Impact of Air Pollution on Tree-Insect Interactions

Research on air pollution impacts on vegetation and interactions among species in ecosystems has evolved in heavily industrialised areas where plants develop visible pollutant stress symptoms. Trees as long-living plants tend to accumulate the pollution load, especially species with long-living foliage. Accordingly, trees will be more impacted by sustained pollution than annual plants. Several observations, the oldest dating back to the year 1832 (Cramer 1951), of the surroundings of industrial point sources have indicated that when forest trees are exposed to "smoke stress",

containing sulphur dioxide (SO_2) as the main gaseous air pollutant, outbreaks of needle mining moths become more frequent (Oksanen et al. 1996; Kozlov 2003). Similar observations of increased densities of herbivorous insects on trees in industrial environments have been made for sucking insects such as the bark-feeding plant bug *Aradus cinnamomeus* (Heliövaara and Väisänen 1986) and aphids (Villemant 1981; Holopainen et al. 1993). Higher aphid population densities and increased growth rates of aphids have also been reported in trees along roads with heavy traffic (Braun and Flückiger 1985; Bolsinger and Flückiger 1987). On the other hand, investigations in industrial areas have indicated that defoliating insects with chewing mouth parts do not usually have mass outbreaks on forest trees exposed to high air pollutant loads (Heliövaara and Väisänen 1986; Holopainen and Oksanen 1995). One explanation for the relatively low incidence of defoliators in polluted atmospheres is that defoliators accumulate potentially harmful particulate pollutants from the leaf surfaces in their bodies, while phloem feeders, cambium-feeding bark beetles and mining species only respond to the quality changes of their host plants under a pollutant load (Holopainen and Oksanen 1995).

Experimental exposures of plants to gaseous, semivolatile and precipitating pollutants such as SO_2, NO_2, ammonium, nitric and sulphuric acid deposition and fluorides have demonstrated a rapid increase in growth rate and number of aphids on woody plants (e.g., Bolsinger and Flückiger 1989; Holopainen et al. 1991; Neuvonen and Lindgren 1987). This indicates that changes in plant quality, such as a decrease in protein concentration and better availability of free amino acids (Bolsinger and Flückiger 1989; Kainulainen et al. 1993) could be the major reason for insect outbreaks on woody plants in areas with high air pollution. Foliar secondary metabolites of trees including terpenoids such as monoterpenes and resin acids (Kainulainen et al. 1993, 1994, 1995a, b) and phenolic compounds such as total phenolics and tannins (Julkunen-Tiitto et al. 1995; Kainulainen et al. 1993, 1994, 1995a, b) have displayed less uniform responses to industrial air pollution stress. The impact of the more ubiquitous pollutant ozone is known to induce the production of simple phenolics and flavonoids (Lindroth 2010) and affect emissions of volatile isoprenoids, especially in interaction with increases in atmospheric CO_2 concentration (Peñuelas and Staudt 2010; Holopainen and Gershenzon 2010).

Besides the effects of air pollution on plant defence and nutritional quality for herbivores, harmful impacts of pollutants on parasitoids (reviewed by Butler et al. 2009) and predators (Sorvari and Eeva 2010; Percy et al. 2002; Zvereva and Kozlov 2010) of herbivores may also result in rapid population growth of herbivores and cause insect outbreaks. The impacts of pollutants on the third trophic level, and in particular on insect parasitoids, have been variable depending on pollutant type and species studied. A review of controlled-condition experiments has indicated that in the majority of the studies, air pollutant load did not affect parasitoids (18 cases) or had negative impacts (also 18 cases), and only in three cases, there were positive effects of air pollutants on the performance of insect parasitoids (Butler et al. 2009). Most frequently, harmful effects on parasitoids were caused by ozone and heavy metal pollution. In particular, extensive field-scale exposures of deciduous forest trees to doubled ambient ozone levels over several years resulted in very

high aphid populations relative to parasitoid densities (Percy et al. 2002). These results suggest that high concentrations of oxidizing pollutants may have a negative impact on tritrophic signalling mediated by herbivore-induced BVOCs in polluted atmospheres.

11.3 Plant Volatiles as Signalling Compounds Within and Across Trophic Levels

11.3.1 Volatile Compounds Induced by Biotic and Mechanical Injury

The "scentscape" of BVOCs created by living plants and associated organisms plays an important signalling function in species interactions and ecosystem processes (McFrederick et al. 2009). When plant cells are damaged mechanically (Brilli et al. 2011; Piesik et al. 2011) by herbivores (Blande et al. 2007; Allmann and Baldwin 2010; Dicke and Baldwin 2010; Holopainen and Gershenzon 2010; Schaub et al. 2010; Holopainen 2011; Piesik et al. 2011; Copolovici et al. 2011; Clavijo McCormick et al. 2012; Trowbridge and Stoy 2013 in this volume), by fungal pathogens (Vuorinen et al. 2007; Toome et al. 2010) or inoculated with fungal symbionts (Schausberger et al. 2012), plants start to emit volatile organic compounds (BVOCs) at higher rates. Mechanical damage of the foliage of herbaceous plants and deciduous trees caused for example by chewing mouth parts, typically results in the rapid emission of various green leaf volatiles (GLVs), C_6 volatile oxidation derivatives of fatty acids with a typical scent of cut grass (Schaub et al. 2010; Clavijo McCormick et al. 2012; Monson 2013 in this volume). Soon after these initial emissions, there are elevated emissions of other compounds such as monoterpenes and sesquiterpenes (Schaub et al. 2010). Of the herbivore-induced terpene compounds, acyclic homoterpenes, 4,8-dimethylnona-1,3,7-triene (DMNT) and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), are among the most widespread volatiles produced by angiosperms (Tholl et al. 2011). Homoterpenes can be found in flower emissions of many plant species (Tholl et al. 2011), but their greatest significance is in attracting parasitoids and predators of herbivores to herbivore-damaged plants (Pinto et al. 2007a). In deciduous trees, DMNT is the most responsive homoterpene in the BVOC bouquet of trees damaged by chewing insect herbivores (Arimura et al. 2004; Blande et al. 2007, 2010b; Staudt and Lhoutellier 2007; Mäntylä et al. 2008). Substantial methyl salicylate (MeSA) emissions in deciduous trees, such as *Betula pendula* and *Alnus glutinosa*, are indicative of aphid infestation (Blande et al. 2010b).

Conifers represent an interesting model system for studies of volatile emissions, as they have a wide range of constitutive and induced compounds, as well as a diversity of stored and constitutively emitted compounds. Among the elicited compounds of conifers are those that are "novel", mostly emitted only after damage

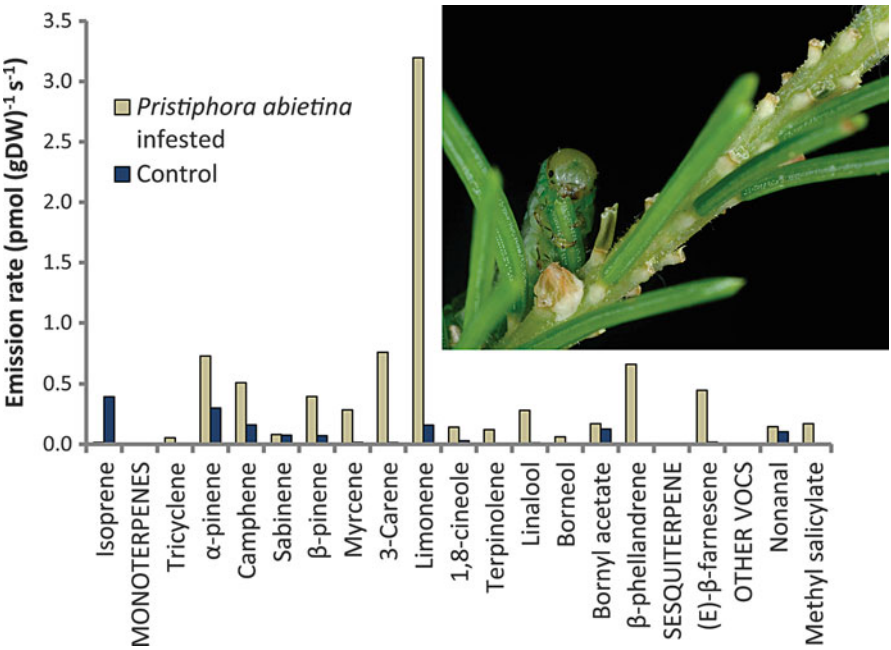

Fig. 11.1 Small spruce sawfly (*Pristiphora abietina*) is feeding on young developing needles of Norway spruce (*Picea abies*) shoots. Herbivore-induced BVOC emission rates (pmol g^{-1} DW s^{-1}) of young shoots have an altered profile with the most distinctive increase in the emission of monoterpenes 3-carene, limonene, β-phellandrene and sesquiterpene (*E*)-β-farnesene (Blande et al. unpublished data). Volatiles were collected from individual branches of *Picea abies* saplings that had been either enclosed for five days in mesh bags containing 5–6 spruce sawfly larvae, or enclosed in a mesh bag without larvae. Branches were enclosed in polyethylene terephthalate (*PET*) bags and samples were collected onto Tenax TA for 30 min. All samples were analysed by gas chromatography-mass spectrometry

of the plant by herbivores (e.g., Kännaste et al. 2008, 2009). The induced emissions also include compounds that are normally emitted constitutively, but are found in much higher quantities after herbivore damage. For example, when young shoots of Norway spruce (*Picea abies*) are damaged by the little spruce sawfly (*Pristiphora abietina*) there is a distinctive increase in the emissions of some constitutively emitted monoterpenes such as limonene, 3-carene, β-pinene and α-pinene (Fig. 11.1). In addition, the monoterpenes terpinolene, linalool, borneol and β-phellandrene and the aromatic compound methyl salicylate are "novel" inducible compounds that are emitted only by branches damaged by sawfly larvae. The sesquiterpene β-farnesene is highly inducible in *P. abies*, but these emissions may also occur in minor quantities in intact control plants. In the shoot emissions of Scots pine (*Pinus sylvestris*) seedlings suffering from pine weevil (*Hylobius abietis*) induced stem bark injury, several monoterpenes and sesquiterpenes were emitted at a significantly increased rate, but monoterpene 1,8-cineole emissions were absent in

shoots of intact plants (Heijari et al. 2011). Significant increase of total monoterpene emissions from herbivore damaged conifer foliage is mostly a result of mechanical damage to needles and the resulting flow of resins from storage organs, and elevated emissions continue until the wound sites are sealed by oxidized and polymerized terpenes (Loreto et al. 2000). Mechanical damage simulating herbivory of needle tissue results in a similar increase in total monoterpene emissions to that induced by actual feeding by an insect herbivore (Litvak et al. 1999).

11.3.2 Volatile Compounds Involved in Within-Plant Signalling

A recently discovered primary signalling function of plant volatiles emitted by branches of herbivore- or pathogen-attacked trees is to protect unwounded branches from the risk of infestation by herbivores (Rodriguez-Saona et al. 2009) or infection by pathogens (Yi et al. 2009). Such inter-branch signalling can result from the full mixture of herbivore-induced BVOCs (Rodriguez-Saona et al. 2009) or from specific herbivore-induced compounds such as methyl jasmonate, methyl salicylate (Heil and Ton 2008) or *cis*-3-hexenyl acetate (Frost et al. 2008). Rodriguez-Saona et al. (2009) showed that intact branches of highbush blueberry (*Vaccinium corymbosum*) exposed to volatiles of gypsy moth (*Lymantria dispar*) damaged branches had as much as 70 % less damage caused by larvae than branches exposed to volatiles from undamaged branches and had greater chemical defences including higher amounts of endogenous *cis*-jasmonic acid. One feature of this within-plant signalling is that non-vascularly connected parts of the same plant may be primed for enhanced defence responses if the given biotic stressor were to attack. Priming undamaged parts of trees with a volatile signal is a more rapid and efficient method of signalling than long-distance vascular signalling in trees (Frost et al. 2007). This allows a systemic (whole plant) response to be achieved, as indicated in holm oak (*Quercus ilex*) damaged by *Lymantria dispar* larvae (Staudt and Lhoutellier 2007). Defence priming could be the primary function of inducible plant BVOCs for within plant signalling.

11.3.3 Volatile Compounds in Intraspecific Plant to Plant Signalling

In dense woody ecosystems, the branches of neighbouring trees of the same species are positioned very close within tens of centimetres to a few metres and signals from damaged trees have been shown to prime undamaged neighbours for enhanced defence responses. The first reports of airborne between-plant signalling, in the 1980s (e.g., Baldwin and Schultz 1983), concerned trees and the phenomenon of increased resistance in intact plants close to herbivore-damaged plants was referred

to as "talking trees" (Baldwin et al. 2006; Heil and Karban 2010). Since that early discovery, between-plant volatile signalling has been detected in many agricultural crop plants (Heil and Karban 2010). It has been suggested that between plant signalling may be a result of 'eavesdropping' by the receiving tree due to the emitting plant benefiting more by signalling to itself, or natural enemies of its pests, rather than to a nearby competitor (Arimura et al. 2010; Karban 2011). Irrespective of the fitness costs or benefits for the emitting plant, it is clear that the receiving plants gain a benefit from being able to pre-empt herbivore or pathogen attack. The genetic relatedness of the receiving plant to the emitter can importantly alter the responsiveness of the receiver plant to the chemical signal. Recent studies have indicated that sagebrush (*Artemisia tridentata*) plants produced clonally from a common parent plant are better able to detect signals from a neighbour of the same clone than from other, genetically more distant *A. tridentata* plants (Karban and Shiojiri 2009; Ishizaki et al. 2012). This suggests that signalling between plants via BVOCs could be far more sophisticated than previously thought with important within population variations and evolutionary implications, and clearly will require further experimental research.

11.3.4 Volatile Compounds in Interspecific Plant to Plant Signalling

The closest association between two plant species is parasitism by parasitic plants, a strategy represented by approximately 1 % of flowering plant species. Constitutively emitted BVOCs of tomato (*Solanum lycopersicum*) are known to attract the parasitic plant *Cuscuta pentagona* (Runyon et al. 2006). However, after infection, emissions of constitutive and herbivore-induced BVOCs are attenuated so efficiently that herbivore-damaged tomato plants become less attractive to parasitic plants (Runyon et al. 2008). Parasitic plants can possibly use BVOCs as cues to avoid herbivore damaged plants, which may have reduced quality as hosts. In addition, generalist herbivores may damage the parasitic plant itself once they attack the infested plant. Interspecific signalling, where volatiles that are released as the result of mechanical damage or in response to herbivory affect the defence traits in another plant species, "associative resistance", has been reported for herbaceous plants (Heil and Karban 2010). Himanen et al. (2010) reported that the woody shrub *Rhododendron tomentosum* releases specific volatile monoterpenes and semivolatile sesquiterpenes and sesquiterpene derivatives that can be adsorbed to the foliage of neighbouring birch (*Betula* spp.) saplings and re-released into the atmosphere when leaf temperatures are elevated in the morning. Furthermore, these semivolatile compounds improved herbivore resistance in the receiver plant (Himanen et al. 2010). This raises the question of an opportunity cost of volatile release in the emitter, i.e., whether the emitters reduce their own competitive status by having healthier *Betula* spp. neighbours that are potentially more able to compete for shared

resources? Or is this an indication of how strictly forest plant species depend upon one another at the community level (Karban 2010)? Earlier, Choh et al. (2004) reported that herbivore-induced semivolatile compounds can be adsorbed to the surfaces of conspecific crop plants, and several studies have further demonstrated certain uptake capacity of several monoterpenes (Copolovici et al. 2005; Noe et al. 2008). However, we do not yet know if these volatiles and semivolatiles adsorbed onto foliage surfaces and/or solubilised in the lipid phase inside the leaves induce defence responses in receiver plants or if they are just increasing defence by passive evaporation and serve as repellents of herbivores or as attractants of herbivore enemies.

11.3.5 Plant Volatile Compounds in Coevolution of Plants and Herbivores – Direct Plant Defences

The coevolutionary arms race, antagonistic adaptations and counter-adaptations between plants and their parasites, pathogens and herbivores, is a traditional way to view plant–enemy interactions and evolution of defence traits in plants. However, this coevolution is only expected when a plant species interacts with a specialist herbivore and with specialist parasites such as fungal pathogens, which have enough continuous pressure to reduce the fitness of the host plant (Agrawal and Heil 2012). For example, a new secondary metabolite produced by a plant should reduce the fitness of a specialist herbivore until a new detoxification trait has evolved in the herbivore species. This detoxification trait is a competitive advantage for the herbivore and it might consequently face less competition from other herbivore species. This will lead to further tightening of the specialization of the herbivore to the specific host plant species. Typically, specialist herbivores have broken the defence barriers of a plant species, while the arms race may increase the plant defence against generalist herbivores (Agrawal and Heil 2012). Generalists, on the other hand, often specialize at feeding on specific plant organs (Schoonhoven et al. 2005) such as fast growing shoots, which may be less defended due to dilution of defence compounds such as phenolic compounds in fast growing tissues (Keski-Saari and Julkunen-Tiitto 2003).

Other plant resistance traits against insect herbivores include the emission of feeding inhibitory or repellent BVOCs, which have impacts on herbivore preference and host selection (Kloth et al. 2012; Clavijo McCormick et al. 2012). The same specific volatile compounds that have primary attractant properties for specialist herbivores act as repellents for other herbivores (Reddy and Guerrero 2004). Species-specific plant BVOCs are most effective against specialist herbivores that specifically target other plant families not constitutively releasing these compounds (Himanen et al. 2010). Many of these species-specific constitutively emitted volatiles are common for one plant taxonomic group, but appear less frequently or not at all in other plant taxonomic groups. For example, the sesquiterpene alcohol palustrol is found mainly only in *Rhododendron* spp. (Jaenson et al. 2005) and

volatile hydrolysis products of glucosinolates in the Brassicaceae (Tansey et al. 2010). Thus, if these compounds are emitted from species not synthesising them, this can significantly reduce the host plant recognition by specialist herbivores. In addition, species-specific plant BVOCs are in many cases effective against generalist herbivore species as well and may even protect neighbouring plant species from generalist herbivores in plant communities (Himanen et al. 2010). More complicated repellent functions have been found for some specific volatiles, such as 4-allyl anisole, a common compound in conifer species, which interrupts bark beetle responses to their own pheromones (Reddy and Guerrero 2004).

11.3.6 Plant Volatile Compounds in Coevolution of Plants and Carnivores – Indirect Plant Defences

Multitrophic signalling by plants is most often defined in a tritrophic context (Vet and Dicke 1992), whereby parasitoids and predators of herbivores use herbivore-induced BVOCs as orientation cues to find suitable herbivore hosts. Even the hyperparasitoids of the fourth trophic level (parasitoids of a herbivore's parasitoids) can utilise herbivore-induced plant volatiles (Buitenhuis et al. 2005). It has also been demonstrated, in a brassicaceous system, that parasitism of feeding herbivores can have a significant effect on plants' susceptibility to oviposition by latterly foraging herbivores (Poelman et al. 2011a). In fact, the identity of the parasitoid species has a greater effect on plant susceptibility than the identity of the herbivore (Poelman et al. 2011a), which may be facilitated by the blend of volatile chemicals induced by the differently parasitized herbivores, or through alternative changes to the plant phenotype (Poelman et al. 2011b).

Most of the detailed research on tritrophic BVOC-mediated communication between plants and natural enemies of herbivores has been conducted with herbaceous plants. There is still ample evidence that attraction of parasitoids (Wei et al. 2008) or predators (Scutareanu et al. 1997) of herbivores by plant BVOCs does occur in woody plants under natural conditions, and is an effective strategy to reduce herbivore damage (Ammunet et al. 2009; Babin-Fenske and Anand 2011; Klemola et al. 2012). However, there has been some criticism that parasitoid larvae inside a herbivore larva may stimulate feeding and increase the size of leaf area consumed by the herbivore (Coleman et al. 1999). It has previously been shown that parasitism of a galling psyllid (*Baccharopelma dracunculifoliae*) by a koinobiont parasitoid (*Psyllaephagus baccharidis*) can stimulate nymph feeding and result in larger galls, which are stronger nutrient sinks for plants (Espírito-Santo et al. 2004). It has also been shown that parasitism by a tachinid (*Thelaira americana*) induces its host caterpillar (*Platyprepia virginalis*) to shift host from lupine (*Lupinus arboreus*) to hemlock (*Conium maculatum*), thus increasing survival rates of the caterpillar and reducing immediate feeding damage to lupine (Karban and English-Loeb 1997). However, parasitism of the frequent defoliator of mountain birch

(*Betula pubescens* ssp. *czerepanovii*), the autumnal moth (*Epirrita autumnata*), by the solitary endoparasitoid *Zele deceptor*, reduced leaf consumption significantly and also hastened the onset of pupation in autumnal moth larvae (Ammunet et al. 2009). The question of whether parasitism of a plant's herbivores ultimately benefits the plant is complex, and only likely to be conclusively answered on a case by case basis. Clearly, those parasitoids that reduce feeding damage by herbivores provide a tangible benefit to the plant. Those parasitoids that promote feeding damage might provide a long-term benefit by removing the oviposition potential of the herbivore. However, if the plant is a short-lived annual such a benefit might not occur. More research is needed to address the long-term health costs or benefits in plants that support herbivores that are parasitized by feeding-promoting parasitoids.

11.4 Polluted Atmosphere Effects on Plant Volatile Signals

11.4.1 Reactivity of Plant Volatiles

In natural environments, many of the oxidative air pollutants appear in lower concentrations than in industrial, urban and in heavy traffic polluted areas, but the reactivity of biogenic BVOCs with air pollutants is probably an integral part of the evolved functions of BVOCs (Lerdau and Slobodkin 2002), giving spatial and temporal dimensions to the signals they convey. The atmospheric gas-phase degradation of BVOCs produced by biogenic or anthropogenic sources is initiated by reaction with hydroxyl (OH) radicals, ozone (O_3) and nitrate (NO_3) radicals or via photolysis (Hallquist et al. 2009). Ozone reacts only by addition to C-C double bonds of alkenes followed by degradation of the resulting ozonide (Pinto et al. 2010; Atkinson and Arey 2003). Many of the biogenic BVOCs emitted by plants, such as monoterpenes and sesquiterpenes, contain one or more double bonds, which explain their reactivity. OH and NO_3 radicals also cleave C-C double bonds, but they additionally affect C-H bonds and to a much lesser extent O–H bonds (Atkinson and Arey 2003). The reactivity of different plant volatiles differs more than 3 (reactions with ozone) to 5–6 (reactions with OH and NO_3 radicals) orders of magnitude (Table 11.1), implying that air pollution can importantly alter the signal strength, composition and dispersal.

11.4.2 Loss of Volatile Signals Versus Possible New Signals

Processes related to degradation of biogenic BVOCs by ozone have been most thoroughly studied (see Pinto et al. 2010 for a review). The concentrations of many herbivore-induced compounds were decreased in ozone-rich atmospheres much faster than in filtered air (Pinto et al. 2007a, b, c). Some of the reaction products

Table 11.1 Atmospheric lifetimes of selected constitutively emitted and herbivore-induced biogenic volatile organic compounds in reactions with major reactive air pollutants

	BVOC	Lifetimes for reaction with oxidants			
Compound	Class	OH[a]	O_3[b]	NO_3[c]	Reference
Typical constitutively emitted compounds					
Isoprene	I	1.4 h	1.3 day	1.6 h	(1)
3-Carene	MT	1.6 h	11 h	7 min	(1)
Limonene	MT	49 min	2.0 h	5 min	(1)
α-Pinene	MT	2.6 h	4.6 h	11 min	(1)
Myrcene	MT	39 min	50 min	6 min	(1)
Longifolene	SQT	2.9 h	>33 day	1.6 h	(1)
Methanol	Alcohol	12 day	>4.5 year	2.0 year	(1)
Typical herbivory-induced compounds					
cis-/*trans*-Ocimene	MT	33 min	44 min	3 min	(1)
β-Phellandrene	MT	50 min	8.4 h	8 min	(1)
Linalool	MT	52 min	55 min	6 min	(1)
β-Caryophyllene	SQT	42 min	2 min	3 min	(1)
β-Farnesene	SQT	52 min	26 min	–	(2)
DMNT (4,8-dimethyl-1,3,7 nonatriene)	HT	40 min	60 min	3 min	(3)
TMTT (4,8,12-trimethyl-1,3,7,11-tridecatetraene)	HT	30 min	30 min	2 min	(3)
cis-3-Hexenyl acetate	GLV	1.8 h	7.3 h	4.5 h	(1)
cis-3-Hexen-1-ol	GLV	1.3 h	6.2 h	4.1 h	(1)
cis-3-Hexenal	GLV	11.2 day	3.0 h	–	(2)
Methyl salicylate	Aromatics	73.5 h	>9.8 year	–	(2)

References: (1) Atkinson and Arey (2003), (2) Arneth and Niinemets (2010), (3) Roger Atkinson, personal communication

BVOC classes: I – isoprene, MT – monoterpene, SQT – sesquiterpene, HT – homoterpene, GLV – green leaf volatile

Pollutant concentrations used in calculations:

[a]Assumed OH radical concentration: 2.0×10^6 molecule cm^{-3} (0.074 pmol mol^{-1}), 12-h daytime average

[b]Assumed O_3 concentration: 7×10^{11} molecule cm^{-3} (26 nmol mol^{-1}), 24-h average

[c]Assumed NO_3 radical concentration: 2.5×10^8 molecule cm^{-3} (9.3 pmol mol^{-1}), 12-h nighttime average

of the initial oxidation step of BVOCs contain one or more polar oxygenated functional groups, such as aldehyde, ketone, hydroxyl or carboxy groups. Oxidation tends to make the products less volatile and more water soluble (Atkinson and Arey 2003; Kroll and Seinfeld 2008; Harley 2013 in this volume). These reaction products may have the potential to function as herbivore repellents when aged and accumulated on plant surfaces (Signoretti et al. 2012a). For example, measurements of the early reaction products of α-pinene show that several organic acids, including 10-hydroxypinonic acid, 10-hydroxypinonaldehyde, 4-oxopinonic acid,

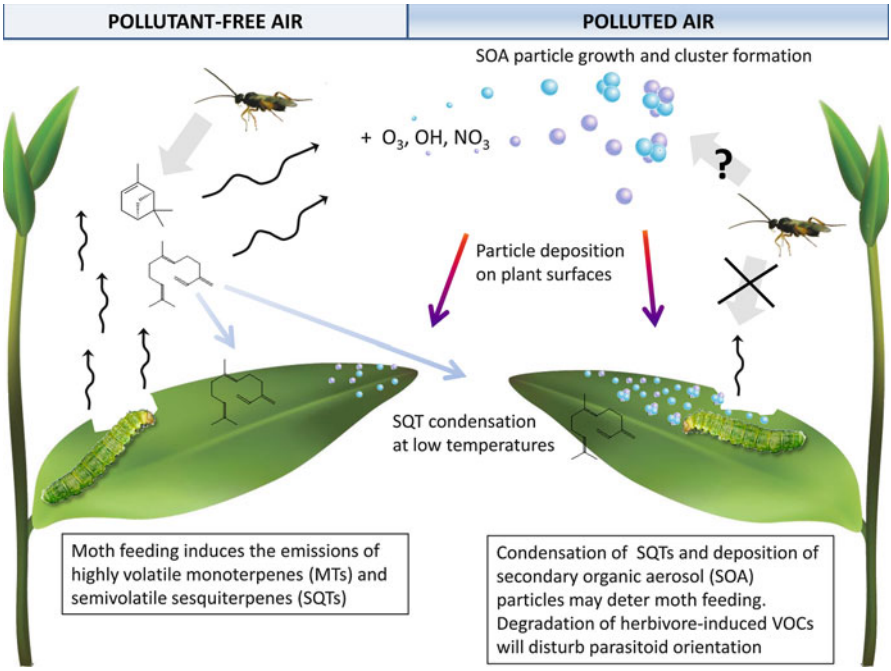

Fig. 11.2 Schematic representation of atmospheric behaviour of monoterpenes (MTs) and sesquiterpenes (SQTs) and their possible impact on multitrophic interactions in clean and polluted environments. The *left side* of the figure illustrates the situation in clean air where most of the experimental behavioural studies have been conducted. These studies have shown that herbivore damage induces emissions of MTs and SQTs from plants, and natural enemies of herbivores use these compounds as orientation cues. The right side illustrates the situation in polluted atmospheres. Formation of secondary aerosol particles from reactions of MTs and SQTs with O_3, OH and NO_3 radicals have been shown to be much higher in elevated oxidant concentrations than in normal background concentrations in reaction chamber studies. There is some evidence that degradation of herbivore-induced BVOCs in polluted air disturbs the orientation behaviour of parasitoids and BVOC-transmitted defence priming in neighbouring plants. So far unexplored area of research is how reaction products of BVOCs, such as oxidized semivolatiles condensed on leaf surfaces and secondary organic aerosol (SOA) particles deposited onto vegetation, may affect herbivore behaviour

and 10-oxopinonic acid are observed in the early stages of atmospheric reactions (Jaoui and Kamens 2001). These organic acids may play an important role in the early phase of secondary aerosol formation. Further oxidation may also lead to formation of solid aerosol particles (Fig. 11.2; Cape 2008; Kroll and Seinfeld 2008; Virtanen et al. 2010) or fragmentation to lower molecular weight volatiles, which finally oxidize to CO_2 (Atkinson and Arey 2003).

The complexity of atmospheric reactions of BVOCs with oxidants is best illustrated by the reaction chamber study by Kundu et al. (2012). They analysed the organic reaction products of the monoterpene limonene mixed with O_3 in a reaction chamber and found approximately 1,200 different organic compounds formed from

this single BVOC. Due to the ubiquitous presence of O_3, and OH and NO_3 radicals in the atmosphere, it is highly probable that some of the reaction products of oxidation of limonene or other major BVOCs emitted by herbivore-damaged plants may have similar biological functionality to the original compounds synthesised by plants. If the olfactory sensors of predators and parasitoids are able to distinguish between the original compounds released by a damaged plant and the oxidant reaction products of the originally emitted compounds as suggested by Pinto et al. (2007a), the ratio of these compounds could be indicative of the distance from the BVOC source and the location of a potential host herbivore. Electro-antennogram (EAG) recordings are needed to elucidate if the ratios of the original herbivore-induced compounds and their reaction products can act as functional signals for insects.

The nitrate (NO_3) radical is formed in reactions of O_3 with NO_X (NO and NO_2) that can be of soil, plant or anthropogenic origin. NO_3 radicals undergo very rapid photolysis (~5 s) in direct sunlight (Atkinson and Arey 2003). Therefore, NO_3 concentrations are usually only at measurable levels during the nighttime (Atkinson and Arey 2003). NO_3 concentrations are found at the highest levels within tree canopies, where nighttime concentrations of NO_3 can reach 20 ppt (McFrederick et al. 2009). This is very important for the signalling capacity of many herbivore-induced BVOCs, since many of these compounds are more reactive with NO_3 radicals than with OH radicals or ozone (Table 11.1, Fig. 11.2). Damaged plants are able to compensate for the high reactivity of some herbivore-induced compounds, such as DMNT, with higher emissions of the compound during nocturnal herbivore feeding compared to daytime feeding (Signoretti et al. 2012b). On the other hand, emissions of herbivore-induced monoterpenes, such as β-ocimene (Copolovici et al. 2011), can be strongly light-dependent (Owen et al. 2002), and accordingly, the way and extent to which atmospheric pollutants alter plant signals can be strongly species-dependent.

11.4.3 Alterations in Signalling Distance

McFrederick et al. (2008) modelled the lifetimes of volatiles released from flowering plants and estimated that the signalling distance of highly reactive floral BVOCs, utilised by pollinators and constituting signals from first to second trophic levels, may have decreased from kilometres during pre-industrial times to 200 m in the more polluted conditions of present times. In their BVOC models, they used the volatile monoterpenes β-ocimene, β-myrcene and linalool, which are common in floral emissions, but increased emissions of the same compounds can be induced by herbivore feeding, e.g., in black alder (*Alnus glutinosa*) (Copolovici et al. 2011), hybrid aspen (*Populus tremula* x *tremuloides*) (Blande et al. 2007), hybrid poplar (*Populus* x *euramericana*) (Brilli et al. 2009), holm oak (*Quercus ilex*) (Staudt and Lhoutellier 2007), silver birch (*Betula pendula*) (Vuorinen et al. 2007), mountain birch (*Betula pubescens* ssp. *czerepanovii*) (Mäntylä et al. 2008) or Scots pine

(*Pinus sylvestris*) (Heijari et al. 2011). Further modelling (McFrederick et al. 2009) showed that with increasing concentrations of ozone, the destruction rate of the sesquiterpene β-caryophyllene was much faster than the destruction rate of the monoterpenes β-ocimene and β-pinene, but with elevated concentrations of OH and NO_3 radicals the destruction rate of β-ocimene was similar to that of β-caryophyllene (Table 11.1, Fig. 11.2). Although the research community tends to think that all sesquiterpenes are very reactive and difficult to detect in plant emissions, some sesquiterpenes such as longifolene have actually relatively long lifetimes in polluted atmospheres (Arneth and Niinemets 2010).

11.4.4 Implications for Signalling Experiments in Polluted Atmospheres

The possibility, that BVOCs exhibiting low reactivity can act as signals when more highly reactive BVOCs have been oxidized, highlights the importance of including other naturally occurring oxidants such as OH radicals in such experiments (McFrederick et al. 2009). Pinto et al. (2007a) hypothesized that methyl salicylate may provide a robust long distance signal, as it is relatively non-reactive with O_3 and OH radicals (Canosa-Mas et al. 2002; Arneth and Niinemets 2010, Table 11.1). So far, the experimental data for reactivity of methyl salicylate with NO_3 radicals are missing. However, assuming that the rate constant of methyl salicylate for the reaction with NO_3 radicals is similar to that for phenol and NO_3 pair, methyl salicylate atmospheric lifetime is predicted to be approximately only 6 min for nighttime oxidation initiated by NO_3 radicals (Canosa-Mas et al. 2002). It may be possible to create and control OH radicals in chamber and olfactometry experiments and thus, simulate pollution-induced degradation of BVOCs. In chamber experiments, this can be done by adding O_3 and OH radical scavengers such as tetramethylethylene (TME), 2-butanol or *trans*-2-butene to control OH and O_3 levels (Arnts 2008; Hao et al. 2011). Behavioural experiments with carnivorous arthropods will be more complicated, because these compounds may directly affect the behaviour of insects and mites, which is something that should be tested first.

11.5 Influence of Air Pollution on Multitrophic Communication

11.5.1 Oxidative Pollutant Effects

As reviewed in Sect. 11.3, there is a multitude of within- and among-species ecological interactions that rely on signalling via plant-emitted BVOCs. Earlier field studies have clearly documented disturbances in many of these type of interactions in forest ecosystems suffering from air pollution (Percy et al. 2002; Butler et al.

2009). Surprisingly few studies have tried to assess the mechanisms underlying the disturbance and clarify the possible role of degradation of BVOC signalling in these interactions (Holopainen and Gershenzon 2010). It is known that exposing plants to higher concentrations of ozone can induce the emission of volatiles that are very similar to those induced by spider mite feeding (Vuorinen et al. 2004a). However, predatory mites are still able to distinguish between ozone and spider mite–induced BVOCs when behavioural tests are run in purified air conditions, whereby there is no loss of volatile signals due to degradation by residual ozone in the system (Vuorinen et al. 2004a, b). Exposure of woody plants to elevated ozone concentrations also induces BVOC emissions (Heiden et al. 1999; Loreto et al. 2004), but the impact of these emissions on the behaviour of herbivores or their natural enemies has not been assessed.

Studies using herbaceous plants and decaying plant material have indicated that air pollutants may affect BVOC-based multitrophic communication between host plant, herbivores and parasitoids or predators. Gate et al. (1995) showed in chamber experiments using a fruit fly (*Drosophila subobscura*), which feeds on decaying plant and fungal material, that searching efficiency of the braconid parasitoid (*Asobara tabida*) was significantly reduced in ozone-rich air, resulting in an approximately 10 % lower parasitism rate of *D. subobscura*. In chambers, exposure to elevated SO_2 and NO_2 concentrations did not affect parasitoid behaviour or parasitism rate of the host fly compared to clean air conditions (Gate et al. 1995). The authors considered this to be clear evidence that increased O_3 levels may interfere with the olfactory responses of the parasitoids.

There are few empirical studies that have assessed the effects of air pollution on the measured levels of BVOCs and the associated behavioural responses of animals of different trophic levels. So far, most of the existing studies have also been conducted with non-woody plants. There is recent evidence from a modelling study of forest trees in Canada that the normal outbreak cycle of the forest tent caterpillar (*Malacosoma disstria*), which lasts approximately 10 years has been disturbed, and in some air-pollution-stressed regions, there has been more severe and frequent defoliation (Babin-Fenske and Anand 2011). Reduced parasitoid efficiency related to chemical cue interference was found to be the most effective parameter explaining the more frequent *M. disstria* outbreaks (Babin-Fenske and Anand 2011). This finding suggests that the role of plant volatiles induced by key tree defoliators in tritrophic communication should be investigated experimentally using the major parasitoids and predators of the defoliators in polluted conditions.

A series of studies with lima bean (*Phaseolus lunatus*) and brassicaceous plants has shown that high ozone concentrations can change the composition of herbivore-induced plant volatiles (Pinto et al. 2007a, b, c, 2008; Himanen et al. 2009) and may alter tritrophic interactions by influencing the behaviour of the natural enemies of herbivores when behavioural tests are run in elevated ozone atmospheres (Pinto et al. 2007c; Himanen et al. 2009). Y-tube assays were used to demonstrate that parasitoids could still use herbivore-induced plant volatiles to locate hosts in the presence of O_3, but given a choice between intact signals and O_3-degraded signals, they preferred the intact signal (Pinto et al. 2007a, c). A field orientation test

with the parasitoid wasp *Cotesia vestalis*, a major parasitoid of the diamond back moth (*Plutella xylostella*), in an open-field ozone-exposure facility with ozone concentrations enriched to double the ambient levels (Pinto et al. 2008) did not indicate significant differences between ambient and O_3-enriched environments either in the number of wasps found in the field, or in the percentages of parasitized larvae. This result suggests that moderately elevated O_3 will not affect the behaviour of this parasitoid over a scale of a few metres, but does not exclude the potential impact of ozone on orientation over longer distances, where the BVOC may be degraded to a greater extent (McFrederick et al. 2009).

McFrederick et al. (2009) suggested that the laboratory results, showing a significant reduction of signal compounds, but still not disturbing the behavioural response of carnivores at the third trophic level, highlight two possible and not mutually exclusive mechanisms that are responsible for the observed O_3 effects. Their first suggestion was that the products of the oxidation of the emitted chemicals are not as effective as signals as the original scents, but can still provide some signal. The second possibility is that BVOCs that exhibit low reactivity can act as signals when more highly reactive BVOCs have been oxidized, and therefore are important compounds for longer distance signalling. Recent chamber experiments to measure plant to plant signalling in ozone rich air (Blande et al. 2010a) suggest a third possible explanation. Blande et al. (2010a) found that receiver plants are able to detect damaged neighbouring plants when grown in low ozone atmospheres. When grown at 80 ppb ozone concentrations, the response of the intact receiver plants at a 70 cm distance from the emitter plants was lost. This was most likely due to several herbivore-induced signalling BVOCs being degraded in the airspace between the plants. Plants do not appear to be very sensitive receivers of BVOC signals and need higher concentrations of volatiles to elicit responses than insects and mites. This suggests that the Y-tube result by Pinto et al. (2007a), showing very low or undetected concentrations of some herbivore-induced BVOCs entering the Y-tube, but still exhibiting behavioural responses by the third trophic level, may indicate that insects and mites are able to detect herbivore-induced plant volatiles in concentrations that are below the detection threshold of the GC-MS method employed.

11.5.2 SO_2 and Sulphur Depositions

Sulphur dioxide (SO_2) is a primary pollutant related to industrial activity. SO_2 concentrations peaked in Europe and North America in the late 1970s (Cape et al. 2003). Due to various clean air policies, the acute and chronic effects of SO_2 on vegetation, which were observed close to the emission sources in industrialised countries and resulted in acid rains in remote areas relatively far from the emission sources, have largely disappeared. However, rapid industrialisation in other parts of the world is leading to localized and regional pollution hotspots with SO_2 concentrations well in excess of thresholds for vegetation damage (Cape et al. 2003).

China has been the world's largest emitter of SO_2 since 2005 (Su et al. 2011). Observations, both anecdotal and scientific, of increased herbivore presence on trees close to pollution sources have indicated an effect of such pollutants, including SO_2, on plant-herbivore interactions. This is particularly true for herbivores that do not feed on the surface tissues of leaves, but rather have strategies such as leaf mining (Oksanen et al. 1996; Kozlov 2003) or phloem feeding (Holopainen et al. 1991; Bell et al. 2011), although some studies indicate increased performance of chewing herbivores at polluted sites (Bell et al. 2011). As sulphur deposition is an important nutrient for plants, these observations are likely due to changes in the nutritional quality of plants. For example, in needles of Scots pine (*Pinus sylvestris*), increased concentrations of sucrose and reduced concentrations of glucose and fructose have been observed (Kainulainen et al. 1995a), reflecting impaired sink-source relations in polluted plants.

Direct impacts of SO_2 on plant secondary metabolites have been studied to some extent. Concentration of some monoterpenes and diterpenes in *Pinus sylvestris* needles were reduced close to an industrial SO_2 point source (Kainulainen et al. 1993). In contrast, Judzentiene et al. (2007) found that diterpene contents of young needles of *P. sylvestris* increased with distance from a point source of SO_2. Exposure of *P. sylvestris* and *Picea abies* seedlings to various concentrations of SO_2, NO_2 or ozone did not affect monoterpene concentrations in needles (Kainulainen et al. 1995b). However, increased concentrations of the monoterpene α-phellandrene and hydroperoxy conjugated dienes were found in the leaves of the peppercorn tree (*Schinus areira*) when grown in an environment with high SO_2 concentration (Wannaz et al. 2003). To our knowledge, modified emissions of isoprenoids or other BVOCs from plants exposed to elevated SO_2 concentrations have not been reported.

The positive effect of SO_2 exposure on herbivore performance does not appear to greatly affect the performance of parasitoids, with most studies so far indicating mixed effects or no effect of SO_2 on parasitoid performance (Butler et al. 2009). However, in polluted atmospheres, SO_2 is eventually oxidized to form sulphuric acid, which in the liquid phase promotes oxidation of isoprenoids and leads to formation of secondary organic aerosols (Hallquist et al. 2009). Sulphates formed from SO_2 may also act as condensation seeds for aerosol particles (Kroll and Seinfeld 2008; Cape 2008) and increase condensation of most volatile organic compounds (Kroll and Seinfeld 2008). These effects may significantly decrease the atmospheric lifetime of herbivore-induced volatiles and thus affect the longevity of the "cry for help" signals of herbivore-attacked trees (Holopainen 2011).

11.5.3 Interactions with Heavy Metal Pollution

Heavy metal pollutants are spread in atmospheric emissions over industrial areas and they are deposited mainly on the soil surface where they are taken up by plant roots. Heavy metals are also deposited on the surfaces of plant foliage. The

direct phytotoxic effects of heavy metal pollution on plants are generally visible as reduced photosynthesis, stunted growth and formation of growth abnormalities, which collectively weaken the plants and their defence mechanisms (Aydin et al. 2012). However, Winter et al. (2012) reported that hydroponically grown maize (*Zea mays*) exposed to either a high or low concentration of either Cu or Cd displayed these visible symptoms, but the higher Cu dose was found to prime for enhanced volatile production that can be triggered by caterpillar feeding. Cu stress correlated with increased levels of reactive oxygen species in roots and priming of herbivore-induced jasmonic acid in leaves. This result suggests that priming in plants exposed to air pollutants in industrial environments rich with heavy metal depositions can partly compensate for the loss of multitrophic BVOC signals in polluted atmospheres by more intense defence response and higher emission rates of herbivore-induced BVOCs.

11.5.4 Multitrophic Interactions Under Elevated CO_2 Atmospheres

Rising atmospheric CO_2 concentration, [CO_2], is affecting ecosystems globally, but impacts of elevated [CO_2] on multitrophic communication have rarely been investigated. There is general knowledge that plant BVOC emissions are in many cases lower in plants grown at elevated [CO_2] levels than those grown at ambient CO_2 concentrations (Peñuelas and Staudt 2010; Fineschi et al. 2013; Calfapietra et al. 2013 in this volume). However, evidence from CO_2 exposure experiments with parasitoids of *Lymantria dispar* suggests that the effects of elevated [CO_2] on the third trophic level are minor (Roth and Lindroth 1995). Experiments with deciduous trees in open-field exposure systems with elevated CO_2 and O_3 concentrations (Percy et al. 2002) indicated reduced population sizes of natural enemies of aphids in elevated [O_3] plots, and no effects of elevated [CO_2] supporting, the observations of Roth and Lindroth (1995).

In small-scale olfactometry experiments, Vuorinen et al. (2004b) demonstrated that *Cotesia vestalis*, a specialist parasitoid of the diamondback moth (*Plutella xylostella*), oriented preferably toward the scent of herbivore-damaged plants of two white cabbage (*Brassica oleracea ssp. capitata*) cultivars grown at ambient [CO_2]. However, under elevated [CO_2], they did not differentiate between volatiles of intact and herbivore-damaged plants of one cultivar. These results suggest that elevated atmospheric CO_2 concentrations could weaken the plant response to feeding by insect herbivores and thereby disturb signalling to the third trophic level. A study with oilseed rape (*Brassica napus*) (Himanen et al. 2009) indicated that lower herbivory by *P. xylostella* larvae reduced herbivore-induced emissions from transgenic, insect-resistant Bt (*Bacillus thuringiensis* insecticidal toxin producing) plants. However, contrary to observations in *Brassica oleracea* plants (Vuorinen et al. 2004b), the study by Himanen et al. (2009) demonstrated that growth under elevated [CO_2] increased emissions of most herbivore-induced volatile terpenoids.

Furthermore, *C. vestalis* always oriented to host-damaged plants independent of plant herbivore resistance or [CO_2] suggesting that elevated [CO_2] effects on plant herbivore-induced BVOC emission can be plant species specific and impacts on higher trophic levels depend on plant species and cultivar. Overall, the evidence of elevated [CO_2] effects on multitrophic communication in tree-based systems is surprisingly limited, calling for future experimental work on tree model systems.

11.5.5 Interactions of [CO_2] Elevation with [O_3]

Polluted air in industrial areas is typically composed of a mixture of primary and secondary pollutants. Primary pollutants, such as CO, SO_2, NO_X and anthropogenic volatile organics that are released from the actual pollution sources, including power plants, industrial complexes and exhaust emissions from vehicles, while secondary pollutants, such as inorganic acids (H_2SO_4, HNO_3), ozone, and PAN (peroxyacetyl nitrate) are formed in the atmosphere (Cape 2008). In highly polluted environments, these pollutants are always present and act together, while in more remote areas, ozone can be the main air pollutant affecting the ecosystems, but OH and NO_3 radicals can be found in photochemically significant concentrations even in "clean" air. Therefore, vegetation is in most cases affected by air pollutant mixtures rather than by a single pollutant compound. Due to the dynamic process of the formation and breakdown of secondary air pollutants, most studies assessing the effects of pollutant mixtures on trees and their trophic interactions have concentrated on situations where only the less reactive primary pollutants, such as CO_2 and SO_2, or end-products with longer lifetimes, such as O_3,are present (e.g., Percy et al. 2002; Vuorinen et al. 2005; Calfapietra et al. 2007). Studies concerning the direct effects of combinations of [CO_2] with gaseous pollutants other than O_3 or gaseous pollutant mixtures on multitrophic communication are not available to our knowledge.

Exposure of trees to elevated CO_2 or O_3 concentrations differentially affect plant responses, with aboveground growth being stimulated by elevated [CO_2] and inhibited by elevated [O_3] (Percy et al. 2002). Exposing deciduous trees simultaneously to elevated CO_2 and O_3 concentrations can result in antagonistic effects. Elevated [CO_2] can stimulate stomatal closure, leading to reduced O_3 flux to the leaves and the associated cellular damage, whereas elevated [O_3] can offset the growth stimulation by elevated [CO_2] (Vapaavuori et al. 2009).

Experiments at the Rhinelander free-air CO_2 Enrichment (FACE) experimental site, where deciduous trees are grown in atmospheres with either elevated CO_2 or elevated O_3 concentration and their combination, have provided evidence of interactive effects of elevated [CO_2] and oxidative pollutant [O_3] (Percy et al. 2002; Calfapietra et al. 2007, 2013 in this volume). These observations suggest that the impact of atmospheric pollutants on BVOC-mediated communication could be very complicated. In addition to the degradation of herbivore-induced plant BVOCs, oxidative air pollutants may have impacts on the behaviour of natural enemies of herbivores by affecting the volatile signals between the herbivore and

the predating carnivore or parasitoid. Aphid alarm pheromones, mostly composed of the sesquiterpene (*E*)-β-farnesene, are released by aphids under attack by predators or parasitoids. Other aphids of the same colony usually start dispersal behaviour in order to escape potential attackers. Mondor et al. (2004) found that the dispersal responses of the aspen aphid (*Chaitophorus stevensis*) to alarm pheromone released by a mechanically damaged aphid, decreased under elevated [CO_2], but increased under elevated [O_3] atmospheres when the host plant of aphids was grown at the Rhinelander open-field exposure site. This study showed that intraspecific olfactory communication may be radically altered in response to elevated concentrations of different air pollutants, but intriguingly, elevated [O_3] did not reduce the efficiency of (*E*)-β-farnesene, a sesquiterpene with a relatively short lifetime at elevated [O_3] atmospheres. The authors did not find an explanation as to why this relatively reactive compound induced a more pronounced response in conditions where it should have been detected at lower concentrations. However, aphid predators such as hoverfly (*Episyrphus balteatus*) larvae and lady beetles (*Harmonia axyridis*) are known to eavesdrop on aphid alarm pheromones and respond in particular to (*E*)-β-farnesene (Vandermoten et al. 2011). It is possible that aphid populations could associate alarm pheromone signals with predation densities depending on their earlier experiences with predators. Percy et al. (2002) reported very low predator densities in elevated [O_3] plots of the same experimental site, while elevated [CO_2] plots had high predator densities. Aphids adapted to high predation rates and continuous exposure to the alarm pheromone (*E*)-β-farnesene, possibly show a reduced dispersal response to alarm pheromone release by individual aphids, because altered dispersal response does not reduce their probability of being consumed by a predator.

11.6 Future Directions

11.6.1 Air Pollution and Volatile Signalling Compounds in Natural Ecosystems

The need to understand the mechanisms, evolutionary role and factors affecting BVOC-based multitrophic signalling in natural and man-made ecosystems is becoming more topical every day. Natural ecosystems are suffering from expanding habitat fragmentation, which will lead to reduced biological diversity and eventually to species extinction in small patches (Hanski 1994). Parasitoids of herbivorous insects have different strategies in searching for populations of their host. For example, the two primary parasitoids *Hyposoter horticola* and *Cotesia melitaearum* of the Glanville fritillary butterfly (*Melitaea cinxia*) larvae have different searching strategies. *Hyposoter horticola* moves long distances to locate herbivores in neighbouring patches, while *Cotesia melitaearum* searches over short distances and mostly stays in the same patch (van Nouhuys and Hanski 2002). In particular,

parasitoid species such as *H. horticola* relying on long distance volatile signals may become endangered in air-polluted and fragmented environments where the BVOC signals from herbivore-damaged plants will become weaker and possibly not reach other distant patches.

11.6.2 Air Pollution and Volatile Signalling Compounds in Plant Production

Exogenous elicitors, such as jasmonates and methyl salicylate are compounds that activate various defence responses in treated plants and induce production of secondary compounds to improve direct and indirect defence in plants (Thaler 1999; Holopainen et al. 2009; Kaplan 2012; Semiz et al. 2012). Foraging efficiency of parasitoids and parasitisation rates of herbivorous Lepidopteran larvae have been increased on plants treated with elicitors over untreated control plants. This is because of induction of emissions of parasitoid-attractive herbivore-induced BVOCs from intact test plants treated with elicitors (Thaler 1999; Qiu et al. 2012). Elicitors have the potential to be used in the seedling production of forest trees, although in conifer seedlings, the response could be extensive, leading to growth of larger resin storage organs and three orders of magnitude higher inducible monoterpene emissions compared to controls (Holopainen et al. 2009). This may have consequences for atmospheric quality by increasing ozone and secondary aerosol formation in polluted environments and consequently, reducing the efficiency of BVOC-based indirect plant defence under pollution episodes.

Push-pull strategies are based on the behavioural manipulation of insect pests and their natural enemies (Cook et al. 2007). This is done by exploiting volatile semiochemicals to repel insect pests from the crop ('push') and to attract them into trap crops ('pull') where the pests are subsequently removed. Trap crops could be attractive and more susceptible cultivars or cultivated separately from the main crop (Cook et al. 2007) or used in intercropping with the main crop (Hassanali et al. 2008). Efficiency of natural parasitoids and predators or released biocontrol organisms to control pest species can be improved by attracting these natural enemies by attractive BVOCs. Elicitors can be used on main crop plants, or synthetic analogues of herbivore-induced BVOCs can be released from dispensers embedded within the crop (Kaplan 2012). This strategy of attracting carnivorous enemies to crops could be based on three different scenarios: (1) parasitoids are directly attracted to released synthetic BVOCs, (2) parasitoids respond to herbivore-induced plant volatiles that were elicited via exposure to a synthetic BVOC, or (3) synthetic BVOCs prime neighbouring plants, and this amplifies the pest-induced volatile production in crop plants when the crop is damaged (Kaplan 2012). Based on our current knowledge of atmospheric behaviour, constitutively emitted species-specific BVOCs (Himanen et al. 2010) and herbivore-induced BVOCs (Pinto et al. 2007c; Himanen et al. 2009), the push-pull strategy to repel pests from crops, e.g.,

by malodorous companion plants in intercropping, is possibly more efficient in environments with a high air pollution load than strategies to attract pest enemies by more reactive herbivore-induced BVOCs.

The potential of semivolatile plant BVOCs which condense on plant surfaces (Himanen et al. 2010; Karban 2010) to protect cultivated plants from herbivores is so far a totally unexplored area of research. If these semivolatiles protect against herbivores, what will be the impact of these compounds for natural enemies of herbivores? The phytotoxicity of these compounds to the receiver plants (Copolovici et al. 2005) should also be considered. Nevertheless, the emissions of *Rhododendron tomentosum* that were dominated by the semivolatile oxidized sesquiterpenoids ledol and palustrol did not show phytotoxic effects on *Betula* spp. (Himanen et al. 2010). Furthermore, oxidation and partitioning of herbivore-induced volatiles into the particle phase in oxidant-rich atmospheres leads to formation of various organic compounds including short-chain organic acids and aldehydes, and condensed long-chain oxidized molecules. These clusters of nanoparticles composed of BVOC reaction products can accumulate on plant surface, but it is not yet known whether they have the potential to serve as protective repellent compounds against herbivores (Holopainen and Gershenzon 2010). The answers to these intriguing questions may open a way to develop new control methods against herbivores or pathogens.

11.6.3 Improved Understanding of Atmospheric Behaviour of Herbivore-Induced BVOCs in Polluted Air

Despite the efforts in recent years, there is still a lot to learn about how atmospheric pollutants affect volatile-mediated interactions. Most of the empirical data available has been attained under laboratory conditions and these data suggest that pollutants most likely reduce the distance over which volatile-mediated interactions occur (Blande et al. 2010a). So far, most studies have involved fumigation with one oxidizing pollutant, which is an experimental short-falling that needs to be addressed in the future. Ozone, due to being both phytotoxic and reacting with numerous plant BVOCs, has received the most attention. However, OH and NO_3 radicals are more reactive than ozone with many BVOCs, including numerous herbivore-induced plant volatiles. Thus, for better understanding of the effects of the atmosphere on volatile-mediated interactions, empirical data need to be collected that combine the effects of realistic combinations of oxidants at realistic proportions and concentrations. Collection of such data will facilitate the development of models that can better estimate the dynamics of volatile-mediated interactions under a range of atmospheric conditions.

At present, controlled generation of OH radicals for experimental purposes is possible and the atmospheric behaviour of herbivore-induced BVOCs can be monitored (Hao et al. 2011). BVOC reactions with NO_3 radicals are important for nighttime emissions when NO_3 radical concentrations can be sufficiently high.

However, the generation of NO_3 radicals is currently less straightforward. Ozone and the potentially dangerous oxidizer dinitrogen pentoxide (N_2O_5) have been used as precursors to produce a variety of reaction products such as pinonaldehyde and alkylnitrates from the reaction of NO_3 with α-pinene (Wängberg et al. 1997). As NO_3 concentrations are generally very low during daylight hours, the time when the majority of volatile-mediated interactions between herbivore damaged plants and natural enemies of herbivores are thought to occur, a strategy involving enrichment of OH radicals and ozone concurrently may constitute a decent interim strategy; the long-term goal being to combine all the major atmospheric oxidants together during experimentation.

It is further important to gain insight into the involvement of atmospheric pollutants in altering the temporal and spatial dynamics of BVOC signalling and how this information is interpreted by receiver organisms. It is known that the ratios of volatile compounds in an herbivore-induced plant volatile odour plume provide information for foraging predators and parasitoids (Pareja et al. 2009). Small changes in those ratios can alter behavioural responses. Increases in certain pollutants, which have different reaction times with different volatiles, can alter the compound ratios, and thereby the informativeness for herbivores and their predators. Such effects may occur at a much faster rate than the general degradation of the volatile blend, and may occur at lower oxidant concentrations than generally used in experiments. The oxidation products may also constitute signals to foraging insects or other plants, and may provide temporal or spatial information related to the emission source. Understanding how pollutants affect the dynamics of a volatile signal and the plasticity of the signal-receiving organism's response are of central importance in understanding how atmospheric pollutants influence multitrophic interactions.

11.7 Conclusions

To understand the full effect of atmospheric pollutants on multitrophic interactions, we need to consider both the direct and indirect effects of the pollutants concurrently. The direct effects include phytotoxicity of oxidizing chemical species and potential direct toxic effects on herbivores or their natural enemies. The indirect effects include the degradation of volatile chemicals and the subsequent effect on multitrophic interactions. These two facets are linked, whereby the combinations of herbivore feeding and oxidative stress may interact to result in an induced chemical bouquet that is different from that induced by herbivore feeding alone. The effects of combined stresses on the volatile emissions of plants and the subsequent effects on higher trophic levels clearly are high priority research questions for the future studies (Holopainen and Gershenzon 2010).

In summary, the key to understanding the effects of pollutants on multitrophic interactions lies in adopting a holistic approach that includes experimentation with multiple co-occurring pollutants, and with consideration of the direct and

indirect effects on the organisms of a multitrophic system. The effects of increased degradation of signal compounds, role of newly created compounds, spatial and temporal signal dynamics and system compensation through adaptation should become the foci of future studies.

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Chapter 12
Leaf-Level Models of Constitutive and Stress-Driven Volatile Organic Compound Emissions

Rüdiger Grote, Russell K. Monson, and Ülo Niinemets

Abstract This chapter provides a review of past and contemporary leaf-level emission algorithms that have been and currently are in use for modelling the emissions of biogenic volatile organic compounds (BVOCs) from plants. The chapter starts with a brief overview about historical efforts and elaborates on processes that describe the direct emission responses to environmental factors such as temperature and light. These phenomenological descriptions have been widely and successfully used in emission models at scales ranging from the leaf to the globe. However, while the models provide tractable mathematical functions that link environmental drivers and emission rates, and as such can be easily incorporated in higher scale predictive models, they do not provide the mechanistic context required to describe interactions among drivers and indirect influences on interactions such as those due to acclimation, accumulated stress and ontogeny. Following a discussion of these issues and the limitations they impose on the current state of model-based prognoses of BVOC emissions, we describe in some detail the knowledge gaps that need to be filled in order to move BVOC emission models into forms that are more directly coupled to physiological processes.

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12.1 Introduction

A diverse range of plant species has the capacity to emit biogenic volatile organic compounds (BVOCs), in particular volatile isoprenoids, in a constitutive manner. These emissions can either come from specialized storage structures, reflecting slow vapourization and diffusion of volatiles synthesized days, weeks and months prior to emission ("storage" emissions such as those from storage structures that play an important role in direct plant defence), or from continuous physiological processes ("persistent physiological emissions" such as methanol emissions from expanding leaves). The key characteristic of constitutive emissions is that they are not dependent on induction by external triggering factors, such as herbivory or abiotic stress. In contrast, induced emissions result from an upregulation of key metabolic pathways in response to external cues, thereby leading to elicitation of BVOC emissions even in species lacking constitutive emissions. Induction of emissions, in particular, includes the upregulation of gene expression to produce additional enzymatic activity, e.g., the induction of genes for terpene synthases in response to insect attack, (Litvak and Monson 1998; Arimura et al. 2000; Li et al. 2002; Babst et al. 2009). In the case of constitutively emitted compounds, such as isoprene and monoterpenes, environmental cues typically alter the level of expression of key controlling enzymes (Wiberley et al. 2008, 2009), complicating separation of stress-triggered and constitutive emission responses. The various timescales across which emissions are influenced, and the interactions of multiple cellular processes in determining the capacity for emissions has created challenges to describing BVOC emissions in mathematical representations, and thus, in producing prognostic models that reflect metabolic and physiological first principles.

In some of the processes that govern emissions we have, in fact, made progress in linking emissions to true physico-chemical theory. For example, chemical properties of volatiles responsible for stomatal control of emissions have been identified (Niinemets et al. 2004; Niinemets and Reichstein 2003), and temperature dependence of many BVOC emissions has been described in terms of fundamental kinetic theory (see Grote and Niinemets 2008; Monson et al. 2012). However, the mechanistic basis for alteration of BVOC emissions by growth in different light or temperature regimes, the influence of drought stress, in either the short- or long-term, and the influence of leaf ontogeny remains largely unresolved (see Monson 2013 in this volume). Furthermore, induction of emissions following biotic or abiotic stress events has not yet been included in emission models, although cellular signalling models have been developed that simulate alterations in gene expression with relatively good success (Vu and Vohradsky 2007; Yip et al. 2010; Muraro et al. 2012).

Apart from the importance of detailed understanding of the emission mechanisms that determine the emissions in the timescale of seconds to minutes, the pathway flux also depends on longer-term emission controls associated with the changes in the capacity of the volatile synthesis pathway. Modelling seasonality has been an especially difficult task, because seasonal variations also involve variations in light and temperature, effects which are hard to disentangle from acclimation responses.

Furthermore, seasonal studies conducted in natural environments inevitably also incorporate long-term stress effects such as soil drought. Embedded within these entanglements are the summed effects of cell birth and death, both of which are controlled by intrinsic 'clocks' as well as programmed responses to stress. We know these synergies exist; we just don't know how to represent them accurately in mathematical representations. The mismatch between our general knowledge of the phenomenology of the processes, and our knowledge about the stoichiometries that determine process rates and feedbacks, has left us with little on which to base our models. In this knowledge gap, and facing pressure from agencies and governments to produce actionable projections of future changes in our environment, we have produced models that work well for replicating observed emissions patterns and dynamics. However, we also know they have limited power to be used in truly prognostic mode, especially if the emission projections have to be made to future conditions and to areas with limited information of species emission characteristics and physiological performance.

In this chapter, we analyse the origin and development of both empirical and semi-mechanistic emission model algorithms to simulate volatile emission responses to immediate variations in empirical drivers. We try to emphasize the gaps in knowledge that cause our projections of BVOC emissions to be bracketed with relatively large uncertainties. Then we consider the knowledge needed to fill some of these gaps, and incorporate especially the long-term influences, such as acclimation mechanisms, and seasonality, in emission models. Finally, we suggest ways to improve our representation of induced emissions in our models – that is, how to design the models to respond to episodic forcing elements in the environment. In assessing the conflicting states of existing knowledge and the need for reliable projections, we end up concluding that the expansion of models to include interactions such as acclimation and ontogeny is a valuable step forward, as it allows for the establishment of a recognized framework within which we must cast our projections. However, we also argue that the limitations of this approach must be broadcast in a more amplified form. It is a dangerous situation to assume that because a model includes a scheme for acclimation or induction, it is in a form capable of more accurate prognosis. We emphasize that new approaches must be developed for assessing the uncertainties created by the gap between knowledge about the existence and basic operation of a certain process, and the exact controls by which the model links emission rates to physiological and environmental drivers.

12.2 Modelling Environmental Dependencies

12.2.1 Brief History of Leaf-Level Emission Models

The complexity of direct emission control represented a big challenge for modelers. Although Sanadze and Kalandaze (1966) reported the dependency of isoprene emission rate on temperature and light long ago, it took more than a decade to

produce the first mathematical relation to describe this dependency (Tingey 1979; Tingey et al. 1981). In developing this first mathematical model, it was noted that isoprene emission in studied broad-leaved species responded to temperature as well as to light, while monoterpene emissions in studied conifers only responded to temperature (Tingey et al. 1980). Thus, monoterpene emission was assumed to originate solely from storage pools within the plant (e.g., oleoresin) that provide an unconstrained source, at least in the case of emissions over minutes to hours to days. Accordingly, most of the control over the short-term monoterpene emission rates was relegated to diffusive resistances. The mechanism of isoprene production was clarified through the studies of Monson and Fall (1989) who highlighted the relationship to photosynthesis (see also Loreto and Sharkey 1990; Monson et al. 1992, 1994). Recognition of this relationship allowed Guenther et al. (1991, 1993) to develop models for leaf-scale isoprene and monoterpene emissions based on the shapes of the light and temperature response curves previously used in photosynthesis models (e.g., Farquhar and von Caemmerer 1982; Tenhunen et al. 1976). Both photosynthesis as well as isoprene emission show an optimum relationship to temperature and a saturation response to increasing light. The temperature optimum of BVOC emission is, however, high compared to most physiological processes (Fig. 12.1a, b). In contrast, the light dependency of isoprene emission is similar to that of photosynthesis (Fig. 12.2a, b). Thus, while the metabolic linkages between photosynthesis and isoprene emission were clear, there was also evidence that isoprene biosynthesis has a unique set of controlling processes that could not be ignored. Later, it was recognized that some species emit monoterpenes that are tightly coupled to their biosynthesis in the chloroplast, and thus, the monoterpene emission mechanisms in these species are similar to isoprene emission mechanisms (Schürmann et al. 1993; Staudt and Seufert 1995). Shortly after the first comprehensive emission models were presented, the main biosynthetic pathway of volatile isoprenoid production in plant plastids, 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway), was discovered (Lichtenthaler et al. 1997; Rohmer et al. 1993; Eisenreich et al. 2001). Given the central role of the MEP/DOXP pathway, the following efforts of mechanistic and semi-mechanistic BVOC emission model development have primarily focused on understanding linkages to photosynthesis and controls over kinetics within this pathway (Niinemets et al. 1999; Martin et al. 2000; Zimmer et al. 2000).

Shortly after the first leaf-scale models were published, global-scale modelers began to incorporate some of the schemes into projections at scales with considerably longer time and greater space intervals (e.g., Guenther et al. 1995). These projections were inherently constrained by the bottom-up approach, because in this framework there was little potential to validate model predictions. Even within the context of atmospheric chemistry, large gaps in our knowledge, for example of the degree of molecular oxidation of isoprene and the deposition of oxidation products, precluded validation of projected global emission rates. Despite acknowledgement of large uncertainties, the models continued to be expanded in terms of the processes they included (e.g., Fuentes and Wang 1999; Guenther et al.

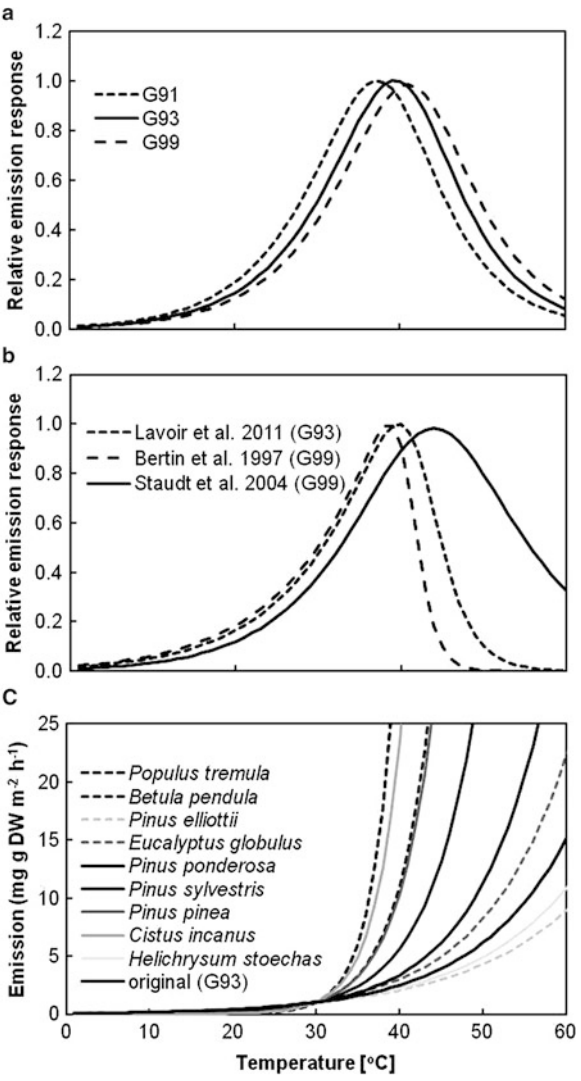

Fig. 12.1 Comparison of shapes of temperature responses of isoprene and monoterpene emissions in species lacking specialized storage structures (**a**, **b**), and monoterpene emissions in species with terpene storage structures (**c**). Panel (**a**) highlights the differences between the various versions of the Guenther isoprene emission algorithm presented in 1991, 1993, and 1999 (G91, G93, and G99), (**b**) compares different monoterpene emission parameterizations in the broad-leaved evergreen sclerophyll (*Quercus ilex*), while (**c**) compares the suggested temperature responses among species (Tingey et al. 1980; Guenther et al. 1991; Holzinger et al. 2006; Ruuskanen et al. 2005; Hakola et al. 1998, 2003; Owen et al. 1997). The *broken lines* correspond to Eq. 12.2 and continuous lines to Eq. 12.3

2000; Müller et al. 2008; Lavoir et al. 2011; Ashworth et al. 2013; Guenther 2013). For example, empirical relationships linking isoprene emission rate to atmospheric CO_2 concentrations (Rosenstiel et al. 2003; Wilkinson et al. 2009) were included in existing global emission models (Arneth et al. 2007; Heald et al. 2009). The 'Model of Emissions of Gases and Aerosols from Nature' (MEGAN) (Guenther et al. 2006, 2012) is currently the most widely used model for projecting global trends in BVOC emissions. MEGAN includes some of the more difficult long-term influences on emissions, such as temperature acclimation, response to drought and

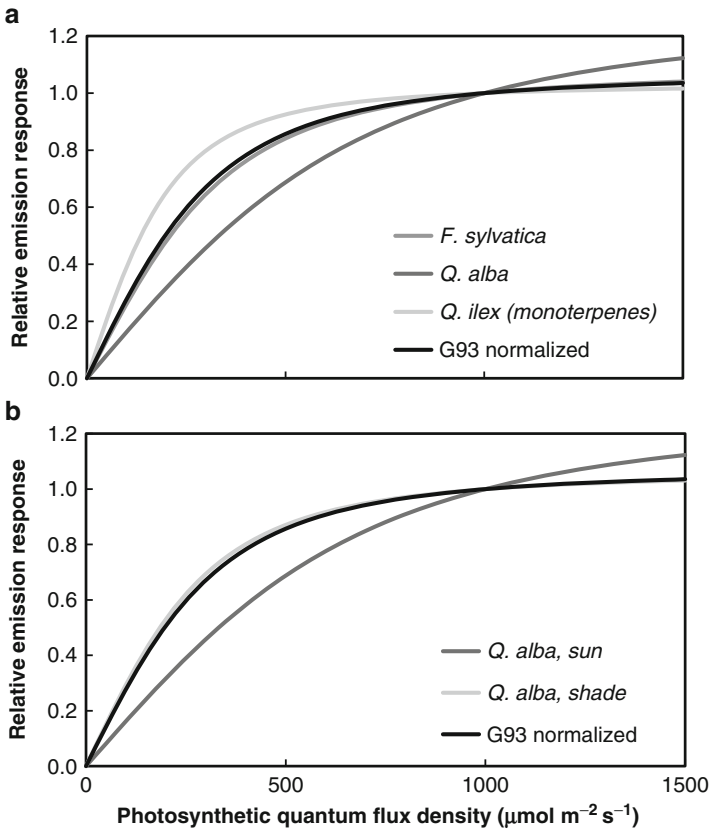

Fig. 12.2 Shapes of light responses of the emissions of isoprene and monoterpenes. Panel (**a**) compares the light response for temperate broad-leaved deciduous trees *Fagus sylvatica* (Schuh et al. 1997), and *Quercus alba* (sun foliage, Harley et al. 2007), and Mediterranean evergreen sclerophyll *Q. ilex* (Lavoir et al. 2011) along with the G93 (Guenther et al. 1993) estimate, while (**b**) shows the light responses for different canopy layers in *Q. alba* from Harley et al. (1997)

ontogeny. However, these schemes are largely non-validated, except for a few case studies. Not yet considered in BVOC emission models are responses to herbivory and pathogen infection, oxidative air pollution stress and soil infertility (Loreto and Schnitzler 2010) and priming of emissions by consecutive and simultaneous stresses or mild stress episode(s) preceding a more severe stress (Niinemets 2010a, b).

One of the more promising approaches to emerge in the past decade with regard to validating model projections and reducing uncertainties is the fusion of satellite remote sensing of formaldehyde and glyoxyl (oxidative products of terpene BVOCs; see Abbot et al. 2003; Palmer et al. 2003, 2006; Ashworth et al. 2013) with emissions models. Inverse modelling of formaldehyde and glyoxyl columns to reveal the locations and magnitudes of BVOC sources and sinks has the potential

to provide new insight into time-dependent interactions between emissions and environmental change, especially at the scales needed to integrate processes from single leaves to regional ecosystems.

In the following sections, we discuss how different environmental drivers are represented in contemporary emission models. Most of these models follow the general idea that there is a certain capacity for the emission of a given compound that depends on the overall diffusion resistance ("storage" emissions) or by the rate of volatile formation ("instantaneous" emissions). The emission capacity can be defined as the maximum emission rate that is physiologically possible (E_{MAX}). For "instantaneous" emissions, this is typically observed at saturating light and a temperature of about 40 °C (see Fig. 12.1a, b; Fig. 12.2). However, no such apparent physiological limitation exists for storage emissions, which are driven solely by volatilization and diffusive resistance. Thus, in emission studies, one often uses a standardized emission rate, E_S (also called the emission factor) that is defined as the emission rate under certain arbitrarily selected environmental conditions (Guenther et al. 1991; Niinemets et al. 2010c). The standard conditions are typically set as the leaf temperature of 30 °C (303.16 K), incident quantum flux density of 1,000 μmol m^{-2} s^{-1} (Guenther et al. 1991, 1993) and ambient CO_2 concentration of 400 μmol mol^{-1} (Wilkinson et al. 2009). Following Guenther et al. (1991), the general form of this approach to estimate the emission rate of a specific compound or compound group, E, can be expressed as:

$$E = E_S f(I_1) f(I_2) \ldots f(I_n), \tag{12.1}$$

where $f(I_i)$ represents different response functions ($i = 1..n$) scaling E_S to environmental conditions other than the standard conditions. These functions are addressed separately in the following paragraphs. Implicit in Eq. 12.1 is that different environmental factors affect the emission rate independently. This is not necessarily valid (e.g., Sun et al. 2012) and will be addressed afterward together with alternative ways of estimating E without the need to specify E_S.

12.2.2 Temperature Dependency

BVOC emissions can originate from specific storages such as resin ducts, or from organelles that are not specifically formed to hold BVOCs (e.g., isoprene from chloroplasts, methanol from cell walls or sesquiterpenes from the cytosol). In some cases, the temperature dependency results from the pathway producing the given compound being sensitive to temperature (here, referring to short-term dynamics in temperature). In other cases, it is the temperature dependency of compound volatility that most determines the emission rate. The first type of temperature dependency is exemplified by the emission of isoprene, methylbutenol, and light-dependent monoterpenes. The second type of temperature dependency is exemplified by the emissions of stored compounds, like the monoterpenes emitted from the resin systems of many conifers. Thus, two separate modelling strategies are developed to represent both types of emission.

12.2.2.1 Temperature Effects on Storage Emissions

These emissions have been described by Tingey et al. (1980) by fitting emissions on a log scale to temperature using a linear relationship:

$$f(T) = \exp\left[p_1 \cdot T + p_2\right] \tag{12.2}$$

with T representing air temperature and p_1 and p_2 being empirical parameters. Guenther et al. (1993) used the following exponential relationship to temperature:

$$f(T) = \exp\left[\beta\left(T_\mathrm{L} - T_\mathrm{S}\right)\right], \tag{12.3}$$

where T_L is the leaf temperature and T_S is the reference temperature (set to 303.16 K = 30 °C) and β is an empirical coefficient. Guenther et al. (1993) set β to 0.09 (K^{-1}) based on observations using 28 species. It should be noted that species-specific estimates ranged from 0.057 to 0.148 in the original Guenther et al. (1993) study, a range that has since been exceeded in other measurements. For example Pokorska et al. (2012) estimated β to be 0.24 for *Abies alba* trees in late summer and Owen et al. (1997) found values larger than 0.3 for *Cistus incanus* plants (see also Fig. 12.1c). In addition, Helmig et al. (2007) showed that β also changes within the canopy. Equations 12.2 and 12.3 have been widely used to describe emissions from storage pools, including those for monoterpenes and sesquiterpenes (e.g., Ormeño et al. 2010).

The implicit assumption in Eq. 12.3 is that the resistance between different types of storage systems and the air is constant. This implies that the storage size is large relative to the emission rate and is not depleted during the emission period – an assumption that has been challenged by the work of Schurgers et al. (2009) who stated that storage emissions in a *Pinus ponderosa* forest could best be described considering a dynamic change in leaf monoterpene concentration during the year.

12.2.2.2 Influence of Temperature on Immediate Emissions

The emissions that are tightly coupled to production, increase with increasing temperature in an exponential fashion up to a maximum rate, thereafter the emissions decrease rapidly, reflecting enzymatic degradation or substrate limitations (e.g., Monson and Fall 1989; Loreto and Sharkey 1990; Monson et al. 1992; Rasulov et al. 2010, 2011). Based on earlier work that relied on chemical kinetics theory such a relation has been postulated by Johnson et al. (1942) to take the form:

$$f(T) = \frac{\exp\left[c_\mathrm{T} - \frac{\Delta H_\mathrm{A}}{R T_\mathrm{L}}\right]}{1 + \exp\left[\frac{\Delta S}{R} - \frac{\Delta H_\mathrm{D}}{R T_\mathrm{L}}\right]} \tag{12.4}$$

where H_A is enthalpy of activation in J mol^{-1}, H_D is the enthalpy of de-activation in J mol^{-1}, S is an entropy term and R is the universal gas constant both in J K^{-1} mol^{-1}, and c_T is a scaling constant. Following the form of this relationship, but substituting the enthalpy and entropy terms with empirically derived parameters, Guenther et al. (1991) rewrote the equation as:

$$f(T) = \frac{\exp\left[\frac{c_{T1}(T_L - T_S)}{RT_L T_S}\right]}{1 + \exp\left[\frac{c_{T2}(T_L - T_M)}{RT_L\ T_S}\right]} \tag{12.5}$$

where c_{T1} (J mol^{-1}), c_{T2} (J mol^{-1}) and T_M (K) are 'tunable' coefficients. Guenther et al. (1999) modified the form of Eq. 12.5 to refer directly to the temperature optimum T_{opt} (K) rather than to T_S:

$$f(T) = c_{T3}\frac{\exp\left(c_{T4}\ x\right)}{c_{T3} - c_{T4}\left(1 - \exp\left(c_{T3}\ x\right)\right)} \tag{12.6}$$

$$\text{where } x = \frac{\left(1/T_{opt}\right) - \left(1/T_L\right)}{R}$$

The parameters c_{T3} and c_{T4} (both in J mol^{-1}) are again empirically determined coefficients. Mathematically, Eqs. 12.5 and 12.6 are equivalent to Eq. 12.4, considering that some differences are absorbed within the coefficients (see Monson et al. 2012).

Equation 12.4 was also applied in the models of Niinemets et al. (1999) and Martin et al. (2000) for describing the temperature response of isoprene synthase and Niinemets et al. (2002c) for describing the temperature response of monoterpene synthases. Zimmer et al. (2000) used it to characterize the temperature dependency of isoprene formation from precursor substances. In later work, the Zimmer et al. (2000) model was applied to other isoprenoids using the same temperature response, but with parameters determined independently for various processes within the MEP pathway (Grote et al. 2006). In their studies, these parameters were derived through an inverse modelling approach whereby the 'best-fit' parameter values were determined after assimilating enzyme temperature dependencies into the model. The temperature dependence of isoprene synthase activity was based on in vitro estimations of synthase activity in crude leaf extracts of *Populus tremuloides* (Monson et al. 1992) and *Quercus robur* (Lehning et al. 1999), and for monoterpene synthase extracts of *Quercus ilex* (Fischbach et al. 2000).

12.2.3 Light Dependency

The original Guenther et al. (1991) model was developed on the basis of numerous studies of Sanadze (1964), Tingey et al. (1981), Monson and Fall (1989) and Loreto and Sharkey (1990) that indicated a functional linkage between photosynthetic CO_2 assimilation and the formation of some BVOCs, especially isoprene. It had

been apparent that absorbed photon flux density is the principal driver for this similarity, suggesting that both processes occurred in the chloroplast, both processes exhibited similar shapes in their light-response curves, and both processes required the input of reductant from the photosynthetic electron transport system. The light dependency of the thylakoid electron transport rate (J, μmol m^{-2} s^{-1}) can be described mathematically as:

$$J = 0.5\, a\, I\, (1 - b) \tag{12.7}$$

where a is the fraction of incident photosynthetic photon flux density (I in μmol m^{-2} s^{-1}) absorbed by the leaf, and b is the fraction of the absorbed photon flux diverted to processes other than photosynthetic electron transport. Implicit in Eq. 12.7 is that J is not saturated by the absorbed I. As electron transport starts to become light-saturated with increasing light intensity, the dependence of J on I will begin to exhibit a curvilinear shape. Recognizing that J is influenced by an upper limit (J_{max}, μmol m^{-2} s^{-1}), and recognizing that the influence of J_{max} on J increases as I increases, the following quadratic equation has been developed for photosynthesis:

$$0 = J^2 - [0.5\, a\, I\, (1 - b) + J_{max} + \Theta]\, J + 0.5\, a\, I\, J_{max}\, (1 - b) \tag{12.8}$$

where Θ is a tunable 'curvature factor' that theoretically can vary from 0 (rectangular hyperbola) to 1 (Blackman type response) with a default value of 0.85 corresponding to moderate curvature. Equation 12.8 describes a rectangular hyperbola in which a continuous transition occurs from $J = 0$ at $I = 0$ to $J = J_{max}$ at saturating I (Farquhar and Wong 1984).

Guenther et al. (1991) used Eq. 12.8 to develop an analogue model to describe the light dependency of isoprene emission (as a multiplication factor for Eq. 12.1):

$$f(I) = \frac{x - \sqrt{x^2 - 4\, b\, aI\, c_{L1}}}{2\, c_{L1}} \tag{12.9}$$

where $x = b\, aI + c_{L1} + c_{L2}$.

The parameters c_{L1} and c_{L2} are tunable coefficients that account for the differences in molar stoichiometry of electron transport between isoprene formation and CO_2 and reflect the curvature coefficient (Θ) and the upper limit of the function formerly defined by J_{max}. Note that the meaning of the coefficients a and b is also different for the isoprene light response than for the light response of J. In later publications, Guenther et al. (1993) used a new form for J, aligning it with a mathematical expression of the so-called Smith's (Smith 1937) equation of photosynthesis (see Tenhunen et al. 1976; Harley et al. 1992):

$$J = \frac{\alpha\, I}{\sqrt{\left(1 + \frac{\alpha^2 I^2}{J_{max}{}^2}\right)}} \tag{12.10}$$

where α is the initial slope of the response carrying the units of mol mol^{-1} photons incident to the leaf (the apparent quantum yield). Equation 12.10 defines the shape of a rectangular hyperbola that approaches an asymptote at relatively high values of I. Adopted from this expression Guenther et al. (1993) described the light dependency of BVOC (isoprene) emission by removing J_{max} and adjusting the function with an additional parameter (c_{L3}). However, as Monson et al. (2012) pointed out, there was a mathematical violation in the denominator in that the square root quantity contains a sum that mixes a unitless constant (1.0) with the product of two terms (α and I) that carry units. Thus the equation should be written as:

$$f(I) = \frac{\alpha_{ISO}\, c_{L3}\, I}{\sqrt{\left(1 + \frac{\alpha_{ISO}^2 I^2}{c_x^2}\right)}} \tag{12.11}$$

where c_{L3} is now in m^2 s μmol^{-1}, and α_{ISO} now carries units mol mol^{-1} photons incident to the leaf, while c_x is in μmol m^{-2} s^{-1}. It can be set to 1.0 to represent the same response as before. The coefficients for α_{ISO} and c_{L3} in Eq. 12.11 were assumed to be constant in the Guenther et al. (1993) analysis, but it was later realized that they can vary within the canopy (Fig. 12.2), partly reflecting the explicit connection between the emission capacity and α_{ISO} (Monson et al. 2012), and partly reflecting acclimation within the canopy (Sect. 12.3.2).

Niinemets et al. (1999) followed a different path and related the emission rate to light using an explicit connection with J. They have therefore used an expression of J limited by ribulose-1.5-bisphosphate (RuBP) regeneration related to net CO_2 assimilation, A, μmol m^{-2} s^{-1}:

$$J = \frac{(A + R_D)\,(4\,C_i + 8\,\Gamma^*)}{C_i - \Gamma^*} \tag{12.12}$$

where C_i is the CO_2 mole fraction in the intercellular air spaces of the leaf, R_D is the rate of non-photorespiratory respiration rate in light (μmol m^{-2} s^{-1}) (mostly mitochondrial respiration), and Γ^* is the hypothetical CO_2 compensation point in the absence of R_D that depends on Rubisco kinetic characteristics. Using this relation, Niinemets et al. (1999) linked the emission rate (E) to photosynthetic electron transport rate. However, expressing J from Eq. 12.12 only yields the rate of photosynthetic electron transport that is needed to reduce CO_2 to the level of immediate photosynthetic product, sugars, and that needed for photorespiration. As isoprene is a more reduced molecule than sugars, additional reductive equivalents are needed to synthesize isoprene. Including this additional electron transport rate, the rate of isoprene emission, E_{iso}, was expressed as (Niinemets et al. 1999):

$$E_{iso} = \varepsilon\, J_T \frac{(C_i - \Gamma^*)}{6\,(4.67\,C_i + 9.33\Gamma^*)} \tag{12.13}$$

where J_T is the total electron transport rate, i.e., that used for CO_2 fixation and photorespiration plus that needed for additional reduction of sugars to the level of

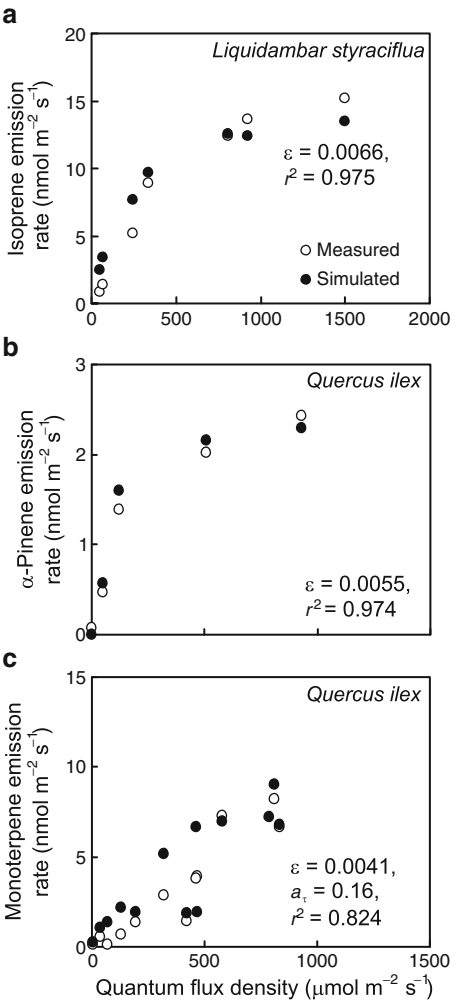

Fig. 12.3 Comparison of measured and simulated light dependencies of isoprene emission rate (**a**), α-pinene (**b**) and total monoterpene emission rate (**c**) in broad-leaved deciduous tree *Liquidambar styraciflua* (**a**), and in evergreen sclerophyll *Quercus ilex* (**b**, **c**). In (**a**), the measurements were conducted at a constant leaf temperature of 25 °C (Data from Niinemets et al. 1999), in (**b**) at 30 °C (Data from Loreto et al. 1996), and in (**c**), the data were filtered from daily time-courses of monoterpene emission measured between Aug. 3–5, 1994, for a temperature range of 25–35 °C (Niinemets et al. 2002c). The data were fitted by Niinemets et al. (1999, 2002c) isoprenoid emission model (Eq. 12.14). Only one leaf-dependent coefficient, ε, the fraction of electrons in isoprenoid synthesis, was used in (**a**), and (**b**), while the dependence of ε on leaf temperature was considered in (**c**) using an exponential scaling coefficient (a_τ (Niinemets et al. 1999, 2002c)

isoprene, and ε is the fraction of J_T required to synthesize isoprene. The dependence of J on I was modelled using Eq. 12.10 and the resultant value of J was inserted into Eq. 12.13. In this equation, the connection between isoprene emission and photosynthetic electron transport results from the assumption that NADPH availability controls the rate of isoprene biosynthesis, although an analogous dependence on ATP availability has also been formulated (Niinemets et al. 1999). Thus, the light dependence of isoprene emission could be explained by only one isoprene synthesis-specific coefficient, ε (Fig. 12.3a). In further model development (Niinemets et al. 2002c; Niinemets 2004), the equation was generalized as:

$$E = \varepsilon\, J_T \frac{(C_i - \Gamma^*)}{\varsigma\,(4C_i + 8\Gamma^*) + 2\,(C_i - \Gamma^*)\,(\vartheta - 2\varsigma)}, \qquad (12.14)$$

where ς is the carbon cost of isoprenoid emission (6 mol mol^{-1} for isoprene and 12 mol mol^{-1} for monoterpenes), and ϑ is the NADPH cost of specific isoprenoid compounds (mol mol^{-1}). Differently from the initial model formulation (Niinemets et al. 1999) where the extra electron transport was assumed to originate from mitochondrial catabolism of the photosynthetically fixed carbon, Eq. 12.14 assumes that the rate of photosynthetic electron transport in photosynthezising leaves is larger than can be predicted by Eq. 12.12, and thus, the extra reductive equivalents rely on this "excess" electron transport (Niinemets et al. 2002c; Niinemets 2004). Overall, the model fit to monoterpene emissions was good (Fig. 12.3b, c), although it was realized that less volatile terpenoids can be non-specifically stored in the leaf liquid and lipid phases, resulting in delays between biosynthesis and emission (Niinemets et al. 2002a, c; Niinemets and Reichstein 2002).

Zimmer et al. (2000) and Grote et al. (2006) modelled BVOC emission on the basis of changes in metabolite pools. Their numerical model is based on reaction rates of various enzymes in the production pathway that are described based on Michaelis-Menten kinetics. Dynamics in the concentration of photosynthates that also serve as primary emission precursors, pyruvate and glyceraldehyde 3-phosphate, are directly related to photosynthesis and then enter the isoprenoid synthesis pathway as substrates. Thus, in this form of model logic, the light dependencies of isoprene and light-dependent monoterpene emission are introduced by the photosynthesis model used to produce emission substrates. Thus, the light relationship is ultimately driven by the same dependence of J on I that was reflected in the Guenther et al. (1991) and Niinemets et al. (1999) models.

As a further simplification, Niinemets et al. (2013 in this volume) directly linked isoprene emission to photosynthesis. In the so-called C-ratio model, isoprenoid emission was calculated as the product of the gross assimilation rate and monoterpene emission to assimilation rate ratio, r_C (Niinemets et al. 2013 in this volume). While being the simplest model, r_C was shown to depend on light and temperature, and thus, required somewhat greater parameterization effort than linking emissions to electron transport rate (Eq. 12.14). Nevertheless, comparison of different model approaches (Eqs. 12.11 and 12.14 and C-ratio model) at canopy level indicated that once correctly parameterized, all models performed similarly (Niinemets et al. 2013 in this volume).

As Monson et al. (2012) pointed out, all these approaches share a common 'quasi-mechanistic' basis in their relation to photosynthesis, with 'quasi-mechanistic' meaning that the dependence is not yet fully understood so that uncertainties remain. Although the overall flux of electrons going into volatile isoprenoid synthesis is small, there is evidence of control of the MEP pathway flux by ATP and/or NADPH status of chloroplasts (Loreto and Sharkey 1993; Rasulov et al. 2009, 2011; Li and Sharkey 2013a, b). This might suggest that the effective Michaelis-Menten constant of MEP pathway for ATP and/or NADPH controls the pathway flux. Given that daytime production of reductant and chemical energy in the chloroplast is driven by J, linking isoprenoid biosynthesis to the assimilation of CO_2 and to the supplies of precursor molecules into the MEP pathway constitutes still a promising way to simultaneously model CO_2 exchange and volatile isoprenoid emission.

12.2.4 CO_2 Responses

The negative relationship between isoprene emission and the concentration of atmospheric CO_2 was first reported in Sanadze (1964). The response of emission rate to changes in intercellular CO_2 concentration (C_i) follows a pattern with an optimum at a C_i of 150–200 μmol mol^{-1} ($C_{i,opt}$) (Loreto and Sharkey 1990; Rasulov et al. 2009; Sun et al. 2012). It has been demonstrated that the CO_2 dependence of isoprene emission is determined by the immediate precursor, dimethylallyl diphosphate (DMADP) pool size over the whole CO_2 range (Rasulov et al. 2009; Sun et al. 2012), but there is still a debate as to why DMADP pool size varies with CO_2 concentration.

Most of the modelling efforts have focused on understanding the decline in isoprene emission at CO_2 concentrations exceeding $C_{i,opt}$. Sanadze (2004) developed a biochemical hypothesis to explain this decline by postulating a competitive partitioning of chloroplast reductant and energy between the reductive pentose phosphate pathway and the MEP pathway. According to the hypothesis, the partitioning in turn depended on the demand for reductant and energy by the reductive pentose phosphate pathway. If this is low, these compounds are more readily available for isoprenoid production, implying that the pathway under these circumstances acts as a kind of excretion system. Conversely, under conditions of high Rubisco activity (e.g., high C_i), the reductant and energy will be diverted predominantly to synthesize and process sugars from photosynthesis.

This logic was to some degree already captured in the model developed by Niinemets et al. (1999) which is based on photosynthetic electron transport rate with isoprene biosynthesis rate defined by the fraction of J that is partitioned to the MEP pathway (Eq. 12.13). In subsequent work, therefore, based on observations, Arneth et al. (2007) introduced an additional empirical relation into Eq. 12.13 characterizing the partitioning as a hyperbolic function of C_i. Following this same concept, the model produced by Martin et al. (2000) represented isoprene emission as driven by competitive partitioning of chemical energy. In this model, as C_i increased, a negative feedback loop was imposed on emission by the decreasing availability of ATP.

On the other hand, experiments by Rosenstiel and others (Rosenstiel et al. 2003, 2004; Loreto et al. 2007) have suggested that the CO_2 sensitivity of isoprene emission can also be explained by competition for carbon substrate between cytosolic and chloroplastic processes. Wilkinson et al. (2009) model is based on this proposed mechanism and follows the assumptions that (1) at low C_i, the availability of recently-produced photosynthates limits isoprene production, although carbohydrate reserves may allow for some emission, and (2) that at increasing C_i, enzyme activity limits isoprene biosynthesis, while carbon precursors are getting more adequate. The overall response to C_i can then be expressed with an inverse sigmoidal function:

$$f(C) = 1 - \left[\frac{E_{MAX} \, (C_i)^{cC1}}{(C^*)^{cC1} + (C_i)^{cC1}} \right] \tag{12.15}$$

C^* is a reference C_i and c_{C1} is a unitless scaling coefficient that forces the right-hand term to be reduced exponentially at low C_i and increase exponentially at high C_i. A similar model that is based on the concentration of dimethylallyl diphosphate, [DMADP] in the chloroplast rather than C_i has been proposed by Possell and Hewitt (2011). Because [DMADP] decreases as C_i increases, in those cases where a negative CO_2 response has been observed, the model takes the following form:

$$f(C) = \frac{V_{MAX}\,[\mathrm{DMADP}]^{c_{C2}}}{K_M{}^{c_{C2}} + [\mathrm{DMADP}]^{c_{C2}}} \tag{12.16}$$

where V_{MAX} and K_M are the Michaelis-Menten constants for isoprene synthase, and c_{C2} is a unitless scaling coefficient. This model was shown to provide good descriptions of the CO_2 response for several species. However, we note that no model is currently able to mechanistically capture the reduction of isoprene emission at CO_2 concentrations below $C_{i,opt}$ of ca. 150–200 μmol mol^{-1} (Loreto and Sharkey 1990; Rasulov et al. 2009; Sun et al. 2012), and empirical fits best describing the entire CO_2 response curve of isoprene emission have been suggested (Sun et al. 2012).

Very recently, Harrison et al. (2013) proposed another relation of isoprene emission to photosynthesis, assuming that isoprene emission depends on excess reducing power, which is increased by the electron transport supply (J), and reduced by the electron transport requirements for the dark reactions of photosynthesis. The excess or deficit of electrons produced by photochemical reactions during photosynthesis can be calculated as the difference between the total photosynthetic electron flux and the total flux of electrons used for carbon assimilation that is determined by C_i, J and maximum carboxylase activity of Rubisco ($V_{c,max}$). The isoprene emission rate is thus given by:

$$E_{iso} = p_3 J - p_4 V_{c,max} \frac{[C_i + 2\Gamma^*]}{[C_i + K_{c,M}]} \tag{12.17}$$

where p_3 and p_4 are empirical parameters that represent the 'baseline' fraction of the total photosynthetic electron flux used for isoprene synthesis (p_3), and the fraction of 'excess' electron flux (i.e., electrons not used in photosynthetic carbon fixation) used for isoprene synthesis (p_4), and $K_{c,M}$ is the effective Michaelis-Menten constant for Rubisco carboxylase activity. The approach is attractive, combining CO_2 and all other direct effects on photosynthesis, but it remains to be validated by mechanistic knowledge concerning the relation of J to E_{iso}, and it does not fully address the combination of both substrate availability and isoprene synthase activity as controls over E_{iso}.

Sensitivity of BVOC emission to the atmospheric CO_2 concentration has, to this point, been described only for isoprene. Yet, we know that the substrate constraints and mechanisms that affect the MEP pathway should affect the production of other terpenoid compounds as well. Thus, in species without specialized terpene storage structures, analogous CO_2-responsiveness of monoterpene emissions is expected. Indeed, a decrease in the rate of monoterpene emissions with increasing CO_2

concentration has been found in *Quercus ilex* (Loreto et al. 2001) and (to a smaller degree) in *Betula pendula* (Vuorinen et al. 2005). In other studies, no effects (Baraldi et al. 2004; Paoletti et al. 2007) or even an increase of monoterpene emission (Staudt et al. 2001; Himanen et al. 2009) have been observed. Clearly more work is needed to gain insight into CO_2 effects on monoterpene emissions, and it remains to be tested if and under which conditions the described models are applicable for direct emissions other than isoprene. Given the large number of terpene synthases and highlighted differences in regulation for some of these synthases (Rajabi Memari et al. 2013; Rosenkranz and Schnitzler 2013), simulating monoterpene emissions under future conditions is currently bound to large uncertainties.

12.2.5 Needs for Future Developments

Implicit in constructing the isoprene emission model as a product of multiplicative type equations (Eq. 12.1), is that environmental drivers such as light, temperature and CO_2 independently affect isoprene emission, i.e., any response function does not depend on other response functions. Recent progress in determining the mechanistic underpinnings of isoprene emission defines DMADP concentration and kinetic controls over isoprene synthase activity as basic determinants. DMADP concentration depends on energy/reductant availability, as well as on temperature and photosynthetic precursors, while the kinetic controls over isoprene synthase activity depend on temperature and DMADP concentration (Monson 2013). However, mixed control by both factors has not yet been fully reflected in models. For example, recent research indicates that the temperature response of isoprene emission depends on DMADP concentrations only at temperatures greater than 30 °C (Magel et al. 2006; Rasulov et al. 2010; Li et al. 2011). The situation is analogous with the CO_2 response. Given that DMADP availability ultimately controls the whole CO_2 response of isoprene emission, and DMADP level is also affected by light availability (Rasulov et al. 2009), CO_2 responses can vary in their dependence on the instantaneous photosynthetic photon flux density. Such a modification in the shape of CO_2-response curve by light has been recently demonstrated by Sun et al. (2012). The interactive effects of key environmental drivers suggests that models based on DMADP pool size may be more accurate for simulating isoprene emissions under co-varying light, temperature and CO_2 conditions.

The models that have been based on cytosol-chloroplast competition for substrate have not been able to explain one aspect of the CO_2 response – the steep reduction toward zero of the isoprene emission rate at a critically low value of C_i (Loreto and Sharkey 1990; Rasulov et al. 2009, 2011; Sun et al. 2012). Typically, this value is 150–200 μmol mol^{-1} that may be occasionally reached under drought in leaves in their native environments. Furthermore, the declining part of the CO_2 response curve below this critical threshold can provide fundamental information on the mechanism(s) responsible for the overall CO_2 dependence of isoprene emission, and clearly, this is an issue in need of further study.

Rasulov et al. (2009) used observations of the response of E_{iso} and DMADP pool size as a function of C_i to argue that the CO_2 effect on E_{iso} is due to variations in chloroplastic ATP supply, not variations in the channeling of PEP from the cytosol to the chloroplast. Both hypotheses rely on the fundamental observation that plastidic DMADP pool size decreases as C_i increases; the debate posed by Rasulov et al. (2009), as a counterpoint to the perspective of Rosenstiel et al. (2004), is focused on the cause of that decrease. Most of the evidence underlying both perspectives is correlative – positive correlations between ATP availability and E_{iso} have been observed (Loreto and Sharkey 1993) and negative correlations between PEP carboxylase activity and E_{iso} have been observed (Rosenstiel et al. 2003, 2004; Loreto et al. 2007; Possell and Hewitt 2011). In a recent study by Trowbridge et al. (2012), proton-transfer reaction mass spectrometry was used to detect the differential kinetics of ^{13}C incorporation into fragments of isoprene presumed to come from cytosolic versus chloroplastic sources. The results during periods of low versus high C_i suggested slower labelling in the fragment originating from cytosolic sources, and this fragment was more highly labeled in the presence of low CO_2, compared to that derived directly from glyceraldehyde 3-phosphate (GAP). These latter results might be used as support for the Rosenstiel et al. (2003) perspective. Once again, this is an issue that needs more study before a definitive model for C_i can be identified.

12.3 Modelling Acclimation and Seasonality

Seasonal dynamics of physiological pre-conditioning have long been either neglected (particularly when only short periods have been investigated) or have been empirically adjusted to time series measurements (e.g., Staudt et al. 2000, 2002). However, instantaneous emission responses to environmental drivers and maximum emission rates depend on the weather conditions days to weeks prior to the emission measurements and on ontogenetic changes in foliage emission capacity. That is why recent weather as an important driver of isoprenoid emission rate is now increasingly included in emission models (Guenther et al. 2006; Keenan et al. 2009; Niinemets et al. 2010a).

12.3.1 Seasonal Changes, Leaf Age Effects and Temperature Acclimation

12.3.1.1 Empirical Dependencies

After establishing that plants emit isoprenoid compounds instantaneously in a manner that is dependent on light and temperature, it was recognized that these dependencies change during the season (Ohta 1986). This has been noted to

lead to considerable biases in total emission inventories and has been related to temperature degree sums in past studies, similar to the metric used to describe phenological development in plants (Monson et al. 1994). Nevertheless, most early attempts on seasonal adjustment related the emission capacity to the day of the year (D). Schnitzler et al. (1997) proposed an asymmetric equation to define the seasonal factor, $f(S)$, which was intended as an additional multiplier in Eq. 12.1, and was described by an equation analogous to those used for enzyme activity modelling:

$$f(S) = \frac{\exp\left(c_{S1}\ D + c_{S2}\right)}{1 + \exp\left(c_{S3} D + c_{S4}\right)} \tag{12.18}$$

where c_{S1-n} are curve fitting coefficients. Pier and McDuffie (1997) used a second-order polynomial with three parameters to describe symmetric seasonal variation of isoprene emission potential observed in white oak (*Quercus alba*):

$$f(S) = c_{S5} + c_{S6} D + c_{S7} D^2 \tag{12.19}$$

Another equation that included parameters with physical meaning was proposed by Staudt et al. (2000) describing a Gaussian (bell-shaped) response with an offset:

$$f(S) = 1 - \rho\left[1 - \exp\left(-(D - D_0)^2/\tau\right)\right] \tag{12.20}$$

with ρ representing the relative annual amplitude of the maximum possible seasonal emission rate (between 0 and 1.0), D_0 the day at which the emission capacity reaches a maximum, and τ the breadth (kurtosis) of the seasonal amplitude in days. Additional asymmetric functions have been used by Lavoir et al. (2011), Keenan et al. (2009) and Niinemets et al. (2013 in this volume). Keenan et al. (2009) compared the seasonality function shapes, asymmetric vs. symmetric, and concluded that an asymmetric function better adheres to the data and is recommended for simulation of seasonal variations in isoprenoid emission.

12.3.1.2 Dependencies Imposing Genetic and Environmental Controls

Early in the history of isoprene emissions studies, it was hypothesized that it is not the day of the year, but the previous integrated environmental conditions that determine seasonal shifts in the isoprene emission rate (Monson et al. 1994). As a consequence, it has been proposed that leaf developmental processes, controlled by genetic-environment interactions, underlie expression of the genes for emission synthases. Two modelling approaches were suggested: (1) isoprene synthase development follows leaf phenology, assuming that only fully-grown and active leaves are able to emit BVOCs at potential rates; (2) synthase activity is subject to continuous

but slow formation and decay processes that depend on environment. Thus, previous environmental conditions are important determinants of E_{max}. Lehning et al. (2001) followed this concept explicitly. The Seasonal Isoprenoid synthase Model (SIM) is split into a description of leaf development and senescence, and an equation that calculates dynamics of enzyme activity. The first mechanism represents the building and decline of emission capacity assuming a linear relation to leaf development (or more precisely, relative canopy leaf area). It has been elaborated to be applicable for evergreen species by Grote (2007) who described leaf development for each leaf age class separately. The second impact is a description of synthase turnover:

$$f(S) = S_0 + [g(S_F) + h(S_D)]\Delta t \tag{12.21}$$

where S_0 is the previous (or initial) state of the seasonality function, $g(S_F)$ is a function that describes the rate of protein synthesis in dependence on past light and temperature conditions and phenological state of the leaves, and $h(S_D)$ is a function that describes the rate of protein degradation (for details see Grote et al. 2010). In contrast to the previous approaches, this model introduces some mechanistic cause-effect relationships by considering the increase of enzyme activity as dependent on absorbed radiation and its decay as a function of temperature.

Another approach has been presented with the Model of Emissions of Gases and Aerosols from Nature (MEGAN) (Guenther et al. 2006). In this model, age effects are described by separating the foliage among new, young, and recently matured leaves. The seasonality aspect was described by adjustment of E_S (Eq. 12.1) independent of phenology in dependence on the temperature of the previous days:

$$f(S) = c_{S8} \exp\left[c_{S9}\left(T_{24} - T_{REF}\right)\right] \exp\left[c_{S9}\left(T_{240} - T_{REF}\right)\right] \tag{12.22}$$

where c_{S8} and c_{S9} are empirical parameters, T_{REF} is a reference temperature (297 K), and T_{24} and T_{240} are average temperatures for the previous 24 and 240 h, respectively.

In MEGAN, the overall response represents a sine function while, the SIM approach follows the general pattern of an exponential response, which generally provides a better fit to data. We present some of the approaches that have been used for seasonal adjustment of E_{MAX} in Fig. 12.4. The shapes of the seasonal responses and their maxima near day of year of 200 are generally conserved. However, the slopes of the responses for the ascending and descending trajectories on either side of the maxima differ, and this is where model-dependent differences are likely to be greatest.

Overall, we note that modelling seasonality remains a challenging task. As acclimation and age effects cannot be deconvoluted, it is important to be aware that the seasonality and age models may partly include acclimation effects. This understanding is relevant especially when trying to incorporate various acclimation, stress, and seasonal controls in multivariate models (Eq. 12.1) to avoid "double-counting" of various factors, thereby over-parameterizing the model (Niinemets et al. 2010a).

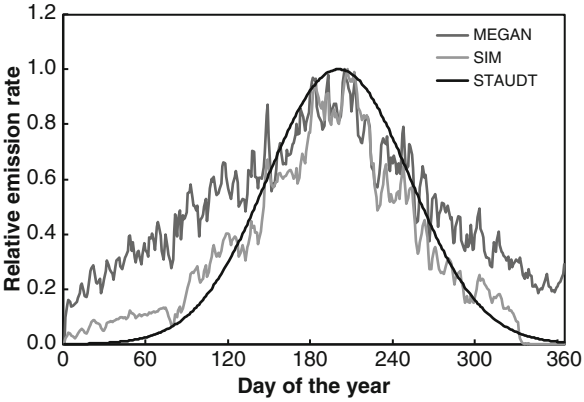

Fig. 12.4 Seasonal adjustment of maximum emission rate in broad-leaved sclerophyll *Quercus ilex* according to an empirical fit to data from Staudt et al. (2000), and the emission capacity predicted according to weather-dependent MEGAN (Guenther et al. 2006) and SIM (Lehning et al. 2001) approaches using the weather conditions at Montpellier, France for 2006 growing season (Modified from Grote et al. 2010). The emission rates were normalized to the highest observed value

12.3.2 Acclimation to Variations in Light Environment

Several studies have demonstrated that both isoprene emission capacity (Harley et al. 1996, 1997; Geron et al. 1997; Hanson and Sharkey 2001; Funk et al. 2006; Niinemets et al. 2010a, b) and monoterpene emission capacity in "non-storage" species (Lenz et al. 1997; Niinemets et al. 2002a; Staudt et al. 2003) increases with increasing long-term light availability. In particular, extensive within-canopy variation in isoprene emission rate of 3-27-fold has been recently demonstrated in broad-leaved deciduous trees (Fig. 12.5). Depending on within-canopy plasticity in isoprene emission potential, model estimates of whole canopy isoprene emissions using a constant emission factor are biased by −8 to +68 % (Niinemets et al. 2010b). Guenther et al. (1999) linked such within-canopy variations at the level of coefficient c_{L3} of the light response function of Eq. 12.11. Thus, the coefficient c_{L3} essentially functions as a scaling factor. As no long-term light measurements were available, c_{L3} was linked to cumulative leaf area index (L_{cum}) as (Guenther et al. 1999):

$$c_{L3} = 1.42 \exp(-0.3 L_{cum}) . \tag{12.23}$$

However, foliage acclimates to long-term quantum flux density, Q_{int}, rather than to L_{cum}, and Q_{int} corresponding to a given value of L_{cum} may vary in dependence on foliage angular distribution and spatial aggregation (Cescatti and Niinemets 2004). Despite species-specific variations in the within-canopy variability of emission capacity, Niinemets et al. (2010b) demonstrated that when all data across the species

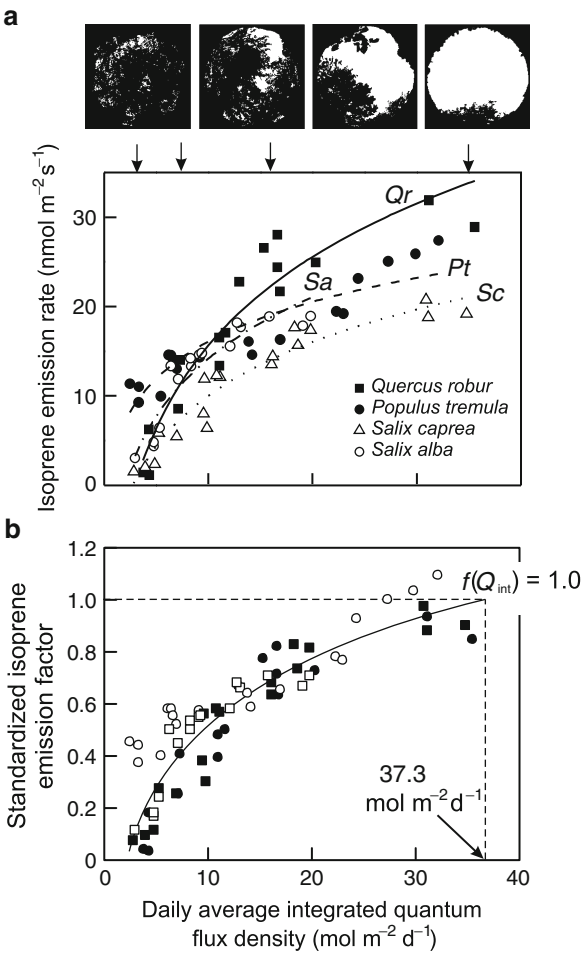

Fig. 12.5 Variation of isoprene emission rate at standard conditions of leaf temperature of 25 °C and incident quantum flux density of 1,000 μmol m^{-2} s^{-1} (emission factor) with seasonal average integrated quantum flux density (Q_{int}) in four temperate deciduous species (**a**), and the emission factor standardized to the above-canopy (average seasonal maximum) quantum flux density of 37.3 mol m^{-2} day^{-1} (modified from Niinemets et al. 2010b). Data in (**b**) are fitted by the so-called canopy function of isoprene emission ($f(C)$, Eq. 12.24). Representative hemispheric photographs demonstrating different light environments within the canopy are also shown (*arrows* denote the Q_{int} values corresponding to the four hemispheric photographs)

were standardized to above-canopy seasonal average Q_{int} (37.3 mol m^{-2} day^{-1} in their study), the variation decreased (Fig. 12.5) and all data could be fitted by a single canopy function, $f(C)$:

$$f(C) = 0.843\mathrm{Log}\,(0.411\,Q_{int})\,. \tag{12.24}$$

The bias of using Eq. 12.24 in estimating whole canopy isoprene emission flux relative to the use of species-specific variation patterns was only −11 % to +6 % (Niinemets et al. 2010b). Thus it would be more accurate to use Eq. 12.24 instead Eq. 12.23 in future emission models.

The other parameter, susceptible to acclimation is the quantum yield (α in Eq. 12.11) used to define the instantaneous light response of immediate emission. This parameter can vary within the canopy (Fig. 12.2b) due to changes in leaf

chlorophyll content (Niinemets 2007). For isoprene and monoterpenes, studies have suggested an increasing apparent quantum yield with canopy depth (Harley et al. 1996, 1997; Staudt et al. 2003), and the apparent quantum yield has thus been expressed in dependence on L_{cum} similar to c_{L3} (Guenther et al. 1999):

$$\alpha_{app} = 0.001 + 0.00085 L_{cum} \tag{12.25}$$

However, we note that there is an explicit connection between α_{app} and c_{L3} as indicated in Sect. 12.2.3 and in Monson et al. (2012). In fact, Harley et al. (1997) fit the light response curves of isoprene emission using Eq. 12.10, and observed only minor within-canopy variation in the true quantum yield. Thus, it remains to be tested to what extent the true quantum yield for isoprene emission does indeed vary in plant canopies.

12.3.3 Needs for Future Developments

Representing acclimation processes of foliage emission at the ecosystem scale is a difficult task since seasonal development of cell-to-leaf level states are not only directly affected by environmental conditions, but are also indirectly influenced by phenological, ontogenetic and structural properties of the emitting plants. This is most obvious for foliage amount which varies during the year due to leaf flushing and senescence, and these effects are particularly obvious in deciduous species. Additionally, ontogenetic changes affect isoprenoid emission potentials (maximum rate under standardized conditions) (e.g., Grinspoon et al. 1991; Kuzma and Fall 1993; Fuentes and Wang 1999). The capacity to emit isoprenoids generally develops gradually during leaf development and reaches a maximum only after full leaf expansion; following maximum leaf expansion, and the emission potentials further gradually decrease with increasing leaf age (Fischbach et al. 2002). With respect to evergreen species, it is thus important that functional activity continuously decreases with increasing leaf age (Niinemets et al. 2006, 2013; Grote 2007). Finally, canopy structure determines microclimatic conditions that affect short- and long-term impacts on emission processes throughout the canopy (Keenan et al. 2011). All of these vegetation processes develop dynamically and simultaneously in response to changes in the seasonal environment. It is difficult to disentangle the direct and indirect seasonal influences on emission potential and to define species-specific differences in acclimation capacity, principally due to the lack of empirical information. For example, surprisingly little information is available on emission potentials in older leaves. Other physiological developments such as seasonal dynamics in isoprenoid storage pools, which are not yet considered in any model (Schurgers et al. 2009) add to these uncertainties. Finally, emissions related specifically to bud, flower, and fruit development are not addressed in models, although modified emission patterns – qualitatively and quantitatively – have been reported for the period of bud burst (e.g., Kuhn et al. 2004).

12.4 Incorporating Stress in Models of Constitutive Emission

Stress can have several effects on volatile emissions. First, in constitutively emitting species, stress may modify the emission capacity and/or the shape of emission responses to environmental drivers. Second, stress can lead to induction of volatile emissions in both emitting and (otherwise) non-emitting species. As natural vegetation is often under stress, even suffering frequently from co-occurring and sequential stress episodes (Loreto and Schnitzler 2010; Niinemets 2010a, b), our ability to predict stress responses on volatile emissions is urgently needed for reliable prediction of emission time series. In this section, we analyse how stress effects on constitutive emissions can be incorporated in emission models focusing on the influences of altered transfer conductances and biochemical modifications as exemplified by drought responses. For pollutant effects on constitutive emissions we refer to Calfapietra et al. (2013) in this volume.

12.4.1 Impacts on Conductances

12.4.1.1 Stomatal Controls

Constitutive emissions are controlled by temperature and the diffusive resistances between storage pools and the atmosphere. Several past studies have focused on stomata as the primary resistance to emission from internal storage pools. In the early studies of foliage isoprene emission, it was recognized that the steady-state isoprene emission rate is independent of stomatal conductance (G_s) (Monson and Fall 1989; Fall and Monson 1992). Fall and Monson (1992) hypothesized that steady-state reductions in G_s were compensated by increases in Δp, the difference in isoprene partial pressure between the intercellular air spaces of the leaf (p_i) and the ambient atmosphere (p_a). Thus, $E = G_s(\Delta p/P)$, where P is the air pressure. The theory underlying this relation and its application to a range of emitted BVOCs requires that for compounds which have relatively high Henry's law constants (gas/liquid phase partition coefficients), perturbations in G_s should result in rapid (within seconds) establishment of a new diffusion steady state (Niinemets and Reichstein 2003). This would not be true for BVOCs with lower Henry's law coefficients (e.g., oxygenated isoprenoids, organic acids or methanol). Niinemets and Reichstein (2003) formalized the theory on these relations by stating:

$$E = G_s \frac{(p_i - p_a)}{P} = G_L \frac{(H\ C_w - p_i)}{P} \tag{12.26}$$

where H is the Henry's law constant for the particular BVOC (Pa m^3 mol^{-1}), C_w is its concentration in the liquid (water) phase of the cell or cell wall (mol m^{-3}), and G_L is the gas-phase equivalent of liquid phase conductance from the site of compound synthesis to the outer surface of cell wall. Implicit in Eq. 12.26 is that compounds

with low H support a lower vapour pressure for given liquid phase concentration, and accordingly, the diffusion gradient, Δp, increases slowly such that changes in stomatal conductance can transiently limit volatile emissions (Niinemets and Reichstein 2003; Harley 2013).

12.4.1.2 Breakage of Storage Structures

Enhancements of emissions of stored BVOCs occur when leaf tissue is wounded and broken epidermis and cuticle strongly decrease diffusive resistances. These effects are particularly relevant in characterizing the impact of logging operations in forests where terpene-filled tissue is destroyed (e.g., Strömvall and Petersson 1991). Similarly, insect attacks can open plant storages of volatile compounds that often act as a defence and serve to poison or otherwise deter attackers (Loreto et al. 2000; Trowbridge and Stoy 2013). We note that past relationships of terpene content vs. emission rate as shown for some conifers (Lerdau et al. 1994, 1995) may reflect the "rough handling problem", i.e., exposure of internal storage structures to ambient air during measurements.

To date, the effects of rapid changes in diffusion conductance from the site of storage to ambient air have not been considered in emission models. However, BVOC pools have previously been quantified (e.g., Llusià and Peñuelas 1998; Llusià et al. 2010) and their release can be simulated according to Eqs. 12.2 or 12.3 with the additional assumption of a limited (and decreasing) storage pool size (Schurgers et al. 2009). Nevertheless, the models likely need to be more complex than just first order decay functions, because the initial rapid increase in emissions is followed by time-dependent reduction of the emission rates as the wound becomes sealed, e.g., as the result of oxidation and polymerization of oleoresin components (Loreto et al. 2000).

12.4.2 Impacts on Biochemistry

The impact of drought has been studied in several investigations as time-integrated, long-term influence on isoprene emission (e.g., Fang et al. 1996; Brüggemann and Schnitzler 2002; Pegoraro et al. 2004; Brilli et al. 2007). However, until recently, drought has not been considered as a modifier in BVOC emission models. Drought can influence the emissions in three ways. First, reductions in leaf evaporative cooling due to constrained leaf transpiration rates, leading to concomitant increases in T_L. Second, decreases in stomatal conductance result in reduced C_i. Finally, there can be direct effects of drought on metabolic processes.

The first effect can be accommodated in the models by considering the deviations between T_L and air temperature. The second influence can be incorporated through Eqs. 12.15, 12.6 and 12.17 when properly parameterized to consider reduced CO_2 growth regime, especially considering the reduction of emissions below a critical C_i.

Modelling the effects of metabolic modifications is most complex. Drought tends to trigger a cascade of metabolic feedbacks that function to balance metabolism with growth potential. Grote et al. (2009) took advantage of previous studies of changes in the concentrations of certain photosynthetic metabolites to represent drought effects on monoterpene emissions through the availability of BVOC precursors. One premise of this approach is that a tight coupling exists between leaf carbon balance, as influenced by leaf photosynthesis rate, and isoprenoid emission. However, this assumption neglects the shift in resources between different biochemical pathways under stress. Within the MEGAN model, Guenther et al. (2006) introduced a drought scaling factor as a linear relation between relative water availability and E as an additional multiplier in Eq. 12.1. This function was defined as:

$$f(W) = \begin{cases} 1, \text{ if } \theta \geq \theta_1 \\ \dfrac{(\theta - \theta_W)}{\Delta\theta_1}, \text{ if } \theta_W < \theta < \theta_1 \\ 0, \text{ if } \theta \leq \theta_W \end{cases} \tag{12.27}$$

where θ is the extractable water content ($m^3\ m^{-3}$), θ_w is the soil water content at leaf wilting point, i.e., the soil water content that cannot be extracted by plant roots, $\Delta\theta_1$ is an empirically-determined soil water limit that can be expressed as $\theta_1 = \theta_w + \Delta\theta_1$. $\Delta\theta_1$ is commonly set as 0.06 following Pegoraro et al. (2004). One of the difficulties with using this type of model is the determination of θ_w as well as $\Delta\theta_1$. Guenther et al. (2006) used the wilting point database of Chen and Dudhia (2001) for global emission estimation. However, there are no studies to date that have established the wilting point as a conserved and relevant determinant of drought stress on photosynthesis or BVOC emission.

The greatest barrier to progressing in our ability to model drought stress effects on BVOC emission is our incomplete understanding of the metabolic connections among drought, expression of BVOC synthase activities, availability of BVOC substrates, and drought-induced changes in the sensitivities of BVOC formation to light, temperature and intercellular CO_2 concentration. Future studies should focus on these connections, which may allow us to integrate drought-stress models more effectively into BVOC models.

12.5 Simulation of Induced Emissions

12.5.1 General Patterns

Consistent with the theory and evidence that BVOC emissions serve primarily as a protection against abiotic stress and for communication among ecological tropic levels (Holopainen 2004; Sharkey et al. 2008). BVOC emissions can be induced by practically any stress factor in species emitting and non-emitting volatiles constitutively (Heiden et al. 2003). The emission of stress volatiles reflects

elicitation of defence pathways, side-products of intermediates of which are volatile, and synthesis of volatile products with known or yet unknown functions in direct and indirect defence (Pare and Tumlinson 1999, Kessler and Baldwin 2001; Peñuelas and Llusià 2003; Owen and Peñuelas 2005, 2006; Niinemets 2010a, b). Induction of volatile emissions has been demonstrated in response to both biotic stresses such as insect herbivory (e.g., Priemé et al. 2000; Miller et al. 2005; Dicke et al. 2009; Copolovici et al. 2011; Blande et al. 2009), and fungal pathogens (Steindel et al. 2005; Toome et al. 2010) and abiotic stress such as UV radiation (e.g., Blande et al. 2009), ozone (e.g., Beauchamp et al. 2005; Blande et al. 2007), heat and frost (Loreto et al. 2006; Copolovici et al. 2012), flooding (Copolovici and Niinemets 2010; Kreuzwieser and Rennenberg 2013), and mechanical wounding (Fall et al. 1999; Banchio et al. 2005; Loreto et al. 2006).

Emissions of early stress volatiles during and immediately after stress reflect activation of signalling at the level of membranes and cell walls and are associated with the release of methanol (Beauchamp et al. 2005; Loreto et al. 2006; von Dahl et al. 2006; Copolovici and Niinemets 2010) and green leaf volatiles (various C_6 aldehydes) (Priemé et al. 2000; Loreto et al. 2006; Copolovici and Niinemets 2010; Copolovici et al. 2011, 2012; Blande et al. 2007, 2009; Kirstine and Galbally 2004; Loreto et al. 2006; Davison et al. 2008; Brilli et al. 2012). These emissions are followed by activation of gene expression and emissions of specific volatile isoprenoids from stressed foliage (Dicke 1994; Paré and Tumlinson 1997; Beauchamp et al. 2005; Toome et al. 2010; Copolovici et al. 2011; Blande et al. 2007, 2009). Furthermore, release of volatiles and synthesis of non-volatile phytohormones in stressed leaves can elicit systemic response in neighboring non-stressed leaves of the same plant and in neighboring different plants, resulting in volatile emissions of apparently healthy leaves (Dicke 1994; Röse et al. 1996; Paré and Tumlinson 1998; Staudt and Lhoutellier 2007; Holopainen et al. 2013; Trowbridge and Stoy 2013).

Characteristic stress-induced volatile isoprenoids are monoterpenes linalool and ocimenes, homoterpenes DMNT and TMTT and various sesquiterpenes (Loivamäki et al. 2004; Herde et al. 2008; Dicke et al. 2009; Toome et al. 2010), and thus, the composition of elicited isoprenoids typically differs from the volatiles released in non-stressed conditions (Loreto and Schnitzler 2010; Niinemets et al. 2010c; Schnitzler et al. 2010). As noted above, in constitutively emitting species, biotic or abiotic stress may result in suppression of constitutive emission rates (Anderson et al. 2000; Copolovici and Niinemets 2010; Toome et al. 2010), but not always (Calfapietra et al. 2007, 2008; Copolovici and Niinemets 2010). Yet, in constitutively non-emitting species, volatile emissions generally increase from low background level by several orders of magnitude even above the level observed in constitutively emitting species (Niinemets et al. 2010c for a review). For instance, temperate deciduous broad-leaved birch (*Betula*) species have been observed to emit mono- and sesquiterpenes at a low level of only 0.1–0.4 $\mu g\ g^{-1}\ h^{-1}$ in some studies and during certain periods during the growing season (Fig. 12.6, König et al. 1995; Hakola et al. 1998, 2001). However, under stress conditions, they have been found to be relatively strong emitters of monoterpenes linalool and ocimenes, and sesquiterpenes, with standardized emission rates (leaf temperature of

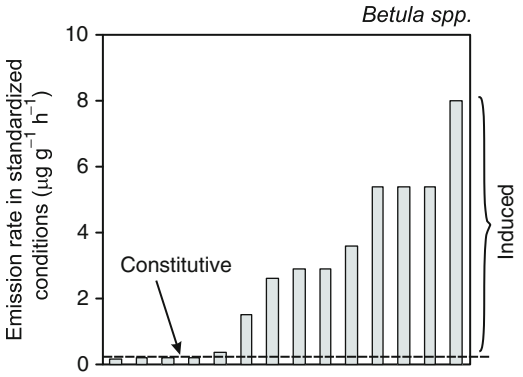

Fig. 12.6 Variation in standardized monoterpene emission factor (leaf temperature of 30 °C, incident quantum flux density of 1,000 μmol m^{-2} s^{-1}) in temperate deciduous birch (*Betula*) species (Data from König et al. 1995; Steinbrecher et al. 1997; Hakola et al. 1998; Hakola et al. 2001; Owen et al. 2003). Sustained emissions under non-stressed conditions are defined as constitutive emissions, while emissions elicited under certain stress periods are defined as induced emissions

30 °C and incident quantum flux density of 1,000 μmol m^{-2} s^{-1}) between 1.5 and 8 μg g^{-1} h^{-1} (Fig. 12.6, König et al. 1995; Hakola et al. 1998, 2001; Steinbrecher et al. 1999; Owen et al. 2003). Analogously, in a constitutive-emitter Mediterranean evergreen conifer *Pinus pinea*, total emissions from stressed plants are several-fold greater than the constitutive emissions from non-stressed plants (Staudt et al. 1997, 2000; Niinemets et al. 2002b, c).

12.5.2 Modelling Induced Emissions

Induction of BVOC emissions can reflect activation of enzymes that are already present or increased expression of the genes that encode various BVOC synthases. Given the growing evidence that a considerable fraction of emission responses are related to stress induction, models that describe these processes are beginning to emerge. Iriti and Faoro (2009) have suggested differentiating between primary and secondary metabolic pathways in the induction process with concomitant modifications in carbon fluxes among the pathways. The mechanism of volatile induction is complex, starting with signal perception that triggers the cascade of events leading ultimately to activation of transcription regulators and onset of expression of volatile synthases (Bolwell et al. 2002; Maffei et al. 2007; Mithöfer and Boland 2008; Loreto and Schnitzler 2010; Niinemets 2010a; Arimura et al. 2011). The mechanisms of signal perception and elicitation of gene expression can differ for different stresses, but there is evidence of a uniform stress response elicitation pathway for both biotic and abiotic stresses at the level of oxidative signalling (Bostock 2005; Fujita et al. 2006). Often, there is also a cross-talk between ethylene-, salicylate- and jasmonate-dependent stress response pathways (Thaler et al. 2002; Traw and

Bergelson 2003; Bostock 2005; Fujita et al. 2006; Mithöfer and Boland 2008). Thus, general stress response models can in principle be constructed (Niinemets 2010a).

From an experimental perspective, there is increasingly more evidence that stress severity and plant volatile emission response are quantitatively related, including positive correlations between the severity of ozone (Beauchamp et al. 2005), heat (Karl et al. 2008; Copolovici et al. 2012) and insect herbivory (Copolovici et al. 2011) stresses. Scaling of volatile emission response with the stress severity has been used in predicting methyl salicylate emissions from a walnut (*Juglans californica* × *Juglans regia*) agroforest on the basis of average temperature preceding the measurements (Karl et al. 2008). While such empirical models based on average level of environmental drivers can be useful once the emissions have been triggered, emissions typically are not induced until a certain stress threshold has been exceeded (Beauchamp et al. 2005; Copolovici et al. 2012), except perhaps for wounding and insect herbivory that essentially always trigger emissions. Thus, the key issue in predicting stress induction of volatiles is to determine when a given environmental driver is sensed as a stress by the plant. The stress thresholds depend on a variety of factors including plant tolerance to given type of stress and past stress history such as stress priming (Conrath et al. 2006; Heil and Kost 2006; Heil and Silva Bueno 2007; Niinemets 2010a, b). Thus, a stress of given severity may or may not result in inductions of volatile emissions.

The second difficulty of simple empirical models is what happens after stress. When the stress is relieved, for how long do the triggered emissions continue? There is evidence that after the stress relief, the induced emissions may reach to a background level in a few days (Copolovici et al. 2011). The emissions may also decrease during the stress as plant acclimates to the stress (Copolovici and Niinemets 2010). However, there is also evidence of sustained emissions once elicited (Staudt et al. 1997, 2000; Hakola et al. 2001; Niinemets et al. 2002b; Copolovici and Niinemets 2010).

Stress signalling models are currently being intensively developed (Vu and Vohradsky 2007; Yip et al. 2010; Muraro et al. 2012), but due to limited knowledge of signal transduction and transcription regulators, completely mechanistic models cannot yet be derived. We suggest that for the time being, the dynamic controls on induced BVOC emissions can be simulated based on the theory of recursive action of regulators on the target gene(s) over time (Vu and Vohradsky 2007; Yip et al. 2010). Thus, the target gene activity change over time, dz/dt, is expressed as (Vu and Vohradsky 2007; Yip et al. 2010):

$$\frac{dz}{dt} = \frac{v_{\max}}{1 + \exp\left(-\sum_{j=1}^{j=n} w_j y_j + c\right)} - kz(t), \tag{12.28}$$

where $v_{\max}$ is the maximum rate of expression, k is the rate constant of degradation, $z(t)$ is the gene product amount at time t, n is the number of gene regulators considered, w_j is the weighting factor for a given control function y_j, and b is the delay factor describing the lag in the transcription initiation. Thus, $v_{\max}$

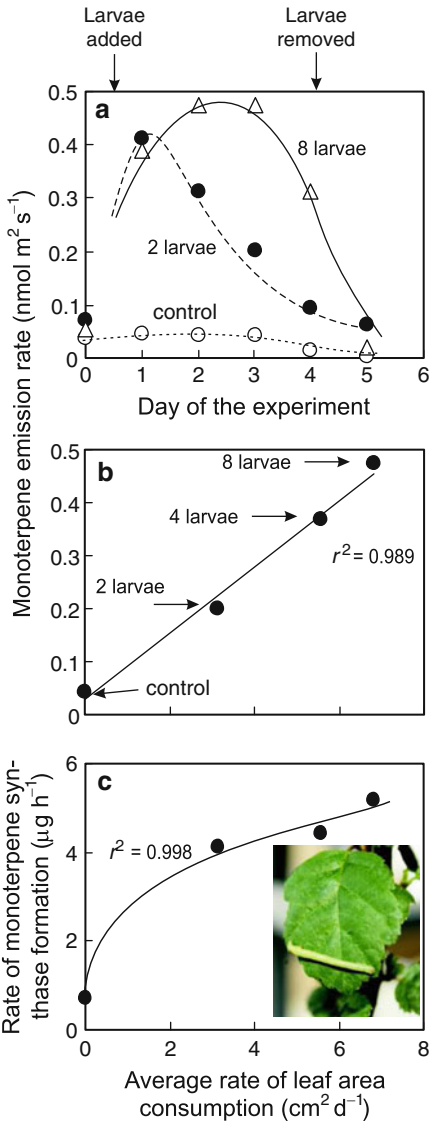

Fig. 12.7 Illustration of elicitation of monoterpene emissions in temperate deciduous tree *Alnus glutinosa* by the common white wave (*Cabera pusaria*) larvae (**a**), and the relationships between the average rate of leaf consumption and monoterpene emission rate (**b**) and the rate of monoterpene synthase formation (**c**) (Data from Copolovici et al. 2011). In the experiment, the plants of *A. glutinosa* were subject to different levels of herbivory by using either 0 (control), 2, 4 or 8 *C. pusaria* larvae per plant. The measurements were conducted at 28 °C. The data in (**a**) were simulated by a model based on dynamic transcriptional control (Eq. 12.28), and the rate of monoterpene synthase formation was found by fitting the data in (**a**) by the model. In calculating the protein formation rate, a specific activity of monoterpene synthase of 94 nmol g^{-1} s^{-1} at 28 °C was used (Niinemets et al. 2002c), and it was further assumed that the induced monoterpene synthases operate in substrate-saturated conditions

and the denumerator determine the onset of gene expression, while kz and $w_j y_i$ functions determine the silencing of the response. This model assumes that the overall regulatory effect on a given gene can be expressed as the combination of all regulators (Vu and Vohradsky 2007; Yip et al. 2010). Highly plastic non-linear transcription control effects can be simulated using various linear or non-linear y_i functions, and it has been demonstrated that Eq. 12.28 provides excellent fits to complex gene expression profiles (Vu and Vohradsky 2007).

Equation 12.28 was applied here to the induction of monoterpene emissions in the temperate deciduous tree black alder (*Alnus glutinosa*) (Fig. 12.7a, Copolovici

et al. 2011). In this study, different levels of herbivory were achieved by using either 0 (control), 2, 4 or 8 larvae of the common white wave (*Cabera pusaria*) on each plant (Copolovici et al. 2011). The rate of leaf biomass consumption scaled positively with the number of feeding larvae, and the rate of induced monoterpene emission was quantitatively related to the rate of foliage consumption (Fig. 12.7a, Copolovici et al. 2011). In the model fit, only one transcriptional control was assumed and the control function was described by a fifth order polynomial.

The model applied here provides excellent fits to the data (Fig. 12.7b), and allows for the estimation of kinetic dynamics in transcription, maximum rates of induced monoterpene synthase formation and rates of monoterpene synthase decay under different herbivory treatments. The maximum rate of monoterpene synthase formation was quantitatively associated with the rate of herbivory (Fig. 12.7c). To our knowledge, this is the first evidence demonstrating that stress signal strength can be quantitatively simulated to project target protein synthesis rate. On the other hand, we also observed differences in the transcription regulation function in different treatments, with the emissions being both elicited and declining earlier in the treatment with two than in the treatment with eight larvae (Fig. 12.7a). Such differences cannot be currently explained, and apart from differences in plant transcription regulation, might reflect differences in the feeding behavior of herbivores in different treatments. Overall, this exercise provides encouraging evidence that models based on dynamic transcription control can be used to simulate induced emissions, and we suggest that simple dynamic regulatory models such as Eq. 12.28 together with quantitative relationships between the severity of stress and maximum plant response have large potential to simulate stress-driven emissions in larger-scale models.

12.6 Conclusions

Considering the different environmental impacts that affect BVOC emission, it has become increasingly apparent that integrated descriptions of processes are beginning to emerge. Such integrated models will permit us to begin examining higher-order interactions between environmental change and ecosystem BVOC emissions, including the feedbacks that control regional- to global-level dynamics in atmospheric chemistry and in the production and lifetime of radiatively-important trace gases such as O_3 and CH_4. In this chapter, we have concentrated on the leaf-scale modelling as the most significant breakthroughs in recent BVOC modelling have been made at this scale. There is now increasing recognition that the mechanistic emphasis that has been in focus at the leaf scale needs to be expanded to consider processes at greater spatial scales and longer temporal scales (Guenther 2013 in this volume). Consideration of the latter, takes us into the need to discover ways of simulating the interactions between environmental cues and gene expression. Simulation of these larger and longer-term processes will allow us to begin tackling some of the regional and global dynamics in air chemistry.

These controls are increasingly recognized as being central components in Earth system models (Ashworth et al. 2013; Kulmala et al. 2013), and we argue that more biological realism needs to be incorporated in these models in near future.

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Chapter 13
Scaling BVOC Emissions from Leaf to Canopy and Landscape: How Different Are Predictions Based on Contrasting Emission Algorithms?

Ülo Niinemets, Paolo Ciccioli, Steffen M. Noe, and Markus Reichstein

Abstract A variety of leaf-level models has been embedded in a canopy model and used to predict monoterpene emissions from canopies and landscapes, but there is no objective basis of choice between different models. Here we analysed the capacity of four leaf-level models and their variations, yielding altogether eight models, for predicting diurnal and seasonal variations in canopy monoterpene emissions. The main models tested were Guenther et al. model with fixed light and temperature dependencies or with optimally adjusted dependencies, two models linking emissions to foliage photosynthetic rate, one to electron transport rate (ETR model) and the other to gross assimilation rate (C-ratio model), and a dynamic model considering non-specific monoterpene storage in leaves. Once parameterized in a consistent manner, all models showed similarly high performance, assessed by explained variance, modelling efficiency and average model deviations for homogeneous canopies. Simulations suggested potentially stronger deviations for landscapes with fragmented vegetation. This analysis indicates that the choice among the models cannot be based on model validation statistics alone, but depends on whether only BVOC emissions need to be simulated (Guenther et al. model) or both photosynthesis and BVOC fluxes are needed (ETR or C-ratio model) or whether one needs data on night atmospheric reactivity (dynamic model).

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13.1 Introduction

Biogenic volatile organic compound (BVOC) emissions play a primary role in tropospheric ozone formation (Chameides et al. 1988; Simpson 1995). Therefore, for mesoscale air pollution modelling it is crucial to have reliable estimates of ecosystem BVOC fluxes (e.g., Simpson 1995; Fuentes et al. 2000). BVOC further participate in the formation of secondary organic aerosols and cloud condensation nuclei, thereby playing a major role in large-scale Earth processes (Huff Hartz et al. 2005; Engelhart et al. 2008; Chen and Hopke 2009; Hallquist et al. 2009; Guenther et al. 2012; Kulmala et al. 2013 in this volume), further emphasizing the importance of accurate estimations of BVOC fluxes. The largest part of BVOC emissions originates from plant leaves (Geron et al. 1994; Simpson 1995; Guenther et al. 2006), and accordingly, the most significant improvement of estimates of large-scale emissions may be achieved through an advancement of leaf- and canopy-scale emission models.

For one of the most volatile plant compounds, isoprene, the synthesis of leaf-level data has led to development of an empirical model that uses a hyperbolic light-dependence and an Arrhenius type temperature response to describe the environmental effects on emission rates (Guenther et al. 1991, 1993; Monson et al. 2012). This model has also been successfully employed to simulate light- and temperature effects on monoterpene emission in species that lack specific terpene storage structures in the foliage such as the Mediterranean evergreen *Quercus* species (e.g., Bertin et al. 1997; Ciccioli et al. 1997) and the broad-leaved deciduous tree *Fagus sylvatica* (Dindorf et al. 2006; Holzke et al. 2006). However, in the case of less volatile monoterpenes, non-specific storage effects have been demonstrated that can potentially introduce time-lags between the synthesis and emissions of these compounds (Niinemets and Reichstein 2002; Noe et al. 2006, 2008, 2010), potentially altering the daily emission dynamics. While these effects have been characterized at leaf scale, their importance at canopy scale has not been assessed.

Although models with fixed response shapes can realistically describe the light and temperature effects on the emission rates in non-stressed conditions and in fully-developed leaves, they cannot adequately predict the emission rates in stress conditions, and they also have limited potential to account for potential changes in the response curves that may occur as the result of leaf acclimation to varying environmental conditions (Berry and Björkman 1980; Mulkey et al. 1991). In particular, drought stress regularly occurs in Mediterranean environments, leading to a gradual decrease in maximal stomatal conductances to water vapour with advancement of soil water limitations, and typically also to mid-day stomatal closure (Tenhunen et al. 1987; Manes et al. 1997b; Peñuelas and Llusià 1999). Decreases in stomatal conductance not only result in decreased net assimilation rates, but also in declines in the emission rates of monoterpenes (Moncrieff et al. 1997; Ciccioli et al. 1999; Hansen and Seufert 1999; Peñuelas and Llusià 1999; Niinemets et al. 2002b). To parameterize such stress effects, a series of empirical modifiers has been recently included in Guenther et al. algorithms (Guenther et al.

2006, 2012; Monson et al. 2012; Grote et al. 2013 in this volume). Stress-related changes in the emission rates might be more adequately described by physiological models resting on the correlations between the emission rate and the rate of synthesis of Calvin cycle intermediates (Martin et al. 2000; Zimmer et al. 2000) or between the emission rate and the supply of reductive and energetic equivalents by photosynthetic electron transport (Niinemets et al. 1999, 2002a, c). However, the ultimate process-level descriptions in these models are limited by the circumstance that the physiological controls on isoprene and monoterpene emissions have still not been fully resolved.

There are also significant seasonal changes in the isoprene (Monson et al. 1994, 2012; Schnitzler et al. 1997; Lehning et al. 2001) and terpene emission capacities (Hakola et al. 1998; Llusià and Peñuelas 2000; Staudt et al. 2000; Sabillón and Cremades 2001; Keenan et al. 2009) associated with developmental changes in the content of enzymes controlling the pathway flux. Seasonality effects have been included in recent emission models (e.g., Guenther et al. 2006; Keenan et al. 2009), although the parameterizations largely differ (Monson et al. 2012; Grote et al. 2013 in this volume). For instance, in the Guenther et al. (2006) model, seasonality is directly related to leaf area development, and aging, but also results from longer-term seasonal changes in environmental drivers. Although the approach used is plausible, the model equations postulated were parameterized on the basis of a limited number of case studies.

Here we compare the potentials of four different leaf-level monoterpene emission approaches to predict monoterpene fluxes at the canopy and landscape levels. In addition to the widely used Guenther et al. (1993) algorithm, two additional approaches linking the monoterpene emission rate either to photosynthetic electron transport (Niinemets et al. 1999, 2002a, c) or to the fraction of assimilated carbon lost as monoterpenes (Martin et al. 2000, current study) were included. Finally, a dynamic model considering non-specific monoterpene storage (Niinemets and Reichstein 2002) was used. At canopy scale, all models were evaluated against relaxed eddy accumulation flux measurements conducted in a Mediterranean evergreen *Quercus ilex* L. stand, while landscape-level estimates were compared in a hemiboreal mixed forest.

13.2 Leaf-Level Monoterpene Emission Models: Emission Algorithms and Scaling

A variety of emission algorithms has been developed to simulate light- and temperature-dependent volatile isoprenoid emissions from the foliage of emitting plants. The model equations have been reviewed in detail recently (Monson et al. 2012; Grote et al. 2013 in this volume), and here the models are only briefly described in order to highlight the characteristic features (Table 13.1) and explain the way they were parameterized in the intercomparison exercise. We consider

separately the steady-state models that assume instantaneous response of emissions to changes in incident quantum flux density (Q) and leaf temperature (T), and dynamic models that consider potential time-lags between the synthesis and emission of monoterpenes. This may be relevant given that due to limited volatility of monoterpenes, they are non-specifically stored in leaf liquid and lipid phases even in species not having specialized storage structures (Niinemets and Reichstein 2002; Niinemets et al. 2004; Noe et al. 2010).

13.2.1 Emission Algorithms

We analysed the performance of three different steady-state emission algorithms and one dynamic emission model for estimating the monoterpene emission rate E (Table 13.1). The selected algorithms cover a spectrum of models including models with fixed response shapes directly simulating emissions, models linking emissions to foliage photosynthetic traits and thus, indirectly simulating emissions and models that consider time-lags between compound synthesis and release (for detailed analysis of emission models see Niinemets et al. 2010c; Monson et al. 2012; Grote et al. 2013 in this volume).

13.2.1.1 Guenther et al. Model

In the Guenther et al. G93 algorithm (Guenther et al. 1993), an estimate of E in standardized conditions (E_S, $T = 30$ °C, $Q = 1{,}000$ μmol m^{-2} s^{-1}) is scaled to combinations of leaf temperature and light using predetermined shapes of temperature (C_T) and light (C_L) response curves (Table 13.1). To describe seasonal changes in E_S, several approaches have been offered (Guenther et al. 2006; Keenan et al. 2009; Niinemets et al. 2010a; Monson et al. 2012; Grote et al. 2013 in this volume). We have tested different symmetric and asymmetric functions and finally we used the following empirical function to describe changes in E_S as a function of day of the year (D):

$$E_S = \begin{cases} E_{S,max}\, e^{(a+b/D+c\ln D)}, & \text{if } D < D_{200} \\ E_{S,max}\, e^{-\frac{(d-D)^2}{2f^2}}, & \text{if } D \geq D_{200} \end{cases}, \tag{13.1}$$

where a, b, c, d and f are empirical best fit parameters and $E_{S,max}$ is the maximum value of the basal emission measured during a year. This model that uses two different equations to describe the emission factor before reaching the maximum and beyond the maximum is extremely plastic and allows both for simulation of symmetric and asymmetric seasonality responses.

Table 13.1 Characteristics of key emission models for simulation of light-dependent monoterpene emission rate (E)

Model[a]	Key equation	Light dependence	Temperature dependence
Guenther et al. (G93) model	$E = E_S C_T C_L$, where E_S is the monoterpene emission rate at $T = 30$ °C and $Q = 1{,}000$ μmol m^{-2} s^{-1}, and C_L is the light and C_T the temperature response function	$C_L = \frac{\alpha c_{L1} Q}{\sqrt{1+\alpha^2 Q^2}}$, where α and c_{L1} are parameters	$C_T = \exp\frac{c_{T1}(T-T_s)}{RT_sT} \Big/ \left[1+\exp\frac{c_{T2}(T-T_M)}{RT_sT}\right]$, where c_{T1} (J mol^{-1}), c_{T2} (J mol^{-1}) and T_M (K) are empirical coefficients, T_s is the temperature in standard conditions (303 K), and R is the gas constant (J mol^{-1} K^{-1})
ETR model	$E = \varepsilon\, J_{CO_2+O_2} \frac{(C_i-\Gamma^*)}{12(4C_i+8\Gamma^*)+2(C_i-\Gamma^*)\Delta_{NADPH}}$, where ε is the fraction of electrons in monoterpene synthesis, $J_{CO_2+O_2}$ is the photosynthetic electron transport rate, ΔNADPH is the difference of NADPH requirements for sugar and monoterpene synthesis, C_i is the intercellular CO_2 concentration and Γ^* the CO_2 compensation point without dark respiration	Results from light dependence of $J_{CO_2+O_2}$	$\varepsilon(T) = \varepsilon_{Tref}\, e^{aT}$, where a is an empirical constant, and ε_{Tref} is the ε value estimated at a reference temperature T_{ref} (30 °C)
C-ratio model	$E = r_C A_G$, where r_C is the ratio between monoterpene emission and gross assimilation rate (A_G, Box 13.1)	Combined non-linear empirical equation describing the dependence of r_C on both Q and T (Box 13.1, Eq. 13.B2)	
Dynamic model	$E(t) = k_1 S_1(t) + k_2 S_2(t)$, where k_1 is the rate constant for the faster pool with size S_1 (Eqs. 13.3 and 13.5) and k_2 that for the slower pool with size S_2 (Eqs. 13.4 and 13.6)	Either G93 or ETR model for monoterpene synthesis rate	Either G93 or ETR model for monoterpene synthesis rate, temperature dependence of k_1 and k_2 is predicted by Eq. 13.7

[a]G93 – (Guenther et al. 1993), ETR model – (Niinemets et al. 1999, 2002c), C-ratio model (Box 13.1), dynamic model – (Niinemets and Reichstein 2002; Noe et al. 2006)

13.2.1.2 Photosynthetic Electron Transport Model (ETR Model)

A correlation between the whole-chain photosynthetic electron transport rate ($J_{CO_2+O_2}$, μmol m^{-2} s^{-1}) and E is employed in the ETR model (Table 13.1, Niinemets et al. 1999, 2002c; Arneth et al. 2007). The primary assumption of this model is that the rate of isoprenoid synthesis is directly related to the content of photosynthetic metabolites and/or NADPH and ATP provided by photosynthetic electron transport (Loreto and Sharkey 1993). Because volatile isoprenoids are more reduced molecules than sugars, NADPH and ATP cost per mol C in given isoprenoid is greater than the cost per C for the formation of sugars in photosynthesis (Niinemets et al. 1999; Sharkey and Yeh 2001; Niinemets 2004). However, because the rate of carbon loss through the emission of volatile isoprenoids is generally much less than the rate of carbon fixation, overall requirement for NADPH and ATP for isoprenoid synthesis is relatively small compared with photosynthetic carbon assimilation and photorespiration. Thus, a direct competition for NADPH and ATP among isoprenoid synthesis pathway and photosynthesis would not explain the control of isoprenoid synthesis by photosynthetic electron transport rate. However, NADPH and ATP may exert the control over isoprenoid synthesis pathway if the effective K_M of volatile isoprenoids for NADPH and/or ATP is relatively large (Rasulov et al. 2009, 2011).

According to the ETR model, a certain fraction of electrons (ε) is available for monoterpene production:

$$\varepsilon = \frac{J_M + J_E}{J_{CO_2+O_2}} \tag{13.2}$$

where J_M is the electron transport rate required to reduce the carbon emitted as monoterpenes to the carbon reduction state in sugars, and J_E is the extra electron transport rate necessary to reduce the sugars to monoterpenes. The ratio ε characterizes the degree to which photosynthetic electron transport is used for monoterpene synthesis, and it depends on total activity of monoterpene synthases (Niinemets et al. 2002c). The conversion from electron to monoterpene units, mol electrons in monoterpene synthesis (mol monoterpene synthesized)$^{-1}$, is calculated according to 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose-5-phosphate (MEP/DOXP) pathway for isoprenoid synthesis (Lichtenthaler 1999). Considering a composition-weighted NADPH cost for the monoterpenes emitted by *Quercus ilex*, a value of 28 mole NADPH per one mole monoterpene emitted is obtained (Niinemets et al. 2002c). The fraction of electrons used for monoterpene synthesis can practically be determined at given leaf temperature from each measurement pairs of $J_{CO_2+O_2}$ and E, and is thus, analogous to the basal emission rate, E_S, in the Guenther et al. algorithm.

In the ETR model, E responds to Q directly through changes in total photosynthetic electron transport rate. Temperature dependence is simulated considering that monoterpene synthesis pathway becomes more competitive for electrons with

increasing temperature. ε at a reference temperature T_{ref} (ε_{Tref}) is scaled to any other temperature using an exponential relationship (Table 13.1). The seasonality in ε is achieved by modifying the ε_{Tref} parameter according to a function with a maximum similarly as in the Guenther et al. model (Eq. 13.1) and in the C-Ratio model (Box 13.1, Eq. 13.B3).

Box 13.1: The C-Ratio Model of Isoprenoid Emission

While linkage of isoprenoid emission rate to leaf physiological activity is relatively complex and/or requires availability of physiological traits that are not routinely available in field studies (Monson et al. 2012; Grote et al. 2013 in this volume), we explored the possibility of directly linking isoprenoid emissions (E) to leaf gross assimilation rate (A_G). There are often correlations between E and assimilation rate (Monson and Fall 1989; Loreto and Sharkey 1990; Peñuelas and Llusià 1999; Llusià and Peñuelas 2000; Staudt et al. 2001, 2003), indicating that a certain foliage photosynthetic activity is associated with limited variation in the fraction of carbon going in isoprenoid synthesis. Given that the assimilation rate can be estimated more easily than either E or the rate of photosynthetic electron transport rate, a simpler model based on A_G may have a large potential in scaling monoterpene fluxes. In this model (the C-ratio model), the monoterpene emission rate is calculated as:

$$E = A_G r_C, \tag{13.B1}$$

where r_C is the ratio between monoterpene emission and CO_2 gross assimilation [mol monoterpene emitted (mol CO_2 assimilated)$^{-1}$]. We note that correlations between photosynthesis and monoterpene emission rate do not necessarily indicate that photosynthetic activity determines monoterpene emission rate, but rather that there are general correlations between overall changes in foliage physiological activity and photosynthesis and isoprenoid emission rate (e.g., Staudt et al. 2003; Sun et al. 2012). Thus, Eq. 13.B1 provides a means to empirically link monoterpene emissions to photosynthetic activity. Given that the activity of monoterpene synthase may increase relatively more with increasing light and temperature than the activity of enzymes limiting photosynthetic electron transport (Niinemets et al. 2002a, c), and that monoterpene synthase activity also strongly varies seasonally (Melle et al. 1996; Fischbach et al. 2002), we expected r_C to be also responsive to these factors. In fact, E was more strongly linked to the fraction of photosynthetic carbon used for isoprenoid synthesis than to A_G in some studies (Niinemets et al. 2010b; Guidolotti et al. 2011), and this has been suggested to be indicative of carbon availability control on isoprenoid emission (Guidolotti et al. 2011).

(continued)

Box 13.1 (continued)

For the Mediterranean sclerophyll *Quercus ilex*, the C-ratio model was parameterized using the enclosure data of Ciccioli et al. (2001). These data demonstrated that r_C increased non-linearly with increasing leaf temperature, T, and incident quantum flux density, Q, (Fig. 13.B1):

$$r_C = r_{C,S} e^{a_1(T-30)} \left(\frac{Q}{1,000} \right)^{a_2}, \tag{13.B2}$$

where $r_{C,S}$ (mmol mol^{-1}) is r_C at standardized conditions ($Q = 1{,}000$ μmol m^{-2} s^{-1}, $T = 30$ °C), and a_1 and a_2 are empirical coefficients.

Data further indicated that r_C varied seasonally, likely reflecting changes in monoterpene synthase activity (Fig. 13.B2). The seasonal variation in $r_{C,S}$ (Fig. 13.B2) was described by an equation with a maximum $r_{C,S}$ (r_{max}) at day of the year (D) D_{max}:

$$r_{C,S} = \begin{cases} r_{max} e^{-k_{up}(D-D_{max})^2}, & \text{if } D < D_{max} \\ r_{max} e^{-k_{down}(D-D_{max})^2}, & \text{if } D \geq D_{max} \end{cases} \tag{13.B3}$$

where k_{up} and k_{down} determine the rate with which $r_{C,S}$ declines before and after D_{max}. Equations 13.B1, 13.B2 and 13.B3 provide the full set of equations needed to simulate E for any combinations of A_g, T, Q and D.

13.2.1.3 C-Ratio Model

As an alternative to the ETR model, we also tested a simpler possibility of linking E directly to the rate of gross carbon assimilation (A_G, Box 13.1). The advantage of the C-ratio model is that it does not require estimation of photosynthetic electron transport rate, but it still makes a connection to overall changes in leaf physiological activity (Table 13.1). Use of gross rather than net CO_2 assimilation increases the correlation between assimilation rate and monoterpene emission rate under conditions of high temperature, when net assimilation rate may decline, but dark respiration rate (Hüve et al. 2011) and monoterpene emission rate (Loreto et al. 1998) increase. Nevertheless, as with the ETR model, the C-ratio model predicts that the fraction of carbon going to monoterpene synthesis (monoterpene emission to A_G ratio, r_C) increases with increasing T, but also with Q, albeit weaker than with T (Fig. 13.B1). Seasonality in r_C is expressed similarly as in Guenther et al. and ETR models (Box 13.1).

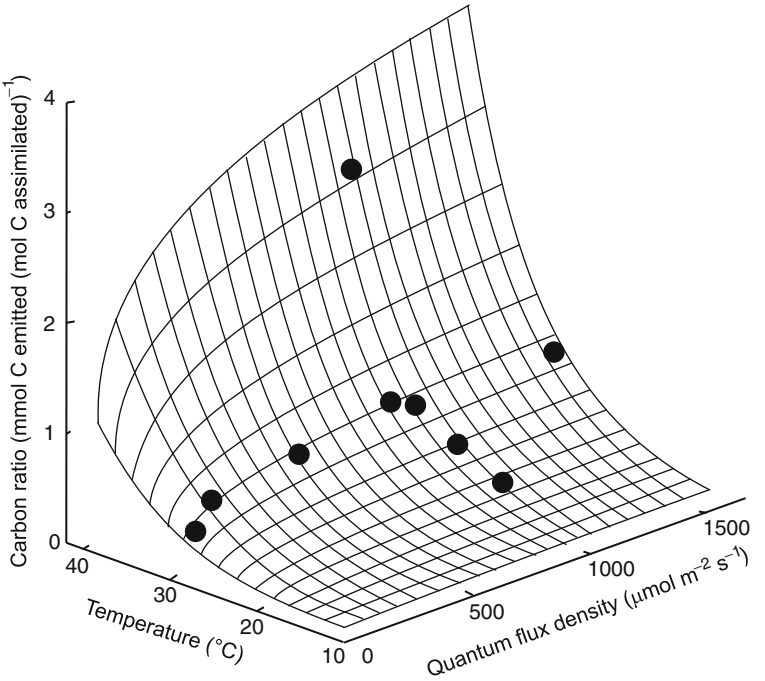

Fig. 13.B1 Dependence of the ratio between monoterpene emission and CO_2 assimilation (r_C) on leaf temperature (T) and incident quantum flux density (Q) in Mediterranean sclerophyll *Quercus ilex* (data of Ciccioli et al. 2001 measured in saplings grown in 50 L pots). The fitted surface corresponds to Eq. 13.B2 with best fit coefficients of 0.131 °C^{-1} for a_1 and 0.210 for a_2 ($r^2 = 0.96$)

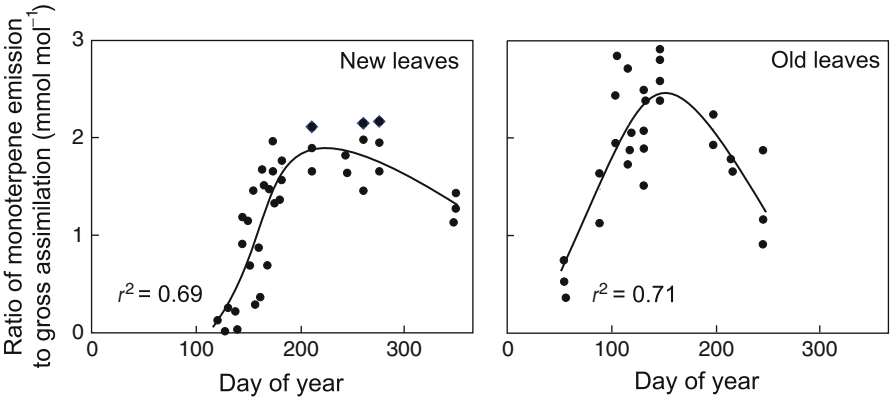

Fig. 13.B2 Seasonal variation of the ratio of monoterpene emission to gross assimilation rate under standard conditions of $T = 30$ °C and $Q = 1{,}000$ μmol m^{-2} s^{-1} ($r_{C,S}$) in current- and 1-year-old leaves of Mediterranean evergreen sclerophyll *Quercus ilex*. The data were fitted by Eq. 13.B2 with $r_{max} = 1.92$ mmol mol^{-1}, $k_{up} = 3.8 \cdot 10^{-4}$, $k_{down} = 1.6 \cdot 10^{-5}$, and $D_{max} = 200$ obtained for current year leaves and $r_{max} = 2.48$ mmol mol^{-1}, $k_{up} = 1.4 \cdot 10^{-4}$, $k_{down} = 7.1 \cdot 10^{-5}$, and $D_{max} = 149$ for 1-year-old leaves (data of Ciccioli et al. 2001 measured in saplings grown in 50 L pots from May 1997 to September 1998)

13.2.1.4 Dynamic Emission Model

To account for non-specific storage of monoterpenes with low volatility in leaf liquid and lipid phases, Niinemets and Reichstein (2002) and Noe et al. (2006) have developed a dynamic emission model (Harley 2013 in this volume for a review). According to experimental data (Niinemets and Reichstein 2002; Noe et al. 2006, 2010), at least two pools, a liquid-phase pool S_1 (nmol m^{-2}) and a lipid-phase pool S_2 (nmol m^{-2}) with differing time-response (time constants k_1 and k_2, s^{-1}) are needed to simulate monoterpene emission rate at time t (Niinemets and Reichstein 2002; Noe et al. 2006). The emission rate is calculated as the sum of the turnover rates of these pools (Table 13.1) with the pool dynamics described as:

$$\frac{dS_1(t)}{dt} = \eta I - k_1 S_1(t) \tag{13.3}$$

$$\frac{dS_2(t)}{dt} = (1-\eta)\, I - k_2 S_2(t). \tag{13.4}$$

where η is the fraction of synthesized monoterpene going in pool S_1 and I the rate of monoterpene synthesis. The analytical solution of the model is (Niinemets and Reichstein 2002):

$$S_1(t) = \left(S_1\,(t_0) - \frac{\eta I}{k_1}\right) e^{-k_1 t} + \frac{\eta I}{k_1} \tag{13.5}$$

$$S_2(t) = \left(S_2\,(t_0) - \frac{(1-\eta)\, I}{k_2}\right) e^{-k_2 t} + \frac{(1-\eta)\, I}{k_2}. \tag{13.6}$$

The rate of compound synthesis, I, can be simulated by any of the three leaf-level algorithms (Sects. 13.2.1.1, 13.2.1.2 and 13.2.1.3), but it is important to consider that the key model parameter determining the emission capacity, E_S in Guenther et al. model, ε_{TRef} in ETR model and r_C in C-ratio model, is not numerically the same as that for the steady-state model (Sect. 13.3.2.1).

Apart from the environmental controls on synthesis, temperature also affects the kinetic constants k_1 and k_2 that depend on monoterpene-specific physico-chemical characteristics (diffusivity in liquid and lipid phases, partition coefficients). The values of the rate constants k_1 and k_2 measured at a given absolute leaf temperature of T_1 (K) were estimated at another temperature T_2 by the van't Hoff equation (e.g., Staudinger and Roberts 1996):

$$k_{i,T_2} = k_{i,T_1} e^{\Delta H_{v,i}/R\left(\frac{1}{T_1}-\frac{1}{T_2}\right)}, \tag{13.7}$$

where k_i is the given rate constant and $\Delta H_{v,i}$ the corresponding enthalpy of volatilization (J mol^{-1}), and R the gas constant (J mol^{-1} K^{-1}). We assume that the key determinant of the liquid-phase rate constant k_1 is volatilization from liquid

to gas phase, while the lipid-phase rate constant k_2 is determined by volatilization of given monoterpene from lipid to liquid phase. Thus, we used the enthalpies of volatilization for Henry's law constant (H, gas/liquid phase partition coefficient) for temperature dependence of k_1 and water/octanol phase partition coefficient ($K_{w/o}$) for k_2. Values for α-pinene (36.9 kJ mol^{-1} for H and 21 kJ mol^{-1} for $K_{w/o}$) were employed for the sum of monoterpenes emitted (Copolovici and Niinemets 2005).

13.2.2 Scaling Up Models from Leaf to Canopy in Mediterranean Evergreen Sclerophyll Quercus ilex

13.2.2.1 Canopy Model

The leaf-level emission models were upscaled to an entire stand with the canopy gas-exchange model GASFLUX (Caldwell et al. 1986; Tenhunen et al. 1994; Reichstein 2001). In this model, the canopy is divided into homogeneous horizontal layers, and for each layer light interception and energy balance is modelled, allowing us to determine leaf temperature and incident photosynthetic photon flux density (Q) at specific canopy depths from above-canopy meteorological drivers. Each layer is further split into sunlit and shaded fractions, and leaf gas-exchange rates are computed according to the biochemical model of foliar photosynthesis of Farquhar et al. (Farquhar et al. 1980; Harley and Tenhunen 1991). The stomatal conductance to water vapour (g) is directly bound to net assimilation rate (A) via the Ball-Berry equation (Ball et al. 1987; Collatz et al. 1991), allowing for simultaneous estimation of A, g and intercellular CO_2 concentration. The computed CO_2, water vapour and monoterpene exchange is summed over the layers to obtain an integrated estimate of canopy gas-exchange. The model does not include turbulence of air, and atmospheric CO_2 concentration is taken constant throughout the entire canopy. The model GASFLUX was parameterized with extensive leaf- and canopy-level physiological data for *Q. coccifera* and *Q. ilex* (Tenhunen et al. 1990; Reichstein 2001).

All the monoterpene emission models were embedded in the layered canopy model using first the independent dataset for parameterization of various model approaches at the leaf scale (Sect. 13.2.2.2) and then embedding these into the canopy model by various approaches, thereby leading to different model versions (Sect. 13.2.2.3).

13.2.2.2 Parameterization and Validation Datasets

As an independent parameterization dataset, we used enclosure experiments carried out on six different *Quercus ilex* leaves belonging to two different plants grown in 50 L pots (Ciccioli et al. 2001). By measuring the basal monoterpene emissions and CO_2 assimilation rates from leaf development to leaf abscission from May 1997

to September 1998, strong seasonal variations in both parameters were observed (Ciccioli et al. 2001). From these data, we have determined E_S and temperature and light response curve parameters (Guenther et al. model), ε_{Tref} (ETR model) and its temperature dependence, r_C and its light and temperature dependencies (C-ratio model, Box 13.1), I and its temperature and light dependence (dynamic model), and seasonality dependencies for E_S (Eq. 13.1), ε_{Tref} and r_C. For E_S seasonality, explained variance (r^2) was 0.81 for increasing and 0.62 for decreasing part of Eq. 13.1 with the fitted parameters being $a = 124$, $b = -4{,}045$, $c = -19.6$, $d = 170.5$, and $f = 75.0$.

The validation dataset for 1997 growing season was obtained using trap-enrichment relaxed eddy accumulation measurements (REA) (Moncrieff et al. 1997) in a typical Mediterranean *Q. ilex* dominated forest at Castelporziano, Rome Italy (41°45′N, 12°22′E, Fig. 13.1 for site details) as described in detail in Valentini et al. (1997) and Ciccioli et al. (2003).

The exchange rates of water (H_2O) and carbon dioxide (CO_2) between the ecosystem and the atmosphere were also measured with the eddy covariance technique as detailed in Reichstein et al. (2002b, Fig. 13.1). The eddy flux data were used to test the validity of the GASFLUX canopy model for calculating CO_2 and H_2O canopy gas-exchange. Overall, the canopy model performance was genuine, and suggested that the parameterization was good and that the basic assumptions of the model were fulfilled. The explained variance was 79 % for the CO_2 flux and 75 % for the water vapour flux, i.e., at the upper limit of what is possible to achieve with the upscaling models fitted to eddy covariance data. Even neural network models do not result in considerably higher r^2-values (Wijk and Bouten 1999; Simon et al. 2005; Boissard et al. 2008). The water vapour fluxes were described slightly worse than CO_2 fluxes by the model, probably because of additional errors resulting from missing descriptions of the evaporation of intercepted water and from soil surface in the model.

13.2.2.3 Tested Leaf-Level Model Versions

Qualitatively different approaches were used to parameterize the four different models, and thus, in the final analysis, we distinguish eight model type/version combinations that all resulted in different model predictions:

(1) Original parameterization of Guenther et al. (1993) with fixed shapes for the temperature and light response curves, i.e., $c_{T1} = 95{,}000$ J mol^{-1}, $c_{T2} = 230{,}000$ J mol^{-1}, $T_M = 314$ K for temperature response curve, and $\alpha = 0.0027$ mol mol^{-1}, and $c_{L1} = 1.066$ for light response curve (Table 13.1 for symbol definition). This set of values has initially been obtained from measurements of isoprene emission in *Eucalyptus globulus*, *Liquidambar styraciflua*, *Mucuna pruriens*, and *Populus tremuloides*, but has also been demonstrated to provide reasonably good fits to monoterpene emissions from the leaves of *Q. ilex*, but with larger deviations at higher temperatures

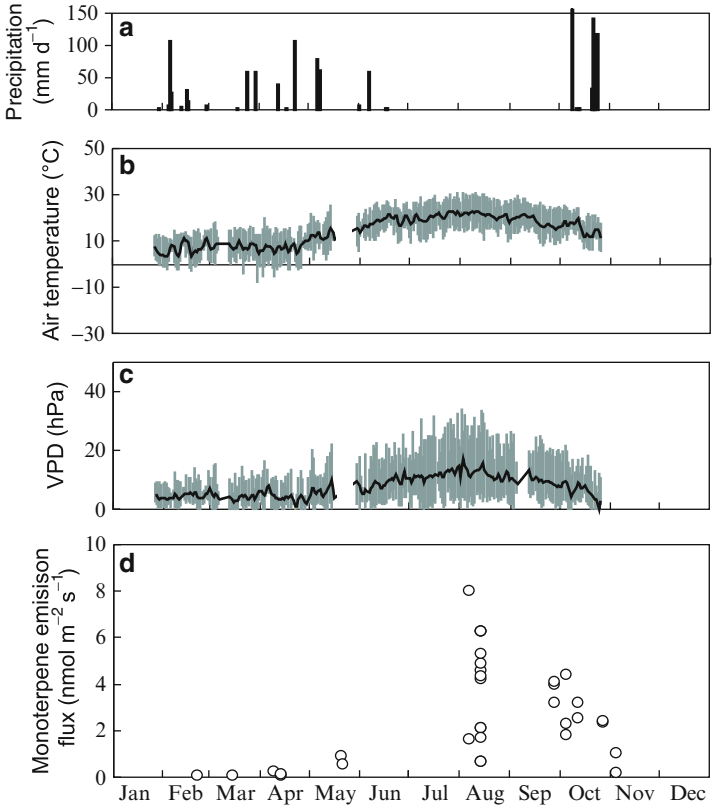

Fig. 13.1 Meteorological conditions and observed ecosystem monoterpene emissions at the Castelporziano site in 1997. (**a**) Daily precipitation above the canopy; (**b**) daily mean (*black line*) and range (*grey bars*) of air temperatures above the canopy; (**c**) daily mean (*black line*) and range (*grey bars*) of water vapour pressure deficit above the canopy; (**d**) total canopy monoterpene emission rate estimated by trap-enrichment relaxed eddy accumulation (REA). The site (41°45′N, 12°22′E) is situated 20 km south-west from the centre of Rome, and is an example of climax forest vegetation of the Mediterranean region with the canopy mainly composed of *Quercus ilex*, and associated (<10 %) *Q. suber* trees. The suppressed understory is dominated by *Arbutus unedo* L. and *Pistacia lentiscus* L. which do not emit monoterpenes. The stand is ca. 10 m tall and has a leaf area index of around 3.5 m^2 m^{-2}. The soil originates from aeolic sands without rocks, and the roots of canopy trees likely reach ground water (Valentini et al. 1992; Manes et al. 1997a), thereby escaping the severest summer drought

($T > 35$ °C) (Bertin et al. 1997; Ciccioli et al. 1997) and with positive or negative deviations for the light response depending on leaf growth environment, in particular, high vs. low growth irradiance (Staudt et al. 2003).

(2) Fully optimized Guenther et al. (1993) model. In the case of full optimization, not only E_S was parameterized, but also the shapes of the light and temperature response curves. As data on within-canopy variations in the emission potential (for within-canopy changes Niinemets et al. 2002a, 2010b) were not available,

models (1) and (2) were initially run with the constant leaf-level parameterization for all leaves in the canopy. This is different from the C-ratio and ETR models where within-canopy variation in $r_{C,S}$ and ε_{Tref} results from changes in assimilation potentials within the canopy. Thus, an inverse modelling approach was used to find the canopy-level $E_{S,max}$ (Eq. 13.1) value that matched best the observed canopy monoterpene flux after scaling with the canopy flux model. A least-squares algorithm was used to perform this model inversion (Visual Numerics 1993).

(3) Standard parameterization of the ETR model with seasonality (ETR model + seasonality) where the parameters of T-dependence of ε (Table 13.1) were set to best approximate the relationship between ε and T in Niinemets et al. (2002c) and seasonality in ε_{Tref} was considered as explained above (similar to Eq. 13.B3). A non-linear fit to estimates of ε in *Q. coccifera* and *Q. ilex* in Niinemets et al. (2002c) data gave a Q_{10} (ε at $T = T_{ref} + 10$ relative to ε at T_{ref}) value of 3.15 ($r^2 = 0.77$, $P < 0.001$).

(4) In the second model modification (ETR model + fitted temperature dependence), the parameters of the temperature dependence of ε (Table 13.1) were optimized by a least squares algorithm to match the relaxed eddy accumulation fluxes measured here. Thus, in this model version, temperature dependence includes both instantaneous and seasonal temperature effects on ε as is often used in modelling VOC emissions (Niinemets et al. 2010c for a review and critique).

(5) The C-ratio model was applied as explained above (Table 13.1, Box 13.1). The contributions for current-year leaves and 1-year-old leaves (Fig. 13.B2) were averaged with equal weights throughout the year and for all canopy layers. The linkage of monoterpene emissions to foliage photosynthetic characteristics in models 3–5 might be considered inconsistent, given that relatively weak correlations may occasionally be observed between CO_2 exchange and monoterpene emissions, especially when the measurements in stressed and non-stressed conditions are pooled (Loreto et al. 1996; Niinemets et al. 2002a). Nevertheless, it is important to recognize here that the way this is done in these models is already considering modifications in the fraction of electrons and carbon going in monoterpene synthesis as affected by temperature and seasonality. Application of the ETR- and C-ratio models requires parameterization of the entire biochemical photosynthesis model of Farquhar et al. (1980) for calculation of the photosynthetic electron transport rate and gross assimilation rate from the flux measurements or predictions of net assimilation. This parameterization is available for the *Q. ilex* forest in Castelporziano (Reichstein 2001).

(6) Optimized dynamic model, where the standard temperature and light dependencies of Guenther et al. model (model 1) were used for monoterpene synthesis and the capacity for monoterpene synthesis was fitted by minimizing the sum of the squares between REA fluxes and model predictions.

(7) Dynamic model running with the rate of synthesis set equal to the input from the optimized Guenther et al. model (model 2).

(8) Dynamic model with the input from the ETR model + seasonality (model 3).

In models (7) and (8) it is assumed that the rate of monoterpene synthesis can be approximated by the steady-state modelled rate of emission. Models (6)–(8) were used only to simulate the diurnal dynamics. The capacity of the dynamic model to simulate seasonal variations was not analysed.

13.2.3 Modelling Landscape-Level Monoterpene Fluxes in a Mixed Hemiboreal Forest

Landscape-level monoterpene emission simulations were conducted at the mixed hemiboreal forest at Järvselja, south-eastern Estonia (58°25′N, 27°46′E) (for detailed site description Noe et al. 2011, 2012). The site is a mosaic of different vegetation types, mainly resulting from differences in soil fertility, but also due to forest management (Fig. 13.5a) and has different coverage of dominating evergreen conifer species Scots pine (*Pinus sylvestris*, Fig. 13.5b) and Norway spruce (*Picea abies*, Fig. 13.5c). In the site, turbulent ecosystem gas-exchange by means of eddy covariance and measurements of ambient concentrations of reactive trace gases such as ozone and nitrogen oxides (NO_x) are carried out within and above the canopy (Noe et al. 2011, 2012).

The emission factors for *P. sylvestris* and *P. abies* were estimated from branch enclosure measurements during field campaigns in summer 2008 and 2009, and standard emission factors E_S, their temperature and light dependencies and corresponding foliage gas-exchange rates were derived (Noe et al. 2011). We follow here the approach of Bäck et al. (2005) and Kulmala et al. (2013) and assume that the monoterpene emissions in these species result from immediate synthesis, i.e., are both light- and temperature-dependent as in *Q. ilex*. This is contrary to several past studies that have assumed that monoterpenes in these species are emitted only from storage structures and only depend on temperature (e.g., Simpson et al. 1995). In reality, the emissions in conifers come both from storage tissues and from immediate synthesis (Shao et al. 2001; Komenda and Koppmann 2002; Niinemets et al. 2010c) whereas the emissions relying on immediate synthesis may even dominate the emissions (Niinemets et al. 2010c). Past studies may have overestimated the contribution of storage emissions due to "rough handling" of branches during measurements (Niinemets et al. 2011 for a discussion). Use of the light-sensitive emission algorithm is consistent with the experimental data at the site demonstrating that light availability throughout the canopy is a main factor determining daytime monoterpene flux (Noe et al. 2012).

In the case of our measurement setup with large branches, we were unable to conclusively separate the different emission sources, although the emissions from darkened branch cuvettes were small (Noe et al. 2011). Thus, we simulated here the emissions only for the light period using the Guenther et al. (1993) isoprene algorithm as for *Q. ilex* using either the fixed response curve shapes (model 1, Sect. 13.2.2.3) or responses optimized to the data (model 2). The upscaling of emission fluxes at different pixels (30 × 30 m) was done with a simple canopy model

as for *Q. ilex* using the leaf area index maps (Fig. 13.5b, c), the percentage share of the species in each grid cell, and incident light and temperature measurements.

13.3 Comparison of Different Model Algorithms

13.3.1 Model Validation Statistics

A number of validation statistics has been used to assess the goodness of model fit. Traditionally, explained variance of the linear regression between measured and predicted variables (r^2) is used to assess how well a model explains the data. However, r^2 measures the association (correlation) between modelled and observed data, and is thus not sensitive to systematic model over- or underestimation. A number of other model validation statistics has been proposed (Mayer and Butler 1993; Janssen and Heuberger 1995; Willmott and Matsuura 2005; Moriasi et al. 2007). Nash-Sutcliffe modelling efficiency (Nash and Sutcliffe 1970) is one of most widely used model validation statistics (Mayer and Butler 1993; Krause et al. 2005; Moriasi et al. 2007):

$$N_{\mathrm{E}} = 1 - \frac{\sum_{\mathrm{i}=1}^{n} (y_{\mathrm{i}} - P_{\mathrm{i}})^2}{\sum_{\mathrm{i}=1}^{n} (y_{\mathrm{i}} - \overline{y})^2}, \tag{13.8}$$

where y_{i} is the i-th measured, and P_{i} the corresponding simulated value of the characteristic, and $\overline{y}$ is the mean of the measurements. The modelling efficiency is an estimate of both the correlation and the coincidence of measured and simulated values, and as such, is sensitive to systematic deviations between modelled and observed values (Smith et al. 1996). N_{E} varies from 1 (perfect fit to the data) to negative infinity (no correspondence with the measurements). A value of $N_{\mathrm{E}} < 0$ indicates that the mean of the measurements is a better predictor than the model. A disadvantage of N_{E} is that the deviations are calculated as squared values, and therefore, N_{E} is more strongly influenced by larger values of y_{i} and P_{i} than by smaller values (Krause et al. 2005).

Additional model evaluation statistics commonly used are the mean absolute error:

$$\sigma_{\mathrm{A}} = \frac{1}{n} \sum_{\mathrm{i}=1}^{n} |y_{\mathrm{i}} - P_{\mathrm{i}}|, \tag{13.9}$$

and the root mean squared error:

$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - P_i)^2}. \tag{13.10}$$

Both these statistics evaluate the average deviation of predicted values from observations. Both σ_A and σ can be normalized by the range of observations (e.g., normalized mean absolute error) or by the average of the observations, thereby allowing one to compare different datasets. The mean absolute error has been proposed as a better estimate of model performance than the root mean squared error, because σ can be biased more by numerically larger values of y_i and P_i and also by $n^{-0.5}$ (Willmott and Matsuura 2005). This disadvantage can be partly compensated by normalization by the standard deviation of the sample (Moriasi et al. 2007):

$$\sigma_{NSD} = \frac{\sigma}{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \overline{y})^2}}. \tag{13.11}$$

Although model comparison studies typically report only a single validation statistic, or in exceptional cases, a few, it is important to recognize that different model statistics provide complementary information about model performance (Krause et al. 2005; Moriasi et al. 2007). Therefore, we recommend to provide always several model validation statistics, including r^2, N_E, σ_A, σ and perhaps also σ_{NSD} and range-normalized σ_A and σ to enable comparisons of model applications in different situations.

13.3.2 Performance of Different Leaf-Level Algorithms in Simulating Canopy Monoterpene Emissions in Quercus ilex

13.3.2.1 Simulation of the Diurnal Monoterpene Fluxes

All models (Sect. 13.2.2.3) provided realistic description of monoterpene emission from the *Quercus ilex* forest at Castelporziano in the standard day with moderate model-to-model differences (Figs. 13.2 and 13.3), and the model validation statistics were similar for different models with optimized Guenther et al. (model 2) and optimized dynamic model (model 6), yielding the greatest values of modelling efficiency (Eq. 13.8) and lowest estimates of model deviation (Eqs. 13.9, 13.10 and 13.11, Table 13.2).

Differently from these two models, the Guenther et al. model with only modified emission factor (model 1) strongly overestimated the emissions in the morning and in the afternoon (Fig. 13.2). If the light and temperature responses were correct,

Table 13.2 Validation statistics of different monoterpene emission models against observed trap-enrichment relaxed eddy accumulation (REA) fluxes during the standard day (Figs. 13.2 and 13.3) in evergreen sclerophyll *Quercus ilex* stand at Castelporziano

	Algorithm[a]							
Validation statistic	Model 1 (Guenther et al., standard)	Model 2 (Guenther et al., optimized)	Model 3 (ETR, seasonal ε)	Model 4 (ETR + fitted ε(T)-function)	Model 5 (C-ratio)	Model 6 (Dynamic, optimized)	Model 7 (Dynamic with Model 2 input)	Model 8 (Dynamic with Model 3 input)
Explained variance (r^2)	0.83	0.89	0.95	0.89	0.85	0.94	0.90	0.91
Modelling efficiency (N_E, Eq. 13.8)	0.83	0.87	0.79	0.78	0.76	0.89	0.63	0.50
Mean absolute error (σ_A, Eq. 13.9)	0.42	0.33	0.50	0.55	0.65	0.26	0.89	1.20
Root mean square error (σ, Eq. 13.10)	0.65	0.61	0.70	0.73	0.77	0.51	0.94	1.10
σ to observed standard deviation ratio (σ_{NSD}, Eq. 13.11)	0.42	0.39	0.45	0.47	0.49	0.33	0.61	0.71

[a]Model details are reported in Sect. 13.2.2.3

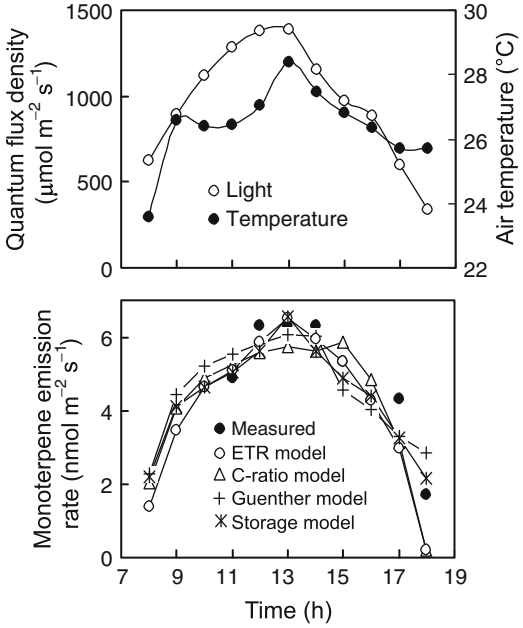

Fig. 13.2 Diurnal variations in light and temperature and corresponding modelled and observed diurnal course of monoterpene fluxes from the *Quercus ilex* stand at Castelporziano (Fig. 13.1 for site details). The potential of various monoterpene emission algorithms to simulate diurnal variability in E was compared using the emission rates and meteorological conditions for July 31, 1997 that was a representative day for the 1997 growing season. The models tested (Sect. 13.2.2.3) were the fully-optimized Guenther et al. model (model 2), standard ETR parameterization (model 3), C-ratio model (model 5) and optimized dynamic model (model 6). Monoterpene emission flux was measured using a relaxed eddy accumulation method (Sect. 13.2.2.2)

such an overestimation in the morning can reflect delayed diurnal upregulation of MEP/DOXP pathway activity as has been observed before (Rasulov et al. 2009, 2010). On the other hand, evening overestimation can be related to constrained foliage physiological activity due to a decrease in mid-day and afternoon stomatal conductance that characteristically occurs in Mediterranean habitats during summer drought (Tenhunen et al. 1987; Reichstein et al. 2002a). Such depressions in monoterpene emissions have been often observed (Bertin et al. 1997; Moncrieff et al. 1997; Staudt et al. 1997; Ciccioli et al. 1999). With an improved algorithm that includes effects of intercellular CO_2 concentration (Wilkinson et al. 2009; Monson 2013 in this volume) such effects of drought can be potentially empirically accounted for.

Alternatively, morning overestimation of emissions can reflect non-specific storage of monoterpenes (Ciccioli et al. 1997; Niinemets and Reichstein 2002; Niinemets et al. 2010c). In the morning, the non-specific pools are small, and thus, the buildup of non-specific storage reduces the emissions. However, non-specific storage effects cannot explain the afternoon overestimation.

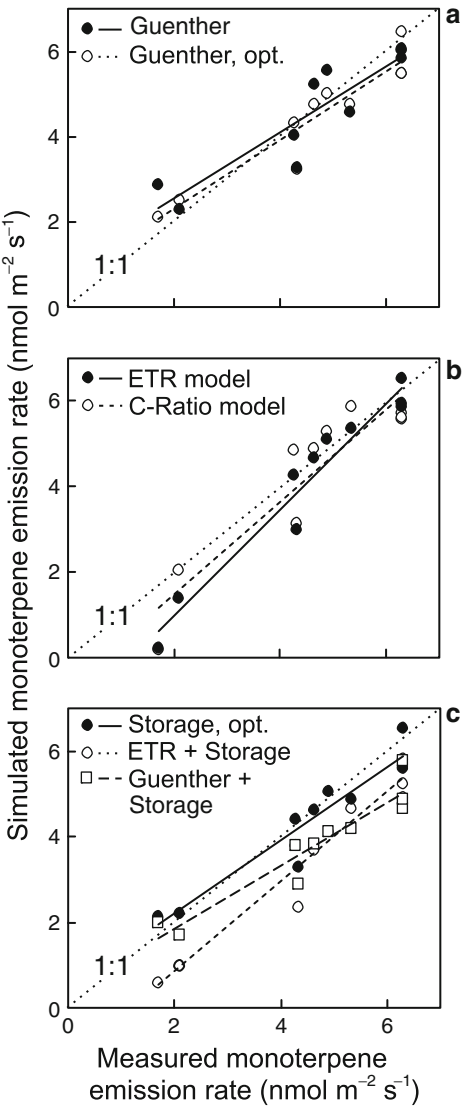

Fig. 13.3 Simulated vs. measured monoterpene emission rates from the foliage of *Q. ilex* in Castelporziano through the standard day (the same simulation as in Fig. 13.2). Canopy monoterpene emission flux by REA measurements was correlated with estimates by (**a**), Guenther et al. model with only fitted E_S (model 1) and Guenther et al. model with all parameters optimized (model 2), by (**b**) standard ETR model (model 3) and C-ratio model (model 5), and by (**c**) fully optimized dynamic model (model 6) or dynamic model using either the input from Guenther et al. optimized model or ETR model (model 7). The models are explained in Table 13.1 and in Sect. 13.2.2.3, and the model validation statistics are provided in Table 13.2. In all panels, 1:1 lines are also shown

Although all the highlighted factors can contribute to the discrepancies between simulated and observed values, the fit to data by the Guenther et al. model could be vastly improved by optimizing the light- and temperature responses of monoterpene emission (Table 13.2, Figs. 13.2 and 13.3). In fact, variations in the shape of temperature and light response curves for monoterpene emission occur (Staudt et al. 2003; Niinemets et al. 2010a, c), and simultaneously modifying E_S and the response curves is a valid approach. Analogous to the results of these simulations, Keenan and Niinemets (2012) were able to significantly improve the performance of Guenther

et al. model in tropical forests. Nevertheless, improving model performance by simultaneous fit of multiple parameters does not rule out the involvement of other missing factors. Thus, fitting all parameters simultaneously could potentially lead to model overparameterization, i.e., in inferior model performance when extrapolated beyond the measurements using other combinations of environmental drivers that were not available for model parameterization.

Both the ETR model versions (models 3 and 4) and the C-ratio model (model 5) performed similarly. In particular, all these models underestimated the emissions in the morning and in the evening, although the explained variance was high, especially for the ETR model with seasonal ε (model 3). However, all these models underestimated the emission rate in the morning and in the evening, reflecting greater light-responsiveness of photosynthesis and, stronger reduction in photosynthetic activity due to limited stomatal conductance in the evening. As with the standard Guenther et al. model, rapid increases in the emission rate in response to increasing light in the morning and rapid reduction in response to decreasing light and reduced stomatal conductance can lead to overestimates of E and reflect non-specific storage effects.

Despite differences in parameterization (ETR models 3 and 4) and in the algorithm complexity (ETR vs. C-ratio model), the similarity in performance of all "physiological" models is striking, and the results provide encouraging evidence that with informed parameterization, canopy monoterpene emissions can be potentially coupled to foliage photosynthetic characteristics, even when using the simplest algorithms such as the C-ratio model (Box 13.1).

Overall, the best model performance in terms of modelling efficiency and model deviation was obtained with the dynamic model (model 6) that included non-specific storage (Table 13.2, Fig. 13.2). This high correspondence between measured and simulated values was only obtained when the maximum rate of synthesis (I, Eqs. 13.3, 13.4, 13.5 and 13.6) was separately fitted. In contrast, the worst correspondence between the predicted and observed values was obtained for the dynamic model that used emissions from either the optimized Guenther et al. model (model 7) or ETR model (model 8) as substitutes for the synthesis rate. In the dynamic model this was improved in the morning and in the evening, i.e., reducing the overestimation for Guenther model and reducing the underestimation for ETR. However, it underestimated maximum fluxes at mid-day, thereby reducing overall model performance. This comparison clearly suggests that the application of dynamic emission models requires a separate parameterization of the monoterpene synthesis component of the model.

13.3.2.2 Seasonal Dynamics of Monoterpene Emission

The seasonal variability in isoprene and monoterpene emission rates (Monson et al. 1994; Bertin et al. 1997, Figs. 13.1d and 13.B1; Staudt et al. 1997, 2000; Guenther et al. 2000) can result from seasonal changes in the activity of enzymes like isoprene

Table 13.3 Validation statistics of different monoterpene emission models against observed REA fluxes in evergreen sclerophyll *Quercus ilex* stand at Castelporziano (Fig. 13.4)

	Algorithm			
Validation statistic	Model 1 (Guenther, standard)	Model 3 (ETR, seasonal ε)	Model 4 (ETR + fitted ε(T)-function)	Model 5 (C-ratio)
Explained variance (r^2)	0.69	0.74	0.72	0.70
Modelling efficiency (N_E, Eq. 13.8)	0.67	0.69	0.68	0.69
Mean absolute error (σ_A, Eq. 13.9)	0.88	0.95	0.91	0.99
Root mean square error (σ, Eq. 13.10)	1.22	1.22	1.26	1.21
σ to observed standard deviation ratio (σ_{NSD}, Eq. 13.11)	0.56	0.47	0.47	0.55

Model validation statistics and tested models as in Table 13.2. In the case of Guenther et al. model, the model version with $E_{S,max}$ (Eq. 13.1) derived from the REA flux measurements by inverse modelling was used (Fig. 13.4a). The performance of dynamic models (models 6–8, Sect. 13.2.2.3) was not analysed

and monoterpene synthases that are responsible for pathway flux (Schnitzler et al. 1997; Lehning et al. 2001). However, temporal changes in enzyme activities may also be confounded by stress-related decreases in the emission rate (Sharkey and Loreto 1993; Bertin and Staudt 1996; Staudt and Bertin 1998), e.g., via changes in the reduced carbon input and limitations due to electron transport or nitrogen availability. Moreover, changes in the environmental conditions may directly trigger alterations in enzyme activities (Sharkey et al. 1999; Geron et al. 2000). In the current study, this complex array of responses was modelled by empirical functions (Eqs. 13.1 and 13.B3). All models reproduced the relative dynamics well and similar to the diurnal variability, the models realistically ($r^2 > 0.69$, $N_E > 0.69$) described the seasonal dynamics of monoterpene emission (Figs. 13.1d and 13.4, Table 13.3).

However, in the case of Guenther et al. model, this high model efficiency was only achieved when the maximum emission factor ($E_{S,max}$, Eq. 13.1) was derived from the REA fluxes by inverse modelling. This may reflect differences in parameterization of Guenther et al. and "physiological" models. For the "physiological models", the seasonal maximum monoterpene emission rate is determined both by the photosynthetic activity and either by the fraction of electrons going to monoterpene emission (ε, ETR model) or by the ratio of monoterpene emission to photosynthesis (r_C, C-ratio model). As the photosynthetic activity is described by a separate model, predicted monoterpene emissions are less sensitive to ε or r_C parameterization than is the Guenther et al. model to accurate parameterization of $E_{S,max}$ (Eq. 13.1). In fact, when an independent estimate of $E_{S,max}$ from leaf-level measurements in different plants was used, the Guenther et al. model significantly overestimated the emissions (Fig. 13.4a, Table 13.3), resulting in low

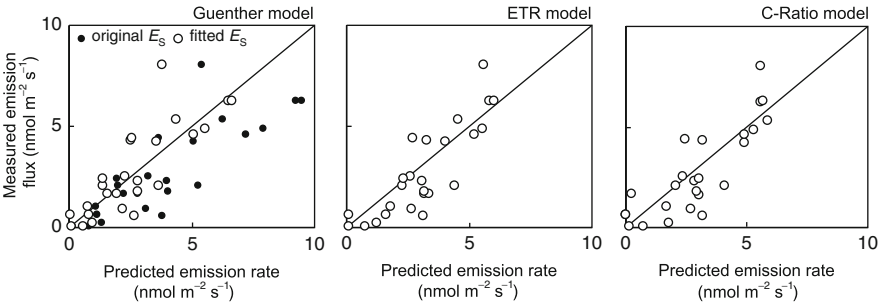

Fig. 13.4 Correlations between the observed (relaxed eddy accumulation, REA, Fig. 13.1d) and modelled monoterpene emission fluxes from *Q. ilex* forest at Castelporziano. The simulations in (**a**) were conducted with the Guenther et al. model (model 1, Sect. 13.2.2.3) either using an $E_{S,max}$ value (Eq. 13.1) estimated from the independent parameterization dataset or deriving an $E_{S,max}$ estimate by inverse modelling using the REA flux data. In (**b**) the emission flux was simulated by the standard ETR model with seasonality (model 3) and in (**c**) by the C-ratio model (model 5). The 1:1 lines are also provided

modelling efficiency (Eq. 13.8) of 0.29 and large mean absolute error (Eq. 13.9) of 1.5 nmol m^{-2} s^{-1}, despite that the explained variance was similarly high ($r^2 = 0.70$) as for the other models.

The mean absolute difference between the modelled and observed fluxes is still about one third of the average flux, suggesting that further improvements of the models, e.g., via more advanced description of underlying physiological mechanisms, might be necessary. With the current modelling schemes, some improvement of model predictions may possibly also be achieved by more detailed parameterization of the vertical variation in foliage physiological characteristics in the layered canopy model (e.g., Lenz et al. 1997; Niinemets et al. 2010b).

Only the ETR model with seasonality (model 3) and C-ratio model (model 5) were calibrated independently of the observations. In the other version of the ETR model (model 4), no seasonality was included, but the temperature dependency of ε was optimized with respect to the observed data, resulting in a very steep exponential dependency of ε on T ($Q_{10} = 7$). Given that experimental values of Q_{10} are below 4 (Niinemets et al. 2002c), the Q_{10}-value determined from a single fit to all data is unrealistically high. Because of higher basal emission rates in summer relative to winter and spring, very high Q_{10} values are possibly attributable to confounding effects of temperature and seasonality on ε. Thus, if we had included the seasonality function as for the other model version, the same Q_{10}-value of 3.15 derived from the data of Niinemets et al. (2002c) could successfully be used for the ε(T) function. This underscores the importance of clearly separating processes that are due to acclimation and lead to changes in the basal emission rate and instantaneous temperature responses (Niinemets et al. 2010a for an extended discussion).

13.3.3 Implications of Model Parameterization for Estimating Landscape-Level Fluxes from a Hemiboreal Forest

Both model approaches, standard Guenther et al. (model 1) and optimized Guenther et al. (model 2) were applied to estimate the monthly average maximum (between 10 and 14 h) monoterpene emission rate from conifer *Pinus sylvestris* and *Picea abies* dominated ecosystems for July 2010 (Fig 13.5d, e). *Picea abies* is a more shade-tolerant species and *P. abies* dominated pixels supported a greater leaf area index (LAI) than the pixels dominated by less shade-tolerant *P. sylvestris* (Fig 13.5d, e). Guenther et al. model with standard parameters for the light and temperature dependencies (model 1) led to higher average daytime emissions than the optimized Guenther et al. model (model 2) (Fig 13.5d, e). The mean normalized deviation between both parameterizations was about 10 % (Fig. 13.5f), but the spatial variation in model deviations was large with the greatest deviations found at pixels with higher LAI. As during the summer months, the daytime temperature remained most of the time between 22 and 27 °C, the difference between the models mainly reflects differences in light parameterization among the two approaches. Especially at places with a high LAI, the change in light will be more prominent than the change in temperature. Strong dependence on LAI also implies that in more shade-tolerant *P. abies* dominated areas with greater LAI, the deviation among the two model approaches was greater than in less shade-tolerant *P. sylvestris* dominated areas with lower LAI.

Overall, this simulation further emphasizes the importance of accurate parameterization of emission models. Differences in model parameterization not only result in biased site average estimates, but also can importantly alter the spatial distribution of emissions. While in this simulation, the bias was of the same direction (sign) across the landscape, both negative and positive deviations can potentially occur for different parameterizations and for more extended temperature range. This would lead to false impression of accurate model prediction when testing against integrated values such as ecosystem-level emission flux measurements by eddy covariance or REA flux measurements. Thus, comparisons of models at different spatial resolution can provide important insight into the performance of emission algorithms (e.g., Ashworth et al. 2010).

13.4 What Model to Choose: Outlook

13.4.1 What Model Performs the Best?

The model intercomparisons presented here demonstrate that models with widely differing structure and mechanisms can be successfully parameterized to effectively predict canopy monoterpene emissions. Once the normalized emission, E_S, was

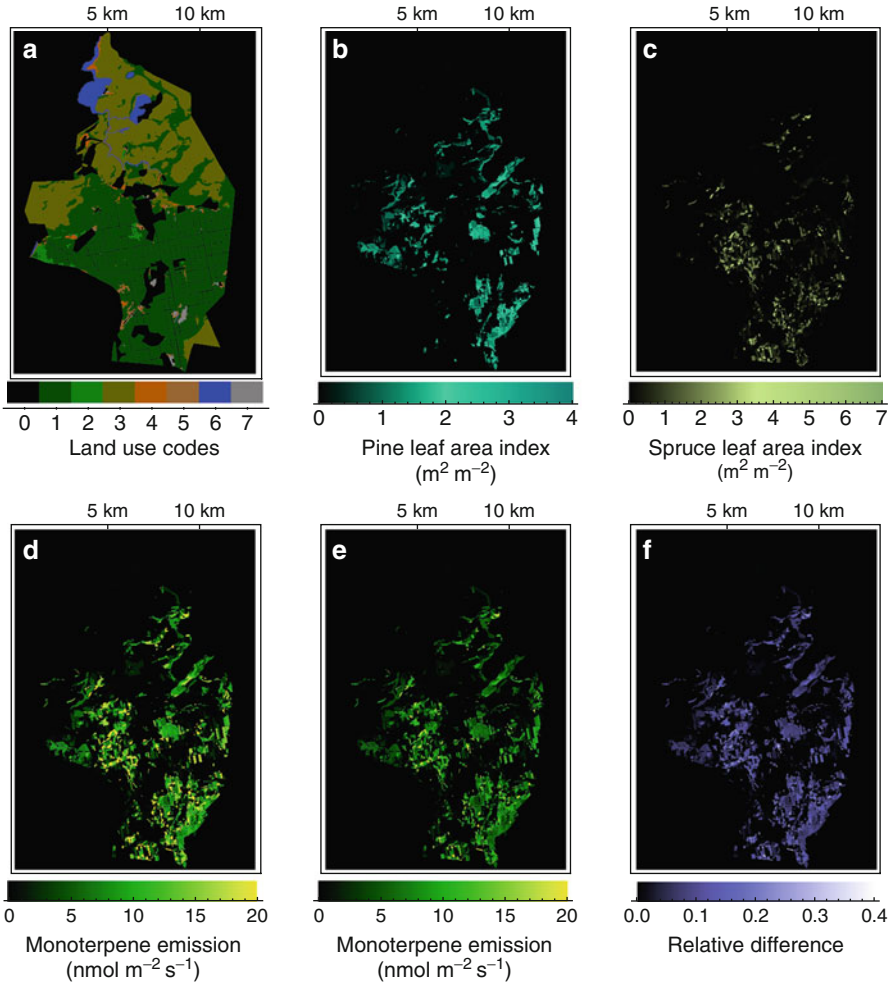

Fig. 13.5 Comparison of monoterpene emissions at landscape scale. The emissions were predicted by Guenther et al. model with original static parameterization (model 1, Sect. 13.2.2.3) and optimized parameterization (model 2) applied to the mixed hemiboreal mixed forest at Järvselja, south-eastern Estonia (58°25′N, 27°46′E) (for detailed site description Noe et al. 2011, 2012). The landcover (**a**) is defined as: *0* = no data, *1* = productive forest, *2* = low productive forest, *3* = swamps and bogs, *4* = shrublands, *5* = grasslands, *6* = waterbodies, *7* = other (croplands and suburban habitats). Panel (**b**) demonstrates the leaf area distribution in Scots pine (*Pinus sylvestris*) and panel (**c**) that for Norway spruce (*Picea abies*). Leaf area index together with species-specific emission factors and incident light and temperature were used to simulate average monthly maximum (10–14 h) monoterpene emission rate in July 2010 according to the original (**d**, model 1) and optimized (**e**, model 2) parameterizations. The difference between the monoterpene emissions estimates by two different models was scaled to the maximum deviation between both models and is demonstrated in (**f**)

correctly described, all models reproduced the seasonal and diurnal dynamics of emission rate with minor differences among model predictions. The circumstance that different models have not been necessarily re-parameterized or the scale of models has not appropriately considered, has been a flaw in many model comparison exercises. Thus, several past comparisons have not necessarily done justice to some of the models simply because of inconsistent parameterization (Niinemets et al. 2010c for a discussion). The bottom-line of this intercomparison (Figs. 13.2, 13.3 and 13.4) is that if all models are parameterized in a consistent manner, it becomes difficult to say which model performs the best.

Overall, the model validation statistics all yielded similar information about the performance of the model. Nevertheless, r^2 was highest for the ETR model that was significantly biased at lower values of emission rate (Figs. 13.2 and 13.3, Table 13.2). In contrast, the greatest values of modelling efficiency in the optimized storage model and optimized Guenther et al. model were associated with the lowest bias in terms of mean absolute and mean squared error (Figs. 13.2 and 13.3, Table 13.2). Thus the modelling efficiency together with estimates of model bias clearly are more informative indicators of model performance than r^2, and they should be routinely included in BVOC model intercomparison exercises.

Another principal difficulty with testing model algorithms against fluxes integrated or averaged over large spatial areas such as REA fluxes is that such tests are only valid if vegetation is homogeneous. In the case of non-homogeneous vegetation (Fig. 13.5) similar fluxes can be predicted with different models, even when the fluxes are differently distributed across the landscape. However, non-homogeneity can also amplify the overall deviation if the bias is in the same direction across the landscape (Fig. 13.5). Thus, analysis of the spatial distribution of deviations among the models can provide important additional insight into model performance.

13.4.2 What Model to Prefer?

Given the similar performance of different models, we suggest that the preference of any one particular model over others depends on the availability of data for model parameterization. The Guenther et al. model is well-established, and its parameterization requires only monoterpene emission measurements. Moreover, as implemented in previous studies, many of the required parameters could be considered as constant for all plants, albeit, as our analysis demonstrates, at the expense of model predictability (Table 13.2). On the other hand, the model results, especially for seasonal predictions, are very sensitive to accurate estimation of the maximum emission factor. Despite this limitation, the Guenther et al. model will be a preferred model if only emissions need to be calculated and when no information of foliage physiological activity is available.

From a different perspective, if the monoterpene emission routine needs to be implemented in a model already predicting stand carbon and water fluxes, linking

the emissions to foliage photosynthetic characteristics is recommended. Especially, because the emission predictions in such models are less sensitive to an independent estimate of emission capacity, and also may more efficiently capture the effects of physiological processes such as stomatal closure on the emission rates. The primary limitation of "physiological" models is that the exact physiological mechanisms of the regulation of the emission rate are currently still not entirely understood. Nevertheless, even very simple models such as the C-ratio model (Box 13.1) performed remarkably well.

The dynamic model that considers non-specific storage of monoterpenes was one of the best models. The dynamic models qualitatively differ from the other models by predicting significant night fluxes of emissions and by moderating the effects of rapid light and temperature fluctuations (Niinemets and Reichstein 2002; Noe et al. 2006, 2010). The model has been successfully validated at the leaf scale (Niinemets and Reichstein 2002; Noe et al. 2006, 2010), and although the validation statistics confirm superior performance of this model at the canopy scale, stand-level monoterpene fluxes cannot be effectively measured by eddy covariance technique at night (e.g., Fisher et al. 2007 for a discussion of eddy flux methodology). Also, the time-resolution of eddy-flux measurements, typically averaged for 30 min. to reduce fluctuations inherent to eddy technology (e.g., Aubinet et al. 2000) is too crude to effectively compare the two best models, optimized Guenther (model 2) and optimized dynamic (model 6). Thus, there clearly are experimental limits for statistical validation of different models (see also the chapter of Guenther 2013 for further discussion on model comparison). Nevertheless, combining canopy models to air chemistry models and air reactivity, O_3 and $NO_3^{\cdot}$ and $OH^{\cdot}$ radical measurements may provide indirect ways to validate night emission fluxes (Di Carlo et al. 2004; Ortega et al. 2007; Sinha et al. 2010).

Given that non-specific storage may be relatively easily implemented in any emission model, and that it may have a potentially large impact on air chemistry, we suggest that future canopy-level emission models should consider non-specific storage. Overall, the existing BVOC emission algorithms are plastic enough to be parameterized to predict emissions with a high degree of accuracy, and the choice between different models is not necessarily dictated by inherent differences in model performance, but rather by practical decisions as driven by the modelling application. This does not mean that wholly mechanistic models should not be used when the emission mechanisms become fully elucidated, but simply indicates the state-of-the-art of BVOC modelling that has reached to a certain level of convergence of different models (Arneth et al. 2008; Ashworth et al. 2013; Guenther 2013 in this volume).

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Chapter 14
Upscaling Biogenic Volatile Compound Emissions from Leaves to Landscapes

Alex Guenther

Abstract The implementation of biogenic emissions in regional air quality and global climate models requires numerical code and input datasets that are compatible with these regulatory and scientific tools. Canopy- and landscape-level emission models can be developed using a scaling up approach where emissions are first calculated on a leaf scale and then scaled up to higher scale using a canopy model that describes the environmental conditions in different canopy locations. Alternatively, big-leaf models can be used that simulate canopy emissions based on the physiological potentials of uppermost leaves in the canopy. Finally, with development of flux technology for measurement of whole canopy emission fluxes, canopy-level emission models have been derived that simulate the emissions on the basis of whole canopy environmental responses. Here the potentials and limitations of different model frameworks are compared and perspectives for future model developments are offered.

14.1 Introduction

After several decades of ozone pollution control strategies met with little success, the US air quality community began to rethink the ozone problem in the late 1980s (NRC 1991). An outcome of this was a heightened appreciation of the role of biogenic volatile organic compounds (BVOC) in ozone production. It eventually became clear that accurate, time varying, gridded BVOC emission estimates were required for air quality models (Pierce et al. 1998). The demand for quantitative BVOC emission estimates for global Earth system models has recently been

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stimulated by studies suggesting a major role of BVOC emissions in controlling aerosol production (Spracklen et al. 2011; Kulmala et al. 2013 in this volume). While BVOC emission modelling approaches have improved in the past decades, it is clear that uncertainties associated with these estimates are still considerable and may be limiting the development of effective air quality and climate management strategies.

At the heart of any BVOC emission model are the leaf-level algorithms and parameters that simulate BVOC emissions from an individual leaf over a wide range of conditions. As described in earlier chapters (Grote et al. 2013; Li and Sharkey 2013; Monson 2013), progress in our ability to describe the processes controlling leaf-level emissions have led to the development of more robust leaf-level algorithms that can account for a substantial part of the BVOC emission variations in response to temperature, solar radiation, soil moisture, and ambient CO_2 concentration. These previous chapters also demonstrate that these algorithms are still not perfect and more work is needed to fully account for the observed variations in BVOC emissions. In addition, the lack of quantitative algorithms to account for stress and other factors that are not included in current models (Niinemets 2010) limits regional model capabilities for quantitatively simulating BVOC emissions.

Accurate leaf-level BVOC emission algorithms are necessary, but not sufficient, for estimating the emission inputs needed for regional to global models. The upscaling from an individual leaf to a whole canopy and then on to an entire landscape is a challenging task that likely dominates the overall uncertainty in regional to global emission estimates. Section 14.2 describes the first step in this process: model approaches for estimating canopy-scale emissions. The second step discussed in Sect. 14.3 is the characterization of the above-canopy environment. The final step described in Sect. 14.4, landcover characterization, completes the task of providing inputs for regional air quality and global Earth system models. Section 14.5 considers the accuracy of BVOC emission models while Sect. 14.6 address questions associated with similarities and dissimilarities in reported BVOC emission model estimates.

14.2 Canopy Environment

Most BVOC emissions are sensitive to changes in leaf temperature and some are also controlled by visible light. Since different leaves within a canopy are exposed to varying light and temperature conditions, the microclimate of the canopy environment must be considered for models of canopy-scale emissions. An obvious implication of the within-canopy variability is that there are much lower light levels on shaded leaves. This is illustrated in Fig. 14.1 along with another important consequence which is the higher leaf temperatures on sunlit leaves and the cooler temperatures on shaded leaves. Given the non-linear light and temperature responsiveness of most BVOC emissions (Grote et al. 2013; Monson 2013), the

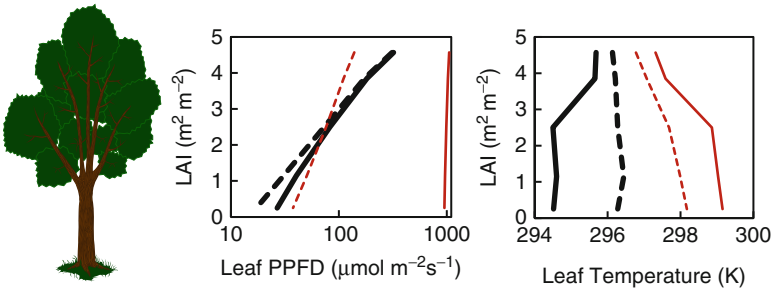

Fig. 14.1 Within-canopy variations in incident leaf quantum flux density (PPFD) and temperature for sunlit leaves (*solid line*) and shaded leaves (*dashed line*) on cloudless (*thin red lines*) and cloudy (*thick black lines*) days

overall impact can be complex and varied for different conditions and canopy types. Of particular importance is that the response of isoprene and other light-dependent BVOC emissions saturates at high light levels. This means not only that assuming above-canopy light levels for the whole canopy would overestimate emissions, by not accounting for the reduced emissions by shaded leaves, but also that the use of a canopy average light level for the whole canopy would overestimate emissions by failing to account for the saturated conditions on sunlit leaves. The two basic approaches used to account for canopy microclimate, bulk (Sect. 14.2.1) and explicit (Sect. 14.2.2) environment approaches, are described below.

14.2.1 Bulk Canopy Environment Approach

The first BVOC emission models scaled to whole vegetation, including Zimmerman (1979) and Lamb et al. (1987), used a bulk canopy approach. This entailed using emission factors and emission response algorithms that were representative of the average of a range of sun and shade leaves. The canopy was treated as a single entity with a canopy-scale emission factor and a canopy-scale emission response. These canopy-scale values were conveniently provided by the branch enclosure measurement techniques that were commonly used at that time (Zimmerman 1979). A branch typically includes a range of sun and shade leaves and so represents a microcosm of the whole canopy. The first temperature and light algorithms were based on whole-plant measurements made in a gas-exchange chamber (Tingey et al. 1981). These algorithms were therefore representative of BVOC emission response of a whole canopy, rather than individual leaves, and so were appropriate to apply using a bulk canopy environment approach, albeit scaling from small plants and single branches to extensive tall canopies inherently necessitates the use of certain correction factors to account for greater environmental gradients and within-canopy variations in emission potentials in mature canopies.

Guenther et al. (2006) used an explicit canopy environment model, with a leaf energy balance model, to simulate the whole canopy emission response to light and temperature for a wide range of conditions and canopy types. The results were used to develop a bulk canopy emission algorithm that was called the Parameterized Canopy Environment Emission Activity (PCEEA) approach. This was done to provide modelers with an option with a lower computational expense. The PCEEA bulk canopy temperature algorithm was similar to the leaf-level temperature algorithm of the explicit canopy model but was slightly less sensitive to temperature. This was because the PCEEA bulk approach accounted for the cooling effect of transpiring leaves. The PCEEA light response algorithm for isoprene was a function of canopy leaf area index (LAI) and photosynthetic photon flux density (PPFD). Emissions increased nearly linearly up to an LAI of 2 but became saturated around an LAI of 3. The PCEEA algorithm increased emissions nearly linearly with PPFD up to full sunlight in contrast to the leaf-level light response where emissions saturated at about half of full sunlight.

In addition to branch/plant measurements and explicit canopy model simulations, a bulk canopy model approach can be based on canopy-scale flux measurements. For example, Schade et al. (1999) used above-canopy flux measurements to develop a numerical description of monoterpene response to temperature and humidity. The increasing availability of above-canopy BVOC flux data has primarily been used to evaluate explicit canopy models, but should also be used as a resource for parameterizing bulk canopy environment models.

The bulk-canopy BVOC emission approach is analogous to big-leaf models of plant photosynthesis (Sellers et al. 1992; Amthor 1994; de Pury and Farquhar 1997), and inherently suffers from potential integration errors due to the spectrum of different light intensities leaves in the canopy receive at any moment of time. This can be overcome by using a two-big-leaf approach, where part of the canopy foliage is sunlit and part is shaded, thereby significantly improving integration of the fluxes (de Pury and Farquhar 1997; Dai et al. 2004).

14.2.2 Explicit Canopy Environment Approach

The recognition that temporal and spatial variations in canopy structure (e.g., leaf area index, species, leaf inclination angles, leaf clumping) and physiological functioning (e.g., maximal stomatal conductance, photosynthetic capacity) control carbon, water and energy fluxes has led to the development of explicit models for quantifying canopy distributions of leaf solar irradiance (e.g., Baldocchi et al. 2002). These models simulate the extinction of solar radiation passing through the canopy using approaches as simple as assuming a logarithmic decrease with canopy depth to models that account for leaf orientation, clustering, penumbra and other effects including three dimensional variability. This subject has been thoroughly reviewed by Cescatti and Niinemets (2004) and readers are referred to this paper for a detailed description of these methods and how they have been

applied to simulate canopy photosynthesis and respiration. This section focuses on the use of explicit canopy environment models to characterize biogenic VOC emissions.

Lamb et al. (1993) introduced the first explicit canopy environment model for estimating BVOC emissions. Their approach was based on the model of Gates and Papian (1971) that was developed to quantify the solar radiation and energy budgets of plant canopies at multiple layers in order to estimate canopy-scale photosynthesis and transpiration. This explicit canopy environment model divides the canopy into multiple vertical layers and estimates leaf-level solar radiation and temperature at each level. Since this approach assumes constant light levels across a given layer, Beer's law can be applied to assume a logarithmic dependence between transmission of global solar radiation through the canopy and the product of an extinction coefficient and LAI as

$$Q_{\mathrm{d}} = Q_0 e^{-k\mathrm{LAI_d}} \tag{14.1}$$

where Q_{d} is the solar radiation at a given canopy depth, Q_0 is the solar radiation above the canopy, $\mathrm{LAI_d}$ is the LAI above the given canopy depth and k is an extinction coefficient that depends on wavelength and canopy structure. Extinction coefficient is greater for visible (photosynthetic, PAR) radiation and smaller for near-infrared (NIR) radiation that penetrates deeper into the canopy and thus, for modelling light effects on BVOC emission fluxes, it is important to partition the solar radiation flux between PAR and NIR components.

In addition to vertical variations in canopy light distribution, there are substantial differences in the light levels on leaves at the same canopy depth. Even in the interior of the canopy, sunlit leaves receive full sunlight due to gaps in the canopy, albeit often for short periods of time (sunflecks). In contrast, shaded leaves do not receive direct sunlight. Any given leaf can change from being a sunlit leaf to a shaded leaf, or the other way around, throughout the day depending on the sun angle and the location of other leaves and cloudiness conditions. Guenther et al. (1995) introduced the sunlit and shaded leaf approach for BVOC emission modelling using the Norman (1982) canopy model which used an approach for estimating the sunlit foliage portion within each canopy layer and calculating the direct and diffuse components of solar radiation incident to sunlit and shaded leaves.

Lamb et al. (1993) were the first to use a leaf energy balance model, to calculate the difference between air temperature and leaf temperature, in an explicit canopy approach for estimating biogenic VOC emissions. A portion of incoming global solar radiation, Q_{abs}, is absorbed along with incoming longwave radiation, $R_{\mathrm{IR,in}}$, from the surrounding environment. As shown in Eq. 14.2, this energy is balanced by outgoing energy which includes sensible heat (R_{S}, convective and conductive heat fluxes), latent heat (R_λ) from transpiration and outgoing longwave radiation ($R_{\mathrm{IR,out}}$) radiated away from the leaf. Fluxes R_{S}, R_λ, $R_{\mathrm{IR,in}}$ depend on leaf temperature, and R_{S} and R_λ also on boundary layer conductances for conductive, convective and water vapour exchange, while $R_{\mathrm{IR,in}}$ depends on surface temperature, in particular, on sky temperature.

$$Q_{\text{abs}} + R_{\text{IR,in}} = R_{\text{IR,out}} + R_{\text{S}} + R_{\lambda} \tag{14.2}$$

An increase in the incoming energy will increase leaf temperature, and thus outgoing energy flux, until the leaf energy fluxes are in balance. The numerical calculation of the leaf energy balance is typically accomplished using iterative numerical solutions which must be efficient or else will result in substantial computational expense.

There are significant uncertainties in approaches for estimating each of the terms in the leaf energy balance shown in Eq. 14.2. Q_{abs} is calculated as the difference between the incoming global solar radiation and the global solar radiation that is reflected back or transmitted (scattered flux). In addition to the uncertainties in estimating the incoming solar radiation on a leaf, there are plant to plant differences in leaf reflectance and scattering coefficients (Goudriaan and van Laar 1994). In addition, accurate estimation of Q_{abs} requires distribution of solar radiation between PAR and NIR components and this partitioning importantly depends on cloudiness conditions that affect the PAR/NIR ratio of global solar radiation (Ross and Sulev 2000). The calculation of $R_{\text{IR,in}}$ is dependent on determination of the fraction of the leaf that is exposed to nearby (1) sun leaves, (2) shade leaves, (3) sky and (4) soil since each of these objects has a different temperature. Estimating the incoming infrared radiation from the fraction exposed to the sky requires an estimate of sky temperature, which is typically not readily available. Leaf sensible heat flux and latent heat flux calculations require heat transfer conductances that depend on wind conditions and plant structure and physiological status. Estimations of these characteristics can introduce some uncertainties (Leuning et al. 1995).

14.2.3 Canopy Model Comparisons

Lamb et al. (1993) compared BVOC emissions estimated using an explicit canopy model with emissions simply calculated as the product of leaf-level emission factor and canopy leaf area (no canopy model). They found that isoprene emissions, which are light- and temperature-dependent, were decreased by a third while monoterpene emissions, which were only temperature-dependent, were decreased by 6 % (Lamb et al. 1993). However, if an emission factor based on branch-level measurements were used with a bulk canopy approach instead of a leaf-level emission factor, there would be little difference in emissions between the two approaches; this is because branch-level emission factors are about a third less than leaf-level emission factors (Guenther et al. 1994).

Above-canopy isoprene fluxes were used to evaluate different canopy models by Lamb et al. (1996) including (1) a bulk canopy approach, (2) a simple explicit model (Lamb et al. 1993), and (3) a more detailed explicit model (CANOAK, Baldocchi and Harley 1995). The Lamb et al. (1993) approach decreases light levels exponentially through the canopy, while CANOAK accounts for the impact of leaf clumping on radiative transfer and the influence of turbulent diffusion on

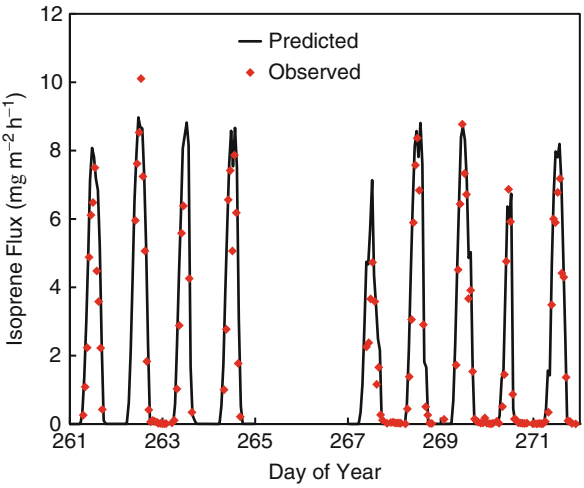

Fig. 14.2 Comparison of eddy covariance observations (*grey line*) and MEGAN model estimates (*red dots*) of isoprene emissions from an Amazon tropical forest canopy. Eddy covariance measurements were conducted using proton-transfer reaction mass spectrometry (PTR MS) and the MEGAN model estimates were simulated using an LAI of 6, an isoprene emission factor of 5.9 mg m^{-2} h^{-1} (Modified from Karl et al. 2007)

canopy exchange. The results showed that the fluxes estimated with the three models were consistent to within about 20 %. The models initially overestimated the mean flux by a factor of two but could be brought into agreement by adjusting the emission factor and biomass density to values that were within the uncertainty range for these factors. It is remarkable that the Lamb et al. (1996) study appears to be the only comparison of different canopy environment models that includes an evaluation with above-canopy flux data. The above-canopy isoprene flux dataset used for the Lamb et al. canopy model comparison consisted of only about 80 relaxed eddy accumulation (REA) and gradient flux measurements (Guenther et al. 1996) which is very small compared to the number of measurements available from more recent eddy covariance studies (e.g., Pressley et al. 2005). Figure 14.2 shows an evaluation of modelled isoprene and monoterpene emissions from a tropical forest canopy (Karl et al. 2007). The results demonstrate the agreement between model estimates and eddy covariance measurements by a proton-transfer reaction mass spectrometer (PTR MS). Partial correspondence between measurements and simulations as demonstrated in this study is often observed when site-specific parameterizations are available (Niinemets et al. 2013). While the model describes the general observed behavior, there are a number of details that are not captured by the model. Overall, we note that it is difficult to achieve full correspondence between the model and measured estimates due to inherent uncertainties in both model algorithms and parameterization and in BVOC flux measurement techniques (Niinemets et al. 2013).

Guenther et al. (2006) conducted a sensitivity study with a range of input values using a simple (BEIS, based on Lamb et al. 1993 and updated by Pierce et al. 1998) and a more detailed (MEGAN, based on Guenther et al. 1995 and updated by Guenther et al. 1999) explicit canopy environment model. The differences were typically within 30 % but were greater in some cases. The impact of

different explicit canopy environment models on estimated isoprene emissions was investigated by Keenan et al. (2011) who found differences exceeding a factor of two and concluded that the large differences in estimated sunlit and shaded leaf area fractions were largely responsible for the observed discrepancies. They also demonstrated that the difference in estimated emissions was highly dependent on the leaf emission algorithm used in the comparison. On the other hand, the discrepancies between different emission algorithms can be greatly reduced by appropriate re-parameterization of given algorithms (Keenan et al. 2009; Niinemets et al. 2013), an approach that is often avoided by BVOC modelers who prefer to use "default" parameterizations.

14.3 Above-Canopy Environment

An accurate simulation of BVOC emissions requires an accurate characterization of the above-canopy environment. This is usually straightforward for canopy-scale flux estimates at a specific site, since direct measurements are typically available, but is considerably more challenging for landscape-scale emission modelling, especially in regions where few observations are available. The above-canopy environmental variables needed include wind and humidity, which can influence leaf temperature, but the most important drivers are soil moisture, solar radiation and temperature. Weather data for driving BVOC emission models are readily available from many different sources including interpolated observations (http://www.cru.uea.ac.uk/data, http://www.metoffice.gov.uk/hadobs/), model predictions (http://www.cesm.ucar.edu, https://esg.llnl.gov:8443/), and model reanalysis that assimilate observations by nudging the model simulation towards the observed value (http://www.esrl.noaa.gov/psd/data/gridded/data.ncep.reanalysis.html, http://www.ecmwf.int/research/era/do/get/index).

Guenther et al. (2006) examined the sensitivity of global annual total isoprene emissions by driving a global model with five different temperature and solar radiation databases and found that global isoprene emissions ranged from −14 to +15 % of the standard case which used the NCEP reanalysis. More importantly, they reported regional differences in isoprene emissions of up to a factor of 3 when using different weather driving variables. Three different global BVOC emission models were compared by Arneth et al. (2011) who also found substantial regional differences in isoprene emissions associated with different temperature and solar radiation inputs. They noted that the impact of changing the weather data differed among the different BVOC emission models. Guenther et al. (2006) and Muller et al. (2008) used the same algorithm, but different soil moisture data, to estimate the influence of soil moisture on annual global isoprene emission. Muller et al. (2008) estimated a 20 % decrease in isoprene due to the soil moisture effect, while Guenther et al. (2006) calculated only a 7 % decrease. This was primarily due to uncertainties in predicting soil moisture. However, even if accurate soil moisture estimates are available, the exact "soil moisture effect" is difficult to simulate because limited

soil water availability can initially increase emissions, while a severe drought can greatly reduce the emissions (Calfapietra et al. 2013; Monson 2013).

Wang et al. (2011) investigated the BVOC emission model uncertainties associated with weather inputs derived from a regional weather model (MM5) simulation of the Pearl river delta in China. By comparing the MM5 output with observations, they determined an average overestimation of $\sim 2^{o}$C for temperature and $\sim$120 W m^{-2} for downward shortwave radiation. These errors are similar to the error values typically observed in weather model simulations in the eastern US (Hanna et al. 2005). Wang et al. (2011) attributed the errors to a lack of aerosol impacts on solar radiation in MM5. These model input errors were associated with errors in predicted isoprene emission fluxes of 23 % due to temperature bias and 45 % due to solar radiation bias. The impact on monoterpene emissions was 17 % due to temperature and 19 % due to solar radiation. Even regional models that account for aerosol impacts on solar radiation, such as WRF (Grell et al. 2005), have difficulties in accurately predicting downward solar radiation. This is primarily due to the challenge of accurately predicting cloud cover as well as difficulties in evaluation of the scattering characteristics of different types of clouds. Accurate prediction of global solar radiation is a problem even in apparently "clear-sky" conditions. Guenther et al. (2012) compared North American isoprene emissions estimated using solar radiation simulated by WRF and solar radiation measured by satellite. The WRF driven estimates were overestimated by 37 % even for "clear sky" cases. They concluded that WRF could not resolve the thin high-level baroclinic shield of cirrostratus or altostratus occurring at 6–9 km above sea level. The situation is even more complicated under cloudy and partially cloudy conditions.

Because the BVOC emission response to temperature and solar radiation is non-linear, BVOC emissions are sensitive to the temporal and spatial resolution of weather input data. For example, the temperature response of most BVOC emissions is exponential. The arithmetic average temperature will underestimate emissions and neglect the high emissions that occur during even a short period of high temperature (e.g., Niinemets et al. 2011). Ashworth et al. (2010) found that global annual isoprene is reduced by 3 % when using a daily average temperature, and 7 % when using a monthly average temperature, instead of using an hourly average temperature. The impact was much greater on local scales with reductions of up to 55 % when using monthly rather than hourly data (Ashworth et al. 2010). Correspondence between spatial and temporal scales is a key issue in modelling (Jarvis 1995) that still receives less consideration than it deserves.

Coarse spatial resolution can also introduce errors due to arithmetic averaging. This was tested by running the MEGAN model (Guenther et al. 2012) over a mountainous domain in eastern Tennessee and western North Carolina in the US. Figure 14.3 shows that the variations in elevation in this region led to large temperature differences, and thus, emission activity differences of more than a factor of four, and yet increasing the spatial resolution from 100 to 1 km^2 had a fairly small impact (−4 %) on isoprene emissions when a constant landscape average emission capacity was assumed. However, Fig. 14.3 shows that the landscape in

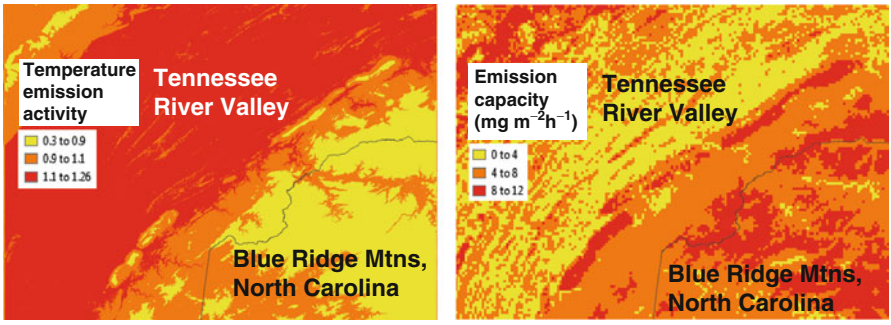

Fig. 14.3 Temperature- and landcover-driven variations in isoprene emissions in a region of variable elevation along the Tennessee and North Carolina, US border for July 2000. The emissions were simulated with MEGAN2.1 (Guenther et al. 2012)

this region is not homogeneous with respect to isoprene emission capacity. The higher (and cooler) elevations tend to have a much higher fraction of oaks which are high isoprene emitters. As a result of the negative correlation between low temperatures with high isoprene emission capacities, there was a 12 % decrease in isoprene emission when spatial resolution was increased from 100 to 1 km^2. An even greater decrease (20 %) in total BVOC emissions with increasing spatial resolution was estimated for central Colorado, USA, where the higher (cooler) elevations are covered by higher emitting forests and the lower (warmer) elevations support lower emitting grasslands.

BVOC emission models are typically driven by a downward solar radiation value from a model or observation. If an explicit canopy model is used, then emission estimates are sensitive to the decomposition of the above-canopy solar radiation into direct vs diffuse and PAR vs NIR components. The uncertainties in the values used to parameterize radiative transfer above and within the canopy make a significant contribution to the overall uncertainties in BVOC emission estimates (Guenther et al. 2012). For example, BVOC algorithms typically require solar radiation inputs in units of μmol photons m^{-2} s^{-1}. Since atmospheric values are typically in units of W m^{-2}, a conversion factor between quantum and energy units is required. Reported values for different sites and conditions range from less than 4 to greater than 5 μmol J^{-1}, and accurate conversion factor cannot be derived without information of solar radiation spectrum (Ross and Sulev 2000). This uncertainty in the PPFD conversion factor leads to an uncertainty in isoprene emissions of about ±13 %. In addition, the value for diffuse PPFD, especially for clear-sky conditions, is considerably less than that for direct PPFD. MEGAN2.1 (Guenther et al. 2012) accounts for this by using different values, 4.6 μmol J^{-1} for direct PPFD and 4.3 μmol J^{-1} for diffuse PPFD. As shown in Fig. 14.4, the decomposition of PPFD into direct and diffuse fractions is also difficult. The updated approach of Guenther et al. (2012) in MEGAN2.1 yields 10–50 % higher estimate of diffuse PPFD than that of Guenther et al. (2006) under cloudy skies and more than a factor of two

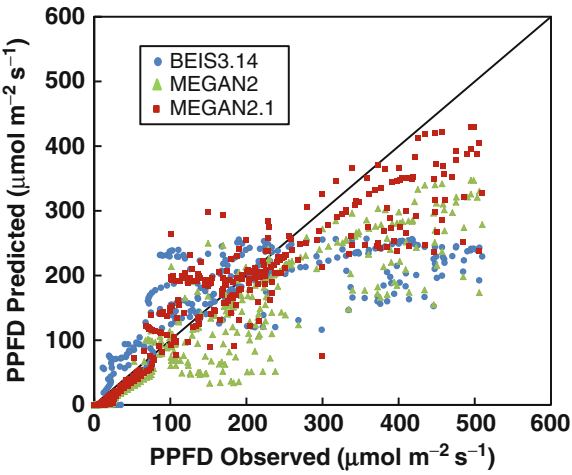

Fig. 14.4 Comparison of diffuse quantum flux density (PPFD) observed at Boulder, CO, USA on June 28, 2008 and predicted using model approaches of BEIS3.14 (Based on Pierce et al. 1998), MEGAN2 (Guenther et al. 2006), and MEGAN2.1 (Guenther et al. 2012). *Solid black line* indicates 1:1 agreement between the observed and predicted diffuse quantum flux density

higher estimate under clear-sky conditions. A higher fraction of diffuse light can increase isoprene emissions by increasing light penetration to shade leaves, as at any moment of time, diffuse light can penetrate through all gaps in the canopy, while direct light only though the gaps that are on the solar beam path. However, since the Guenther et al. (2006) leaf-level algorithms assume that shade-adapted leaves are not very responsive to increases in PPFD, a 25–50 % increase in diffuse PPFD results in only 5–10 % increase in isoprene emissions under cloudy skies and a factor of two increase in diffuse PPFD under clear skies results in only ~5 % increase in emissions. The impact could be greater using other canopy environment models. For further discussion on the global modelling uncertainties the reader is referred to the chapter of Asworth et al. (2013) in this volume.

14.4 Landcover

The pioneers of BVOC emission modelling had few options for obtaining landcover data for estimating regional- to global-scale BVOC emissions. The available scaling approaches consisted of simply multiplying a branch-level emission to a rough estimate of the regional or global total biomass (e.g., Rasmussen and Went 1965). The resulting emission estimates are surprisingly similar to the output of current models that use detailed emission algorithms, emphasizing the important role of amount of biomass in determining canopy, region and global estimates of BVOC emission.

BVOC emission models have assumed that foliage is the dominant BVOC source and scaled BVOC emissions to an estimate of the amount of foliage, either LAI or foliage mass per ground area. Foliage mass was used in earlier studies because it was easier to measure. As a result, most leaf and branch emission

measurement data were normalized to leaf dry weight. Representative data on standing foliage biomass were available for broad ecosystem types and constant values were assigned based on literature compilations (e.g., Box 1981). Seasonal variations in foliage amount could be predicted using empirical algorithms driven by precipitation and temperature (e.g., Lieth and Box 1977). The availability of satellite observations of ecosystem greenness in the late 1980s provided a potentially better alternative for quantifying LAI variations within a given vegetation type. The initial data based on Advanced Very High Resolution Radiometer (AVHRR, http://nsidc.org/data/avhrr/), a weather satellite, had relatively large uncertainties and so they were used to drive monthly variations, but the peak LAI was still a constant assigned to each ecosystem type (e.g., Guenther et al. 1995).

The deployment of improved satellite landcover sensors and several decades of refining satellite algorithms provides more confidence in satellite landcover products although considerable uncertainties remain especially due to saturation of reflectance-based information at relatively low LAI values and difficulties in estimating spatial aggregation of foliage from remote sensing products. Current global LAI products include the NASA MODIS data (http://modis.gsfc.nasa.gov/data/) and the ESA SPOT/VEGETATION data (http://www.spot-vegetation.com). Garrigues et al. (2008) compared these two products with ground observations and found that each product performed better in some ways. SPOT generally agreed better with observations from lower LAI ecosystems such as shrublands and savannas, but MODIS estimates were superior in high LAI ecosystems such as forests. MODIS is available globally for 2003 to present while the SPOT LAI is currently only available from October 2009 to present. A significant advantage of remote sensing products over other approaches is the potential to characterize LAI changes associated with disturbances and later regrowth. For example, Fig. 14.5 illustrates satellite-based observations of the decreased LAI associated with wildfires and bark beetle outbreaks in Colorado. Satellite derived seasonal variations in LAI should also be an improvement over empirical algorithms, e.g., in wet tropical regions where light limitations may be more important. Although initial satellite databases provided monthly average data, higher-resolution data, e.g., 8-day data, are now widely available. However, the higher-resolution data availability will depend on cloudiness and air clearness conditions in given areas, limiting the use of these data for some areas or for some periods such as periods of rain or vegetation burning. Monthly and ten day average MODIS LAI data are compared in Fig. 14.6. The 8-day data often appear to be relatively noisy and so may not present a significant advantage over monthly data. However, Fig. 14.6 also shows that the 8-day data capture some features in landscapes dominated by deciduous vegetation, especially fast growing plants such as some crops.

Regional to global land surface models quantify the variability in landscape characteristics by using either a landcover approach or a plant functional type (PFT) approach. The landcover approach categorizes entire landscapes (e.g., mixed forest, savanna, mixed woods and urban) while the PFT approach categorizes the individual components that occur within a landscape (e.g., temperate cool conifers,

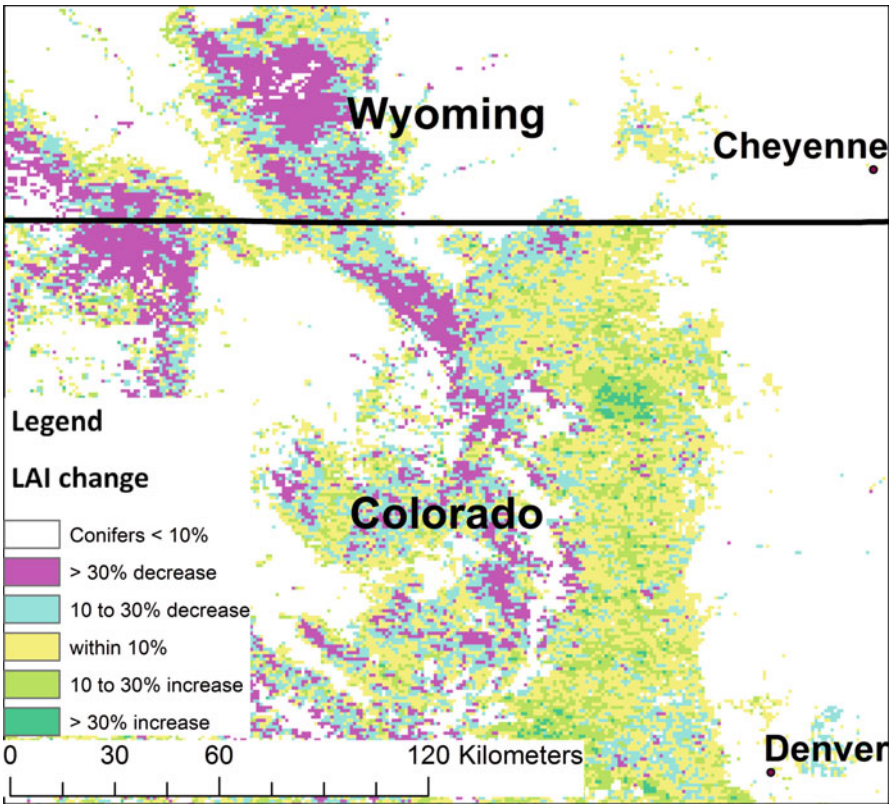

Fig. 14.5 Percent change in July 1–8 leaf area index (LAI) of Colorado Rocky Mountain forests between years 2003–2005 compared to years 2010 and 2011 based on MODIS satellite data products used as MEGAN2.1 input data (Guenther et al. 2012). Areas of increased LAI may indicate forest regrowth after wildfires, while areas of reduced LAI may indicate areas impacted by mountain pine beetle (*Dendroctonus ponderosae*) or other disturbances. State borders and city names are shown for reference

C_4 grasses). Guenther et al. (1995) assigned isoprene and monoterpene emission factors to the 72 landcover types in a global database. Most of these landcover categories did not represent specific isoprene or monoterpene emission types, for example, temperate deciduous forests do not all have the same isoprene emission capacity. Other, e.g., coastal mangrove category with limited number of species, more closely represented specific isoprene or monoterpene emission categories. Since the measurement data on isoprene and monoterpene emissions were only available at locations representing half of these landcover types, there was no need for a more detailed approach. Wang and Shallcross (2000) converted the Guenther et al. (1995) landcover type emission factor scheme into the PFT approach that was becoming increasingly common in Earth system models. The availability of

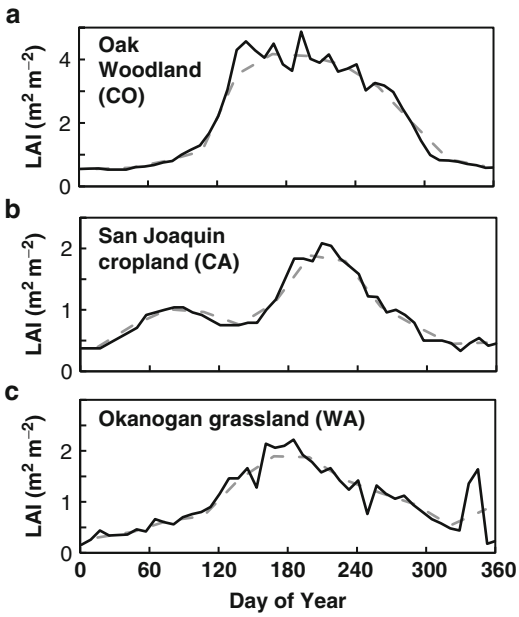

Fig. 14.6 Comparison of monthly (*dashed thick grey line*) and 8-day (*solid thin black line*) average leaf area index (LAI) for three regions of the western US in year 2008. The leaf area index data were derived from MODIS satellite data products used as MEGAN2.1 input data (Guenther et al. 2012)

high-resolution satellite-based landcover data and additional BVOC measurements enabled some advancement for BVOC emission modelling in the following decade. Guenther et al. (2006) devised a flexible global framework that integrated the PFT and landcover approaches with a high resolution ($\sim$1 km^2) suitable for regional modelling. Each location was associated to one of several thousand ecosystem types. In addition, the fraction of each of 6 PFTs were assigned to each location. For regions where data were available, quantitative tree inventories were combined with species-specific emission factors. This was implemented in the model through gridded emission factor maps for individual compounds for each PFT type (e.g., an emission factor map for isoprene emissions from broadleaf trees). This approach combined a large number of landcover and emission measurement data which made it difficult to reference the source of information for the emission factor assigned to each location. Guenther et al. (2012) extended this approach to 16 PFTs to make it more compatible with Earth system models. Since the Guenther et al. (2012) model also has 19 emission categories, over 300 gridded emission factor maps would be required to specify emission factors for each emission and PFT type. Future approaches should extend the PFT scheme to a larger, but limited (<50), number of emission types. This would facilitate efforts to provide more transparency in describing the basis for each emission factor. It may also be beneficial to link the parameterizations to dynamic vegetation models. This would make it possible to predict changes in emission categories through vegetation succession, e.g., from greater isoprene emissions in early successional forests towards greater prevalence of monoterpene emissions in late-successional forests (Harrison et al. 2013).

14.5 Assessing the Accuracy of BVOC Emission Models

BVOC emission modelling began more than 50 years ago with a simple calculation that Went (1960) outlined to characterize the potential contribution of BVOC emissions to petroleum formation. A decade later, Rasmussen (1972) asked the question "what do the hydrocarbons from trees contribute to air pollution?" and addressed it by integrating a forest inventory with species-specific emission factors for isoprene and α-pinene. The result suggested that biogenic VOC emissions from just forests were about six times greater than anthropogenic sources. Zimmerman (1979) and Winer et al. (1982) used branch enclosure emission measurements to characterize emissions from important North American species and integrated these into regional BVOC emission estimates. Gridded landcover data were generated for specific locales (e.g., San Francisco Bay area, southwestern Virginia) and emission inputs were calculated for ozone model simulations (Salop et al. 1983). The US EPA began using time varying, gridded BVOC emissions for regional air quality modelling in 1986 (Pierce and Waldruff 1991). The procedures were adapted from Lamb et al. (1987) which had a domain limited to the continental US and included three emission categories: isoprene, α-pinene and other non-methane hydrocarbons. Five wildland landcover types were used including oak forest, other deciduous forest, coniferous forest, scrubland, and grassland. Many different crop types were included in the model, although they only accounted for 3 % of the estimated emissions. An uncertainty of 210 % (about a factor of three) was associated with these BVOC emission estimates based on the propagation of uncertainties in emission factors, emission algorithms, amount of biomass, and land use distributions. However, this uncertainty estimate neglected many of the components that are now routinely found in BVOC emission models. For example, the Lamb et al. (1987) isoprene emission variations due to changes in solar radiation were simulated by assigning zero emissions at night and assuming constant solar radiation during the day.

The first US EPA biogenic emission model, called BEIS (Pierce and Waldruff 1991), was released in 1988. The second version of the model (BEIS2), released in the mid 1990s, predicted dramatically different estimates of isoprene emission rate, by about a factor of five higher than BEIS (Pierce et al. 1998). BEIS and BEIS2 differed in many aspects including leaf-level emission algorithms, biomass densities, and landcover distributions. However, the major driver of the difference was the emission factors which were based on Guenther et al. (1994) rather than Zimmerman (1979). Guenther et al. (1994) concluded that the Zimmerman (1979) measurements underestimated isoprene emission factors through the use of shaded branches and overestimated monoterpene emission factors due to disturbances associated with the measurement technique. As discussed in Sects. 14.2, 14.3 and 14.4, there are a number of individual BVOC emission model components that each contribute uncertainties of 10–30 %. These uncertainties, if they are all in the same direction, can add up to a factor of two or more. And yet, the emission factor remains

the dominant contributor and can result in uncertainties of a factor of five or greater in regions were emissions from the dominant vegetation are not well characterized.

Hanna et al. (2005) used a Monte Carlo probabilistic approach to estimate uncertainties associated with BEIS3 BVOC emission model outputs and their impact on regional ozone concentrations. The assessment considered the area-averaged emission factor which integrates both plant species specific emission factors and plant species composition, nine emission algorithm parameters, and three model inputs (LAI, temperature and solar radiation). The 95 % confidence range on the calculated uncertainty in isoprene emission was about one order of magnitude, while the calculated uncertainty for monoterpenes and other BVOC was only ±20 %. This is contrary to what is expected since our understanding of isoprene emission is greater than that of other BVOC. The reason for their assignment of higher uncertainty to isoprene seems to be that there were more parameters associated with the isoprene emission algorithm. This emphasizes the need to consider not only the uncertainties due to the factors that are considered in BVOC emission models, but also the potentially larger uncertainties associated with processes that are not considered in BVOC emission models.

While comparisons of BVOC emission models provide little information about the accuracy of these models (see also Niinemets et al. 2013), the availability of independent observations can inform us. Comparisons of canopy-scale fluxes and emission models tend to agree within ~30 % when site-specific parameters are used (Lamb et al. 1996), while comparisons of canopy-scale flux measurements when scaled to regions or globe and compared with regional and global model output often differ by a factor of two or more (Muller et al. 2008; Arneth et al. 2011). It should be noted that these are not direct comparisons due to the difference in scale between a canopy flux measurement and the resolution of a global model.

Aircraft measurements provide the means to directly evaluate BVOC emissions models and have the potential to dramatically improve assessments of the accuracy of landscape average emissions. One approach is to use ambient concentration measurements and infer the fluxes required to maintain the observed concentration distributions. The limitation of this approach is the requirement for accurately describing chemical losses and dispersion. Warneke et al. (2010) used an extensive aircraft database to examine the performance of two BVOC emission models, BEIS3 and MEGAN2. They concluded that MEGAN2 isoprene emissions tended to be higher than the observations and BEIS3 isoprene emissions tended to be lower, but both models were within the factor of two uncertainty of the measurement approach. In addressing the question of whether this should be considered a "good" agreement, Warneke et al. (2010) point out that anthropogenic emission estimates are often off by more than a factor of two. Karl et al. (2009) have successfully demonstrated a PTR MS eddy covariance flux measurement approach that can provide high-resolution BVOC flux measurements. Aircraft flux measurements systems can be used to accurately quantify BVOC emission fluxes at the scales required to evaluate regional and global models.

Satellite-based estimates of formaldehyde distributions over specific regions have been used to evaluate BVOC emission models (Barkley et al. 2009;

Stavrakou et al. 2009; Marais et al. 2012). However, the uncertainty associated with the satellite approach is ~40 % for high NO_X regions and 40–90 % for low NO_X regions (Marais et al. 2012). In most cases, satellite-based estimates are within ~50 % of BVOC emission models which indicates good agreement, especially given the uncertainties associated with the two approaches (Stavrakou et al. 2009; Marais et al. 2012). This also gives us some confidence that regional-scale isoprene emission estimates are within ~50 % of the "true" value, although there are exceptions (e.g., Barkley et al. 2009).

Our limited ability to quantify the accuracy of BVOC emissions precludes a detailed quantitative assessment of BVOC emission model uncertainty, but local canopy flux tower data, regional aircraft concentration distributions, and global satellite-based emission estimates all suggest that isoprene emission estimates are usually within a factor of two of the "true" emission flux. Higher uncertainties are expected for specific locations where the isoprene emission capacities of the dominant vegetation are unknown and also for regions impacted by stress. The impact of this uncertainty on the accuracy of ozone simulations is highly dependent on the chemical regime of a given region. BVOC uncertainties are important in BVOC-sensitive regions but less important in other areas. The growing recognition of the role of BVOC in secondary organic aerosol formation will increase the requirement for more accurate BVOC emission estimates. The best approaches for accurate assessment of regional BVOC emission rates are based on airborne direct eddy covariance flux measurements. These measurements have advanced from relaxed eddy accumulation (Greenberg et al. 1999) and variance (Karl et al. 2004) techniques to direct eddy covariance methods that can be applied at very high (~2 km^2) resolution (Karl et al. 2009). More widespread application of aircraft eddy flux techniques, which would benefit from the development of an approach for low cost light aircraft, could enable verification of BVOC emission models and provide observations for improving parameterizations.

14.6 Why Are Estimates of Global Isoprene Emissions So Similar (and Why Is This Not So for Monoterpenes)?

The large difference in BEIS and BEIS2 isoprene emissions, discussed in the previous section, emphasized the high uncertainty in these model estimates. In contrast, not all comparisons of BVOC emission models should be expected to reflect the uncertainty in the emission estimates since the models may be based on the same or similar parameterizations and approach. Arneth et al. (2008) found that the annual global isoprene emission estimates reported by 15 studies were "surprisingly" similar, and yet the monoterpene emission estimates were quite different, and posed the question "Why are estimates of global terrestrial isoprene emissions so similar? (and why is this not so for monoterpenes)?". They noted that the standard deviation in these isoprene emission estimates was only a little more than 10 % of the mean value. They recognized that part of the reason was

that all of the models used at least some of the algorithms or emission factors from Guenther et al. (1995), but they expected larger differences due to the various driving variables used by the models. Table 1 of Arneth et al. (2008) lists the annual global emission estimates of the 15 studies and states that the same global value (503 Tg C year^{-1}) was reported by both Guenther et al. (1995) and Guenther et al. (2006). This is incorrect as Guenther et al. (2006) only report a range of values for different simulations and give an approximate value for their base case which is ∼10 % higher than the Guenther et al. (1995) estimate. While not exactly the same estimate, this is still a small difference and does not significantly change the standard deviation of the results from the 15 studies.

Arneth et al. (2008) note that the range in the annual global isoprene emission estimated by the 15 studies, 189 Tg C year^{-1}, is similar to the range reported by Guenther et al. (2006) for results using a single model with 24 different sets of driving variables. The variability in the model simulations listed in Table 4 of Guenther et al. (2006) is 13 % which is similar to that reported for the 15 studies by Arneth et al. (2008). The answer to the first part of the question seems to be that all 15 studies used the same general approach with similar emission activity algorithms and emission factors. While driving variables can result in large differences for a specific location and time, they tend to result in differences of ∼15 % on the annual global scale.

In addressing the second part of their question, Arneth et al. (2008) conclude that "There is no apparent reason that the spread in monoterpene emission rates should be so much larger compared to isoprene emission rates." They go on to argue that BVOC emission modelling is in the "illusion phase: a lack of observations prevent independent model evaluation and the models have the propensity to not depart greatly from previously published estimates", but that the divergence of the monoterpene emission estimates indicates that monoterpene emission modelling has advanced to the "chaos phase where model results diverge freely, reflecting more candidly the lack of observational constraints and of true process modelling". Another explanation is simply that, as shown in Fig. 14.7, light-independent monoterpene emission estimates scale almost linearly with foliar biomass, while the isoprene emission estimates become saturated at certain LAI due to increasingly stronger light-limitation on emissions. If we eliminate the three studies reporting lower monoterpene emission rates (Levis et al. 2003; Naik et al. 2004; Schurgers et al. 2009) in Table 1 of Arneth et al. (2008) then the variability (∼9 %) is less than that for isoprene. The difference between the Levis et al. (2003) and Guenther et al. (1995) estimates of foliar biomass in tropical forests results in about a factor of three difference in monoterpene emissions, but a relatively small difference in isoprene emission.

The difficulty with monoterpene emissions is that there are light-independent emissions from storage in species such as conifers, and light-dependent emissions in species lacking storage structures such as many broad-leaved emitting species (Grote et al. 2013; Niinemets et al. 2013). This is complicated further by the presence of both emission behaviors in some species (Taipale et al. 2011). Schurgers et al. (2009) showed that the difference in monoterpene emission algorithm used

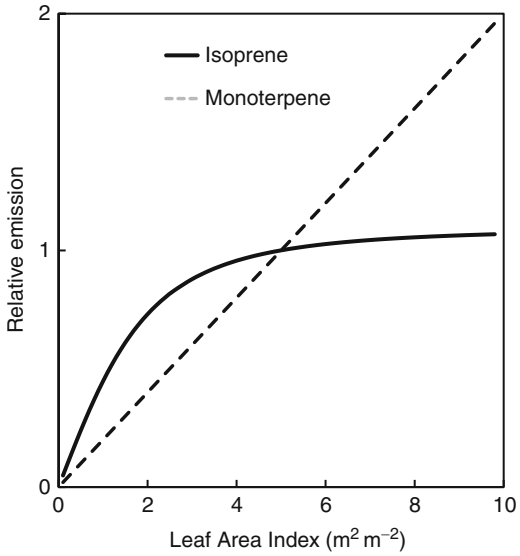

Fig. 14.7 Isoprene and light-independent monoterpene emission responses to vegetation leaf area index (LAI) simulated by MEGAN2. Emission is normalized by the emission at a LAI = 5

resulted in only a 7 % difference in emissions, although clear separation of species into storage and non-storage monoterpene emitters (Fineschi et al. 2013; Grote et al. 2013; Niinemets et al. 2013) and consistent landcover categories might be difficult. In addition, differences in emission factors appear to play a small role since Levis et al. (2003), Schurgers et al. (2009), and Naik et al. (2004) values were also based on the emission factors of Guenther et al. (1995). There are some apparent errors in the way in which Naik et al. (2004) and Schurgers et al. (2009) adapted these emission factors. Guenther et al. (1995) included categories for tropical seasonal forest and drought deciduous landscapes. The drought deciduous landscape was intended to represent shrublands but Naik et al. (2004) used this value for tropical broadleaf drought-deciduous trees and Schurgers et al. (2009) used it to estimate emissions from tropical broadleaf raingreen trees. These errors did not result in large differences in global emission rate estimates, but if they had we should not take it as an indication that the models are advancing to an improved state where they are diverging freely. That would only be the case if the models were diverging because of some new understanding such as improved emission algorithms, emission factors or approaches for upscaling emissions.

The statement that the lack of variability in BVOC emission estimates could create the illusion of overly accurate BVOC emission estimates is a valid concern although the tendency for the atmospheric chemistry modelling community to modify BVOC emissions by 20–50 % (e.g., Houweling et al. 1998, Poisson et al. 2000; Ehhalt and Prather 2001), presumably because they are considered one of the more uncertain parts of the model system, would seem to indicate otherwise. In addition, there is a potential for model deviations to introduce unintended errors. An example is the use of the Guenther et al. (1995) tropical forest emission factors in models with different canopy environment models and foliar densities. The

leaf-level emission factors recommended by Guenther et al. (1995) were based on estimates of above-canopy concentrations, i.e., they represent the leaf-level emissions required to give the observed above-canopy flux, assuming a certain canopy environment model. Thus, these emission factors are only valid when used with the Guenther et al. (1995) canopy environment model and foliar biomass. Even for temperate and boreal regions, where the Guenther et al. (1995) emission factors were based on leaf enclosure measurements, there was a potential confusion since some models assumed that the emission factors were for sun leaves (Guenther et al. 1995), while others assumed a canopy average value (Pierce and Waldruff 1991). To address this concern, Guenther et al. (2006) introduced a canopy-scale emission factor so that, at least for standard environmental conditions, the emission rate estimates of different models would be the same regardless of the canopy model and LAI used. Disadvantages of this approach include obscuring the importance of an accurate representation of canopy environment, which is needed for simulating emission variations, and creating a false confidence in the accuracy of BVOC emission models. A solution to this shortcoming is the reporting of both leaf- and canopy-scale emission factors and a community effort to expand the observations, including aircraft eddy covariance measurements, for assessing the accuracy of BVOC emission models. Ideally these data would be available in a model testbed that would streamline the process of testing and evaluating BVOC emission modules against measurements over a wide range of spatial and temporal scales. This testbed would consist of a suite of tools that allows extracting and comparing the predicted BVOC fluxes or concentrations with a wide range of field measurements and/or previous model predictions. The performance of new BVOC emission treatments could then be quantified and compared to existing treatments before they are used to assess the impacts of BVOC on regional air quality and the global Earth system.

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Chapter 15
Scaling Emissions from Agroforestry Plantations and Urban Habitats

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Abstract Agroforestry plantations and urban habitats contribute importantly to atmospheric volatile compound fluxes in densely populated areas. Simulation of emissions from such habitats is associated with several key challenges, including high spatial heterogeneity due to habitat fragmentation and high diversity of planted tree species. On the other hand, plants in urban habitats and in agroforestry plantations commonly receive more nutrients and water than species in natural communities, resulting in higher production and potentially greater capacity for volatile production per unit of land area. This chapter reviews the strategies for simulation of biogenic volatile organic compound (BVOC) fluxes from urban habitats and agroforestry plantations and provides an outline for parameterization of volatile emission models for densely populated areas with high vegetation fragmentation and large number of gardened, often exotic, tree species.

15.1 Introduction

In 2008, the global urban population exceeded the non-rural population for the first time in history (United Nations 2008), and is currently more than seven billion people. The increasing demands of a growing world population for food, fibre, fuel, water, and shelter, is causing rapid land-use changes with global declines in

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natural forests and increases in agroforestry and urbanization (Foley et al. 2005). It is predicted that by 2050, 70 % of the world's population will live in towns or cities (Seto and Shepherd 2009). The ecological and environmental impacts and implications of rapid land-use change are well documented in the research literature (e.g., Foley et al. 2005 and references cited therein). In this chapter, we focus on the effects of these changes on fluxes of reactive biogenic volatile organic compounds (BVOCs), which can have subsequent impacts on regional tropospheric chemical reactions related to ozone and aerosol formation. Although the total urbanized area is still relatively small, BVOC emissions from urban green areas significantly contribute to the air quality of urbanized areas (Owen et al. 2003) with immediate effects on human health. Furthermore, rapid expansion of urbanized environments, agroforests and crop plantations implies that the effects of anthropogenic landscapes to total emission budgets are expected to increase in the future. Urban environments and agroforest have their specific environmental limitations that may significantly differ from natural environments, and species compositions can also vastly differ. Therefore, separate consideration of BVOC emissions from urbanized environments and agroforests is warranted.

15.2 Importance of Urbanized Areas and Agroforests for BVOC Emissions

15.2.1 Global Landcover of Urban Areas, Plantations and Natural Forests

An overview of landcover of natural and plantation forests and urban land in the major continents is shown in Table 15.1. Although the land footprint of cities occupies less than 0.5 % of the Earth's total land area (Schneider et al. 2009), cities make a large contribution to the fluxes of reactive gases to the atmosphere. Consequently, they are an important source term in the modelling of regional- to global-scale biogeophysical processes such as atmospheric chemistry, redistribution of atmospheric nitrogen oxides and aerosol production (Atkinson 2000). The urban heat island effect influences local- to regional-scale climates (Quattrochi and Ridd 1994), and sensible and latent heat fluxes are modified by impervious surfaces (Offerle et al. 2006), which probably affects precipitation regimes (Shepherd 2005). Despite the growing importance of urban land area in regional- to global-scale environmental issues, estimates of urban coverage vary with mapping method, and the complexity and homogeneity of the urban land surface mosaics can propagate large uncertainties (Schneider et al. 2009). Alvey (2006) states that "the urban forest, which includes vegetation along urban streets and in urban parks, woodlots, abandoned sites, and residential areas, can comprise a significant percentage of a nation's tree canopy", suggesting that this may amount to 25 % of mainland US total tree canopy cover (Dwyer et al. 2000; Alvey 2006). As the result of rapid urbanization worldwide, the importance of urban forests is expected to increase (Alvey 2006).

Table 15.1 Estimates of landcover (in 10^6 km^2) for natural forests, forest plantations and urban areas

	Total forest area (2010)	Forest annual change rate (%) (2000–2010)	Planted forest area (2010)	Planted forest annual change rate (%) (2000–2010)	Urban area (2009)
Africa	6.74	−0.49	0.154	+1.75	0.069[a]
Asia and Pacific	7.40	+0.19	1.20	+2.85	0.215
Europe	10.1	+0.07	0.693	+0.6	0.149
Latin America and Carribean	8.91	−0.46	0.150	+3.23	0.095
Near East	1.22	+0.07	0.151	+1.49	
North America	6.79	+0.03	0.375	+2.46	
World	40.3	−0.13	2.64	+2.09	0.13

The data for forests and forest plantations are from FAO (2011), while the urban area data are from Schneider et al. (2009) that provides MODIS satellite data at 500 m spatial resolution
[a]Includes Near East

Biogenic volatiles can contribute significantly to total hydrocarbon flux from a city. For example, Wang et al. (2010) reported that biogenic isoprene can contribute more than 12 % to the non-methane hydrocarbon flux during August in Beijing. Similarly, Saito et al. (2009) estimated that biogenic isoprene contributed about 40 % of the total non-methane hydrocarbon propylene-equivalent concentration measured in summer in Nagoya, Japan. These biogenic sources of hydrocarbons in a city can contribute in a significant way to the regional atmospheric chemistry. For example, Harrison et al. (2006) estimated that biogenic isoprene removed around 16 % of the available OH in summer at an urban site in Birmingham, UK.

As human population and urbanization increase, so does the amount of land used for agroforestry activities. The world's total forest area is just over four billion hectares (FAO 2010; Table 15.1), representing ∼31 % of total land area. Forest plantations are being established at an increasing rate throughout much of the world, and they now account for >6 % of global forest area (FAO 2011). There are huge regional variations, with the UK having ∼68 % and New Zealand ∼24 % of forest cover as plantation of exotic species. China has ∼16 % and France ∼13 % of forest cover as plantation of native species (Brockerhoff et al. 2008). The species grown in plantations are often fast-growing trees used for biofuel feedstocks and wood pulp, and they are often high isoprene emitters (e.g., species of *Populus*, *Salix*, and *Eucalyptus*; Harley et al. 1999).

15.2.2 Implications of Biodiversity for BVOC Emissions from Urban, Plantation and Natural Forests

Agroforestry plantations and urban areas are considered to be species poor, although this may not necessarily be the case (Brockerhoff et al. 2008). Ecosystem-sensitive

management practices on plantations and greening of towns and cities may result in higher biodiversity than expected, which at times can reach or even surpass the biodiversity found in natural forests (Alvey 2006). Brockerhoff et al. (2008) report that longer-rotation plantation forests, especially those managed with conservation objectives, may differ little from managed natural forests (e.g., Keenan et al. 1997).

The type and amount of BVOCs synthesised and emitted are highly specific to individual plant species, so the species composition of canopies within agroforestry plantations and urban areas exerts a large influence on the type and amount of BVOC flux from that canopy. Changes in plant species composition over a large region due to new plantations or large-scale greening of urban space can therefore have far-reaching impacts on the amount of BVOCs emitted and on the chemical reactivity of these emissions, and hence have major impacts on tropospheric chemistry.

Since the emissions of BVOCs are species-specific, in order to construct a "bottom-up" emission inventory, it is necessary to estimate the species composition for a given habitat or canopy. In the case of an agroforestry plantation, the composition will be dominated by the managed crop species. However, even in this situation, understory or riverine species may make important contributions to the total BVOC emissions, and hence should not be ignored.

In the case of the urban environment, the huge and rather unpredictable diversity of tree species makes the integrated estimation of BVOC emission rates difficult. Sun (1992) used the inverse of the Simpson's diversity index (Simpson 1949) to characterize the urban tree diversity:

$$S_{\mathrm{DI}} = \frac{N\,(N-1)}{\underset{i=k}{\sum_{i=1}} n_{\mathrm{i}}\,(n_{\mathrm{i}}-1)} \tag{15.1}$$

where n_{i} is the number of individuals of species i, k is the total number of species in the particular area, and N is the total number of individuals of all species together. Hence, the greater the S_{DI} the higher the species diversity. The S_{DI} can be considered as the "adjusted" number of species in a population based on species composition (Sun 1992).

Sun (1992) reviewed the S_{DI} of 21 towns and cities, principally in the US and UK, and found that the S_{DI} was less than 10 in 12 cases, and above 20 in one city with a mean S_{DI} of 9.5. This is very high diversity compared with tree diversity in natural forests where the values of S_{DI} are typically between 2 and 3 (Onaindia et al. 2004; Singh et al. 2005; Sharma et al. 2009), and may reach 8–10 in exceptionally species-rich forests in biodiversity hotspots (Gimaret-Carpentier et al. 1998; Sharma et al. 2009). Despite the high diversity, S_{DI} may not be a useful metric when assessing BVOC emissions, since BVOC emissions from individual tree species vary enormously, and in any given urban area, may be dominated by very high emission rates from a few individual specimens belonging to a few species. For example, in Longyan County, Fujian, China, Sun records a total of 1,084 individual trees (N) from 20 different tree species, with an S_{DI} of 6. The most abundant six

species accounted for 941 individuals, with the remaining 143 individuals belonging to 14 species. It is possible that these less common 13 % of individuals might contain some very high BVOC emitting species, although this is impossible to ascertain from currently published information as emission measurements have not been made on most of these species.

Owen et al. (2003) used tree census data for different "urban morphology types" (residential, industrial, transport, open space and commercial) together with landcover maps to estimate the tree species present in the West Midlands conurbation of the UK. However, this statistical representation of species diversity was not tested by field surveys and may not include exotic species which might make a relatively large contribution to overall BVOC emissions. Thus, choosing how many, and which specific tree species to make measurements from is an integral and necessary first step in any attempt to quantify BVOC emission rates from a habitat, especially in the urban environment where the prevalence of exotics may be high.

Nevertheless, the implications of the degree of species richness for BVOC emissions if combined with information on vegetation coverage may still be straightforward. A plantation of a high BVOC-emitting crop species, such as willow (*Salix* spp.), oil palm (*Elaeis guineensis*) or poplar (*Populus* spp.), will result in a canopy flux much greater than natural mixed forest. A highly built-up urban area with few green spaces or plantings will have lower BVOC fluxes than the equivalent areas of natural mixed forest, while an urban area rich with green spaces and gardens might produce BVOC fluxes equivalent to or even exceeding some natural habitats (Owen et al. 2003).

15.2.3 Exotic Species and BVOC Emissions in Urban and Plantation Forests

An "exotic" species is a species growing outside its natural area of dispersal. Richardson and Rejmánek (2011) performed an exhaustive literature survey, presenting a "global list of invasive alien trees and shrubs and discussing taxonomic biases, geographical patterns, modes of dispersal, reasons for introductions and key issues regarding invasions of non-native woody plants around the world". While woody plants were not widely considered to be important invasive alien species until fairly recently, Richardson and Rejmánek (2011) report that thousands of species of trees and shrubs have been moved around the world, and that many have spread from planting sites, and some are now among the most widespread and damaging of invasive organisms. This is relevant because invasive exotics may emit more than natives in the local flora (Llusià et al. 2010).

In an urban environment, it is doubtful whether the foliage and flowers of exotic planted species are likely to alter the regional-scale BVOC flux, although they may do so locally. Widespread garden and park plantings, and the escape and

proliferation of large-flowered exotic species, might result in changes in regional BVOC flux for the short periods of time when such a species flowers (e.g., Baghi et al. 2012), but little work has been done to quantify these effects. For example, the *Rhododendron* species are now widespread in the UK following introduction in the nineteenth century, but to date, it is not known to what extent their foliage or flowers contribute to total emissions of BVOCs. Large-scale monoculture plantings of exotic species in agroforestry plantations have the same potential for contributing to emissions of regional significance as their native counterparts, and the degree to which a plantation species emits BVOCs with potential to cause detrimental changes in air quality should be included in responsible decisions about the choices and extent of agroforestry crops in any particular region.

The issue of exotics in urbanized areas is likely further gaining in relevance, especially in Northern Hemisphere higher latitudes where greatest rise in temperature is predicted in the future with concomitant possibilities of many warmer climate exotic species to be grown in urban areas (Niinemets and Peñuelas 2008).

15.2.4 Management Practices and Their Potential Effects on BVOC Emission

Plants respond to biotic and abiotic stresses using a number of strategies, including a change in BVOC production and emissions. The BVOC emission potential of a non-stressed plant varies, depending on past environmental conditions, plant physiological status, and phenology (Niinemets et al. 2011). Management practices discussed in this section may cause physiological stress in plants for a period of time. Other stresses associated with the urban and agroforestry environments, but not a direct result of management strategies, are discussed in the next section. The directions and magnitude of the BVOC response to any stress depends on plant species, the health of the plant before the onset of stress, and on the severity and duration of the stress, and the potential for synergies among several coincident stress factors (Niinemets et al. 2010a).

Management practices in urban green spaces and agroforestry plantations may include application of inorganic or organic fertilizers, irrigation, and cropping, felling, pruning or mowing. These practices may be responsible for additional emissions, and also restrict the vegetation, e.g., managed to an age limit. The effects that these practices have on BVOC emissions are likely to be plant species specific, and are likely to be different for isoprene and other compounds emitted immediately following synthesis, and those compounds emitted by vapourization from stored pools within the plant tissues (Grote et al. 2013). Some likely responses of BVOC emissions to different management practices are summarised in Table 15.2.

Fertilizers appear to have different effects on BVOC emissions. Results vary with plant species, amount of fertilizer applied and the pre-fertilized status of the growing medium. For example, Blanch et al. (2007) found that fertilizer treatments

Table 15.2 Review of responses of plant BVOC emissions in response to management and other stresses

Management practice/ Stress	Response (Study) Isoprene/ instantaneously emitted terpenes	Monoterpenes from stored tissue pools	Sesquiterpenes	Oxygenated compounds
Fertilizer	↑(4, 5); no change (6)	↓(1) ↑(2, 4)	↑(3, 4)	↑(13)[d]
Irrigation	↑(21)	↑(21)	↑(27); ↓ (27)[c]	↑(25); no change (25)[b]
Cropping, felling, pruning or mowing	↑(26)	↑(9)	↑28	↑(7, 8)
Plant age	↑(12)	↑(10, 11)	↑(33)[c]; no change (32)	↓(34); ↑(34)[b,c]
Drought/desiccation stress	No change (14); ↓(15, 16, 17, 18, 19, 22)	↓(20 23, 31); ↑ 31[a]	No change (31); ↓(24)	No change (25)[b]; ↓(25)
Herbivory stress in plantations	↑ short-term (35); ↓ long-term (35)	↑(29, 30)	↑(29, 30)	↑(29)
Overcrowding/shading	↓(37)	No change (39)[b,e]; ↓(38, 39)	↓(40)	↓ (41)

Studies: (1) Blanch et al. (2007); (2) Blanch et al. (2012); (3) Rinnan et al. (2011); (4) Ormeño et al. (2009); (5) Possell et al. (2004); (6) Funk et al. (2006); (7) Seco et al. (2007); (8) Davison et al. (2008); (9) Räisänen et al. (2008); (10) Kim et al. (2005); (11) Street et al. (1997a); (12) Street et al. (1997a); (13) Hörtnagl et al. (2011); (14) Steinbrecher et al. 1997; (15) Tingey et al. 1981; (16) Sharkey and Loreto (1993); (17) Fang et al. (1996); (18) Lerdau et al. (1997); (19) Brilli et al. (2007); (20) Lavoir et al. (2009); (21) Peñuelas et al. (2009); (22) Pegoraro et al. (2004); (23) Bertin and Staudt (1996); (24) Ormeño et al. (2007); (25) Filella et al. (2009); (26) Brilli et al. (2011); (27) Llusià and Peñuelas (1998); (28) Piesik et al. (2011); (29) Schaub et al. (2010); (30) Staudt and Lhoutellier (2007); (31) Staudt et al. (2008); (32) Agelopoulos et al. (2000); (33) Hakola et al. (2001); (34) Bracho-Nunez et al. (2011); (35) Loreto et al. (2006); (36) Brilli et al. (2009); (37) Harley et al. (1996); (38) Owen et al. (2002); (39) Tarvainen et al. (2005); (40) Staudt and Lhoutellier (2011); (41) Folkers et al. (2008)

[a]Depends on severity of stress
[b]Depends on compound
[c]Depends on plant species
[d]Soil with vegetation
[e]Depends on the site of emission

reduced the monoterpene emissions from *Pinus halepensis* by 40 %, yet Blanch et al. (2012) found five-fold increased monoterpene emissions from phosphorus-stressed seedlings of *Pinus pinaster*. Both of these species emit monoterpenes from tissue pools, and both experiments were conducted on tree seedlings growing in pots. Rinnan et al. (2011) found a stimulation of the sesquiterpene β-selinene from *Salix phylicifolia* growing in field plots, after administering annual additions of NPK fertilizer. This was contrary to their expectations that increased soil nutrient availability would decrease BVOC emissions, assuming an increased allocation of carbon to growth (Bryant et al. 1983; Sorensen et al. 2008). Ormeño et al. (2009) reported that mono- and sesquiterpene basal emissions from *Rosmarinus officinalis* (a terpene-storing species) and *Quercus coccifera* (a non-storing species) increased with a moderate application of organic fertilizer at 50 t ha^{-1}, which the authors proposed were optimal N conditions. Possell et al. (2004) also found that increasing nutrient availability increased isoprene emissions from *Quercus robur* growing in pots. Analogous increases have been observed in velvet bean (*Mucuna* sp.) by Harley et al. (1994) and in white oak (*Quercus alba*) by Litvak et al. (1996). Yet Funk et al. (2006) found that leaf-level emission at a given canopy height did not differ between fertilized and unfertilized 6-year-old *Eucalyptus saligna* forest trees. They proposed that this was linked to the leaf nitrogen content, which also did not vary with fertilizer treatment.

The emission responses to fertilizer treatment at ecosystem scale are even more difficult to predict. For example, Hörtnagl et al. (2011) reported that application of organic fertilizer significantly increased methanol emissions from a grassland. However, these emissions probably reflected enhanced microbial activity in the soil associated with the applied manure (Hörtnagl et al. 2011). A meta-analysis of published results is needed to attempt to tease out the underlying cause and effects, and more manipulation experiments are needed to understand better the role of nutrients, and hence fertilizer treatment, on BVOC emissions not only from the plant tissues, but also from the soil substrate.

Cropping, felling, pruning, mowing etc. can result in a burst of the emissions of a wide range of BVOCs, particularly the oxygenated compounds. In a comprehensive overview of short-chain oxygenated volatiles, Seco et al. (2007) reviewed emissions of formic and acetic acids, acetone, formaldehyde, acetaldehyde, methanol, and ethanol, some of which have been observed as a response to cutting, harvesting or mowing (e.g., Davison et al. 2008; Fall et al. 1999, 2001). Hörtnagl et al. (2011) also reported high emissions of methanol during and after cutting a grassland meadow, which they attributed to the wounding of the plant material and subsequent depletion of the leaf internal aqueous methanol pools. Räisänen et al. (2008) measured higher atmospheric concentrations of monoterpenes at felled sites compared to non-felled sites within a Scots pine (*Pinus sylvestris*) plantation, and considered that the logging residue was the most important factor explaining the elevated monoterpene concentrations. These practices are carried out regularly on agroforestry plantations and within many different urban green spaces. Episodes of maintenance practices will contribute significantly to the BVOC flux from these canopies.

BVOC emissions from agroforestry plantations or urban green spaces may also be modified if these spaces are managed to constrain the age of the vegetation growing there. Niinemets et al. (2011), reviewing a number of studies about the factors affecting BVOC emission potentials, report that age (leaf age, plant age) is one of the factors that can alter species-specific emission potential values by more than an order of magnitude. An emission potential is the emission rate of a particular compound at a standard set of environmental conditions, usually 30 °C and 1,000 μmol m^{-2} s^{-1} light. Guenther et al. (2006) added further parameters to the definition of standard conditions, including leaf age and soil water conditions. The direction in which the age of a plant alters BVOC emission potential is species-specific, and it may also depend on environmental and biotic conditions. For example, Kim et al. (2005) found that total emission rates and speciation of monoterpene compounds varied significantly with tree species, age, and season. In their study, total emissions from *Cryptomeria japonica* and *Pinus koraiensis* were higher for older trees than for younger trees. Yet, significantly higher emissions were found from younger trees for *Chamaecyparis obtusa* (Kim et al. 2005). Street et al. (1997a) found that BVOC emission potential of mature *Pinus pinea* forest trees was twice that of young plantation trees growing nearby in similar environmental conditions ($P < 0.05$). Wiberley et al. (2005) found that in kudzu (*Pueraria lobata*), the age dependency of the capacity to emit isoprene was in turn controlled by environmental growth conditions.

Taking account of leaf age when extrapolating BVOC emissions from leaf- and branch-scale to canopy-scale flux is possible if the species composition and age structure of the canopy is known, and if investigations have already been made on the effect of leaf age on BVOC emissions to provide the species-specific functional relationships. Simulation of changes in isoprene emissions due to age can be made using algorithms which can be found embedded in e.g., the Model of Emissions of Gases and Aerosols from Nature (MEGAN) for global isoprene flux emissions (Guenther et al. 2006, 2012; Guenther 2013). Leaf age is also indirectly embedded in some of the seasonality functions used to characterize seasonal variations in emissions (Grote et al. 2013; Niinemets et al. 2013). These approaches (species-specific measurements and simulation algorithms) are likely to be feasible for monoculture agroforestry plantations, but they can be complex to apply for a highly fragmented urban green-space canopy.

15.2.5 Biotic and Abiotic Stresses and Their Potential Effects on BVOC Emissions

Because most plants are sessile, they need to be able to cope with many changes in their environment, such as light intensity, temperature, water availability and other abiotic factors, as well as biotic factors such as herbivory and competition. When these factors shift out of the normal range, due to management strategies in plantations for example, then plants experience stress that can result in slower

growth rate, impaired reproduction and even death. Environmental and biotic stress can also select for shifts in ecological traits (both short-term and long-term), with potential impacts on biological diversity, ecosystem functioning and carbon sequestration (Vickers et al. 2009). Urban vegetation and trees in particular are likely to experience several different types of stress. For example, street trees may be growing in concrete and paved surfaces where they may be exposed to drought stress, which has different effects on BVOC emissions depending on tree species, type of BVOC emitted, severity and duration of stress, and general conditions of the tree. This is comprehensively reviewed in Laothawornkitkul et al. (2009) and Possell and Loreto (2013). A review of multiple stresses on BVOC emissions is provided by Holopainen and Gershenzon (2010). Urban vegetation may be nutrient stressed because their fallen leaves are swept away and are not allowed to decompose in situ and recycle soil nutrients. They may be exposed to high levels of pollutants, and may even be used as natural pollutant filters alongside roads. Trees and other plants growing in towns and cities are also vulnerable to mechanical damage caused by heavy management strategies and vandalism.

In contrast, trees and other plants growing in agroforestry plantations and gardens are more likely to be protected against stress, as productivity depends upon good growing conditions. Agroforestry managers will aim for optimum fertilizer application and watering regimes. However, planting closer than the prescribed optimum can result in competition for resources, therefore trees and plants in badly managed plantations may be stressed. In most cases, prolonged moderate stress and strong rapid stress result in reduction of the constitutive BVOC emissions for all stresses studied so far (Niinemets 2010; Fineschi and Loreto 2012). Thus, an urban green-space canopy or a plantation canopy suffering any of a range of biotic and abiotic stresses will result a change in magnitude and chemical speciation of the BVOC flux from that canopy. Some effects on BVOC emissions from different stresses likely to be experienced by plants growing in an urban environment and in agroforestry plantations are summarised in Table 15.2 and we also refer to chapters from Calfapietra et al. (2013), Grote et al. (2013) and Possell and Loreto (2013) in this book.

15.3 Estimating BVOC Emission Rates from Urban and Plantation Canopies

An overview of BVOC flux estimates for agroforestry plantation species and urban canopies is presented in Table 15.3. In this section, we discuss some methods, complexities and uncertainties associated with these emission estimates. For monocultures and for canopies with very few species of uniform age, simple extrapolations still can sometimes produce estimates that are comparable with canopy-scale measurements (Guenther 2013). Advanced canopy parameterizations account for light extinction and changes in emission potential through the canopy,

Table 15.3 BVOC canopy-, branch- and leaf-level emission fluxes reported from agroforestry plantations and urban canopies

15.3a Canopy BVOC emission estimates (mg C m^{-2} h^{-1}) for dominant species

Dominant species	Flux	BVOC	Notes	Reference
Acacia nilotica	0.02	Isoprene	[a],[b], Lext, aa	Harley et al. (2003)
Acacia nigrescens	2.7	Isoprene	[a],[b], Lext, aa	Harley et al. (2003)
Elaeis guineensis	0.36	Estragole	E, 24m	Misztal et al. (2010)
Elaeis guineensis	26.5	Isoprene	E, md	Misztal et al. (2011)
Hevea brasiliensis	0.15–1	Monoterpenes	E	Baker et al. (2005)
Picea abies	0.65–1.08	Monoterpenes	Cmod, md	Forkel et al. (2006)
Pinus sylvestris	0.15	Monoterpenes	E, ms	Räisänen et al. (2009)
Pinus sylvestris	0.92	Monoterpenes	[b], E	Räisänen et al. (2009)
Salix viminalis	3.1	Isoprene	[b], C	Olofsson et al. (2005)

15.3b Leaf- or branch-level BVOC emission estimates[c]

Species	Flux	Notes	Reference
Isoprene (μg C m^{-2} h^{-1})			
Acacia nigrescens	11–86	[b], L	Possell and Hewitt (2011)
Eucalyptus globulus	560–3,760	[b], [f], [g], L	Guenther et al. (1991)
Eucalyptus globulus	10,800	[b], [g], L	Monson et al. (1991)
Eucalyptus globulus	3,530–4,410	[b], L	Street et al. (1997b)
Eucalyptus globulus	6,100	[b], [g], L	He et al. (2000)
Eucalyptus globulus	440–1,300	L	Winters et al. (2009)
Eucalyptus saligna	5,400–6,700	L	Funk et al. (2006)
Picea abies	74	[b], L	Forkel et al. (2006)
Populus trichocarpa	9,720	[b], L	Guidolotti et al. (2011)
Populus deltoides	5,400	[b], L	Guidolotti et al. (2011)
Populus × *euramericana*	5,400–6,050	[b], L	Guidolotti et al. (2011)
Populus nigra	3,900–8,200	[b], L	Guidolotti et al. (2011)
Populus deltoides x Populus trichocarpa	2,590	[b], L	Ryan et al. (2009)
Quercus serrata	6,050	[b], L	Tani and Kawawata (2008)
Quercus mongolica	6,050	[b], L	Tani and Kawawata (2008)
Quercus dentata	6,480	[b], L	Tani and Kawawata (2008)
Quercus aliena	3,900	[b], L	Tani and Kawawata (2008)
Quercus robur	650–3,880	[b], L	Lehning et al. (2001)
Isoprene (μg C g^{-1} h^{-1})			
Morus alba	[d,i]1.8–53	[d], [i], L	Singh et al. (2007)
Picea abies	[d]0.01–0.07	[d], L	Filella et al. (2007)
Picea abies	0.6	L, md	Grabmer et al. (2006)
Picea abies	[b,h]0.32–1.7	[b], [h], L	Grabmer et al. (2006)
Picea mariana	[b]6.7	[b], L	Fulton et al. (1998)
Picea sitchensis	[b]11.5	[b], L	Hayward et al. (2004)
Picea sitchensis	0.004–1.3	L	Street et al. (1996)
Populus tremula	[b]45	[b], L	Hakola et al. (1998)
Nandina domestica	[b]17.5–22	[b], L	Winer et al. (1983)[j]

(continued)

Table 15.3 (continued)

Species	Flux	Notes	Reference
Robinia pseudoacacia	[b]118–192	[b], L	Geron et al. (2001)
Salix phylicifolia	[b]28	[b], L	Hakola et al. (1998)
Monoterpenes (μg C m^{-2} h^{-1})			
Eucalyptus globulus	475–6,400	[b], [e], [f], [g], L	Guenther et al. (1991)[g]
Eucalyptus globulus	175–530	[b], L	Street et al. (1997b)
Eucalyptus globulus	2,920–3,320	[b], L	Winters et al. (2009)
Picea abies	300	[b], L	Forkel et al. (2006)
Quercus coccifera	2,350	[b], L	Staudt and Lhoutellier (2011)
Monoterpenes (μg C g^{-1} h^{-1})			
Cupressus forbesii	1.5	[b], L	Arey et al. (1995)
Hevea brasiliensis	1.8–83	[b], L	Wang et al. (2007)
Larix sibirica	4.6–15.4	[b], L	Ruuskanen et al. (2007)
Picea abies	0.09–0.9	[d], L	Filella et al. (2007)
Picea abies	1.8	L, md	Grabmer et al. (2006)
Picea abies	0.5	[b], L	Grabmer et al. (2006)
Picea mariana	2.4	[b], L	Fulton et al. (1998)
Picea sitchensis	2.6	[b], L	Hayward et al. (2004)
Picea sitchensis	0.01–1.9	L	Street et al. (1996)
Pinus sylvestris	0.05–3.3	L	Komenda and Koppmann (2002)
Pinus halepensis	27.8	L	Blanch et al. (2007)
Populus tremula	4.1	[b], L	Hakola et al. (1998)
Salix phylicifolia	0.3	[b], L	Hakola et al. (1998)

15.3c Leaf- or branch-level BVOC emission estimates[c]

Location	Flux or concentration	Notes	Reference
Isoprene (mg C m^{-2} h^{-1})			
Frankfurter Stadtwald, Germany	0.75	C	Steinbrecher et al. (2000)
Houston, USA	up to 0.0.6	C	Park et al. (2011)
London, UK	0.11	C, ma	Langford et al. (2010)
Las Vegas, USA	0.14–0.21	[b], Lext	Papiez et al. (2009)
West Midlands, UK	0.04–1.46	Lext	Owen et al. (2003)
Greater London, UK	1	Lext	MacKenzie et al. (1991)
Tucson, Arizona, USA[k]	2.5	Lext	Diem and Comrie (2000)
Isoprene (ppbv)			
Changping district, Beijing, China	0.7	C	Wang et al. (2010)
Nagoya, Japan	0.7	C	Saito et al. (2009)
28 cities in the US	0.05–2.5	C	Baker et al. (2008)
Bilbao, Spain	0.12	C	Durana et al. (2006)
Birmingham, UK	0.2	C	Harrison et al. (2006)
43 cities in China	0.02–0.8	C	Barletta et al. (2005)

(continued)

Table 15.3 (continued)

Location	Flux or concentration	Notes	Reference
Monoterpenes (mg C m^{-2} h^{-1})			
Frankfurter Stadtwald, Germany	0.02	C	Steinbrecher et al. (2000)
Las Vegas, USA	0.09–0.4	[b], Lext	Papiez et al. (2009)
West Midlands, UK	0.02–0.09	Lext	Owen et al. (2003)
Tucson, Arizona, USA[k]	0.3	Lext	Diem and Comrie (2000)

Notes key
[a]Assuming 100 % canopy cover for the species
[b]Standardised to 30 °C (and 1,000 μmol m^{-2} s^{-1} light for light-dependent emissions)
[c]Emission rates expressed per mass of leaf material cannot be converted to leaf area based emission rates without specific measurements of leaf mass per unit area
[d]At different temperatures
[e]Depending on compound
[f]Depending on leaf age
[g]Cited in Winters et al. 2009
[h]Depending on model used
[i]Field data in different seasons
[j]Cited in http://bai.acd.ucar.edu/Data/BVOC/index.shtml
[k]Urban vegetated residential area
24m – 24 h mean of measured flux, md – mean midday measured flux, ms – mean summer measured flux, ma – mean autumn measured flux, aa – area-averaged, C – canopy, Cmod – modelled canopy; E – ecosystem, L – leaf/branch, Lext – leaf measurements extrapolated to canopy-scale flux

as well as changes in physiology and phenology of the vegetation (Guenther 2013). However, there remain substantial uncertainties associated with extrapolating leaf emission measurements to whole canopy-scale flux which are summarised by Guenther et al. (2006): "(1) a limited understanding of chemical sinks and deposition losses within vegetation canopies (2) artificially disturbed emission rates due to the enclosure, (3) differences between the functioning of individual ecosystem components (e.g., leaves) and the entire ecosystem, and (4) limited sample size within the enclosure (relative to the whole landscape), as well as uncertainties associated with canopy microclimate models themselves." Furthermore, a specific limitation of available BVOC models in agroforest plantations is the high degree of foliage clumping in many fast-growing agroforest species, implying that accurate parameterization of radiative transfer in such canopies would require more complex models (Cescatti and Niinemets 2004). Ideally a combination of bottom-up and top-down approaches to quantifying canopy flux is likely to give the most accurate estimates of BVOC flux and its speciation from an urban or agroforestry plantation canopy.

In general, two different approaches are possible for estimating emissions from plantations or urban habitats. The first method is to construct a "bottom-up" emissions inventory, analogous to that used to estimate emissions from anthropogenic pollutant sources. Until now, this method is by far the most widely used for constructing BVOC inventories. Briefly, it requires the identification of individual

emission sources (i.e., which tree species emit which compounds), an emission factor for each tree species and each BVOC species at standard environmental conditions, per unit leaf area or unit biomass, an "activity" factor (i.e., the amount of leaf area or biomass present), and finally a mathematical description of how temperature, light intensity and all other external factors moderate the "standard" emission rates. These are then combined to give a "canopy-scale" emission rate, which may be moderated by "in-canopy" losses due to chemical reactions or deposition. The "bottom-up" approach with its various ramifications is discussed further in the next section. The second, alternative, method is to use a direct "top-down" canopy-scale measurement of the integrated emission rate from the habitat using one of the several flux measuring methods that are currently being developed, based on an understanding of boundary-layer micrometeorology. Indirect 'top-down' approaches using atmospheric concentrations to infer emission rates by inverse modelling are also possible, utilizing measurement data from aircraft (e.g., NASA's Global Hawk aircraft) or satellites (e.g., GOSAT, SCIAMACHY or OMI) (Ashworth et al. 2013 for a discussion on satellite-based estimates).

15.3.1 "Bottom-Up" Approach

A description of the "bottom-up" approach is given by Keenan et al. (2009b), and summarised here in the most simplified form as:

$$F_c = \sum_{i=1}^{i=k} E_{psi} B_{si} f_e(T, Q, \ldots), \tag{15.2}$$

where F_c is the canopy flux, E_{psi} is the species-specific emission potential (emission factor) per unit dry mass, B_{si} is species biomass and $f_e(T,Q,\ldots)$ is a function describing the effects of environmental drivers on the emission rate. This function routinely includes instantaneous temperature and light effects, but it may also include the effects of CO_2 concentration (Wilkinson et al. 2009), drought, leaf age and other factors depending on the model parameterization (Guenther et al. 2006; Grote et al. 2013). The function $f_e(T,Q,\ldots)$ can be a composite of best-fit environmental dependencies (Guenther et al. 2006), or link the emissions to photosynthetic light (Niinemets et al. 2002) or dark reactions (Martin et al. 2000). The emission model is typically driven by incident light and air temperature, and can consider the canopy as a big leaf or a two-big-leaf with sunlit and shaded leaf area fractions (Dai et al. 2004; Guenther et al. 2006; Keenan et al. 2009a). Models with layered canopy have also been used simulating the vertical variations in temperature, light and E_{psi} (Guenther et al. 2006; Guenther 2013; Niinemets et al. 2013) However, the current key limitation of these models is the lack of information of species-specific variations in emission potentials within the canopy (Niinemets et al. 2010b).

Because these models are driven by light, temperature, and may include water availability and other key environmental drivers, it is potentially possible to run these models under different climate change scenarios (Keenan et al. 2009a; Schurgers et al. 2009; Young et al. 2009). It is also possible to perturb the vegetation data to simulate land-use changes, thereby predicting possible changes in BVOC emissions resulting from future climate, planning and management changes.

Estimating correct leaf-level emission potentials for the component species of a canopy is of paramount importance for parameterizing these models. In the urban environment, trees from a large number of species are present, including both native and exotic species, as individual specimens (for example in domestic gardens or common areas), as linear plantings along streets, as groups in squares or other smaller open spaces, or in larger numbers in parks or other large open spaces. In size, they vary from small shrubs and saplings in gardens or as new plantings, to fully-grown specimens which may reach 40 m or more in height in large gardens or parks. The number of different species present in an urban area varies with land use with suburban residential areas typically containing many more exotic species than, for example, dense inner-city areas. Agroforestry plantations typically contain far fewer species. In both urban and plantation canopies, the extrapolation process also requires knowledge of the contribution in terms of biomass and leaf area for each component species.

15.3.2 Estimating Urban and Plantation Canopy Species Composition and Biomass by Remote Sensing

Remote sensing is the remote measurement of reflected radiation, by a sensor, often for a range of different wavelengths, and it can provide data on vegetation cover and type for the 'bottom-up' approach to estimating canopy flux of BVOCs. Remote sensing datasets vary in:

Type of sensor e.g., optical, radar or Light Distance And Ranging (LiDAR).
Spectral resolution – number and location of wavelengths that are sampled.
Spatial resolution – size of the area on the ground represented by a pixel in an image.
Radiometric resolution – precision with which radiation is observed.
Temporal resolution – frequency with which repeated images of a site are acquired.

Consequently, the information that can be derived from the remote sensing data varies considerably depending on sensor specifications and mode of sampling. A range of techniques have been explored for characterizing urban greenspace and the urban canopy from remote sensing data. The techniques can be grouped in several ways, but for the purposes of this chapter, they are grouped according to spatial resolution:

High resolution – pixel size <5 m (often <1 m), includes data from LiDAR, aerial photography and high-resolution satellite data from satellites such as IKONOS,

Quickbird and Worldview. High-resolution sensors are typically designed and applied to small test sites, where high spatial detail is required for a single-point in time.

Medium resolution – pixel sizes of roughly 10–30 m. They are principally designed to meet regional mapping requirements, including mapping change over time.

Coarse resolution – defined here as pixels >250 m that are suitable for large-scale or global mapping and suitable for monitoring change over time.

In addition to these methods, tools such as Google Earth and Google Streetview increase the level of information about species and management that can be gained remotely. However, the data are limited both in temporal coverage and, in the case of Streetview, its spatial coverage, but it may provide a useful supplement to field data in some cases.

There are potential synergies between different datasets, so, for example, combining LiDAR, aerial photography and field measurements enables the delineation of tree crowns and identification of species and height estimation. Clearly, the best approach is a combination of remote sensing to provide very high resolution raster and landcover data, with classification and survey work for ground-truth, and for obtaining plant-level measurements and observations which are important for BVOC emission estimates.

15.3.2.1 LiDAR

LiDAR is a high-resolution technique that uses laser pulses to measure the distance from the sensor to the ground. Post-processing of the data then enables construction of a digital surface model, which maps the height of features, such as buildings, vegetation and the ground. When the laser pulse reaches a vegetation canopy, it will typically penetrate some distance into the canopy before being reflected back to the sensor. Consequently, LiDAR is likely to underestimate the true height of the canopy, unless a calibration or correction is applied (Gaveau and Hill 2003; Anderson et al. 2006). LiDAR can be segmented to automatically delineate tree crowns (Shrestha and Wynne 2012). Segmentation is the use of an algorithm to identify homogeneous areas, in this case corresponding to tree crowns. Once tree crowns are identified as discrete objects, then object-based processing methods enable other attributes derived from remote sensing datasets or field observations to be attached, such as species, tree height and crown diameter. The identification of individual trees is especially useful in urban areas, as species, age and management may be highly variable over small areas (Ardila et al. 2012). Once species are known from aerial photography or field data then relationships between the LiDAR data and tree height, crown diameter and biomass can potentially be developed for the main tree species, if field calibration data are available (Shrestha and Wynne 2012).

15.3.2.2 High-Resolution Datasets

High-resolution datasets are currently provided by three sources, aerial photos, high-resolution aircraft measurements such as those taken with AVIRIS (hyperspectral imaging) and high-resolution satellite data, such as Quickbird and IKONOS. Aerial photographs are able to provide higher resolution images than the satellite sensors. High-resolution aircraft and satellite data can be processed in the same manner as aerial photographs or medium-resolution satellite data. Manual interpretation, by an expert, of aerial photographs can yield information about a range of urban canopy parameters including location and size of tree canopies, tree species in very fine-resolution images (Valerie and Marie-Pierre 2006) and, with stereo-photography, estimates of tree height (St-Onge et al. 2004). Identification of features in aerial photos is based on interpreting subtle differences in shape, texture, size, colour, shadow and spatial context (Morgan et al. 2010). It is often difficult to apply automatic processing methods to aerial photographs, because of the limited spectral information, whereas expert manual interpretation may produce better results, but can be time-consuming. High-resolution hyperspectral aircraft data can compensate for these limitations yielding extreme level of detailed information, albeit automatic data processing is still somewhat difficult due to lack of generalized algorithms (Somers and Asner 2012).

15.3.2.3 Medium-Resolution Satellites

Medium-resolution satellite sensors are valuable for mapping urban areas and greenspace in urban areas, because a single image is able to cover an entire city or conurbation. For example, Landsat images are approximately 180 km × 170 km. This enables large areas to be mapped relatively easily. There are two main methods for classifying urban landcover, either image classification where each pixel is assigned a landcover type based on its spectral characteristics, or spectral mixture analysis (SMA) methods which assign a percentage of different landcover types to a pixel. The key limitation of medium-resolution satellite data for urban areas is that their pixel size is poorly suited to the highly heterogeneous urban landscape. Individual pixels will usually contain a complex mix of buildings, roads, shadows, trees and grass. Spectral mixture analysis methods are often preferred over image classification, as they offer a more flexible method for dealing with mixed pixels. The number of sub-pixel components that can be estimated using SMA is limited by the spectral resolution of the sensor, and so typically three components are used for urban mapping: green vegetation, substrate and dark surface (Small and Lu 2006). However, the SMA results can be augmented by image classification results, where more classes are typically resolvable, including coniferous, deciduous and grass classes in parks or densely vegetated suburban areas.

15.3.2.4 Coarse-Resolution Satellites

There are two main advantages of coarse-resolution satellite data. First, the data are typically free, although there may be licensing restrictions and costs for certain products or data delivery charges in some cases. Second, the temporal coverage is high, meaning that change over short time periods can potentially be monitored. Variation in vegetation indices derived from coarse-resolution data have been shown to track increasing urbanization (Dallimer et al. 2011), and can be used to characterize changes in vegetation seasonality (Guenther 2013).

The availability and cost of the different types of data varies widely and is an important consideration. Most remote sensing datasets have to be purchased from satellite operators or resellers of aerial photo archives, with the exceptions being data from coarse-resolution sensors and data from the Landsat series of sensors. The availability of archived datasets differ widely across sensors, with aerial photographs providing a long, but often sparse, time-series extending over 60 years in some cases. Conversely, satellite archives cover shorter time periods, but have many more acquisitions per site.

15.3.2.5 Use of High-Resolution Remote Sensing Data to Identify and Quantify Agroforest Canopies

The "bottom-up" approach to mapping tree species and modelling emissions is also appropriate for agroforestry canopies. Remote sensing of agroforestry is typically simpler as the relatively large, homogeneous, monospecific, even-aged stands are more suited to the current capabilities of satellite sensors, than the heterogeneity of the urban environment. The techniques for agroforestry include the methods discussed in the section on remote sensing of the urban canopy, but also include radar methods. Radar is sensitive to the structure of the woodland and Interferometric radar (InSAR) is particularly useful for estimating canopy height or biomass (Balzter et al. 2007), especially if a good quality digital terrain model (DTM) exists or can be derived from the InSAR data.

15.3.3 Urban Land Classification and Ground Survey

An alternative or supplementary approach to remote sensing are ground surveys of the vegetation. This approach requires at least 1 km^2 resolution raster data of land- and vegetation-cover characteristics (for example %woodland, % roads, %suburban, %dense urban, %inland water, etc.), but provides a wealth of ground-truthed data. Briefly, the land- and vegetation-cover characteristics for each pixel (e.g., km^2) in the raster dataset for the area of interest are analysed using PCA and cluster analysis to generate a classification of the urban landscape. This classification is then used for a stratified sampling of vegetation. Depending on available resources,

sample plots are randomly allocated within each characteristic cover-type of the classification strata, and weighted to the number of pixels in each class. Details are recorded, such as species, age, height, diameter-at-breast-height, crown spread, LAI, and leaf dry mass per unit area (LMA). The health/stress status is also noted as this is likely to affect BVOC emissions. It is also worth recording any obvious management practice or damage, such as branch trimming, coppicing, damaged bark. When the tree inventory of the sample plots is complete, the data can then be extrapolated to the sampled area or forest footprint using the knowledge of total coverage for each land- and vegetation-cover characteristic contributing to that square. The classification of each sampled square then enables simple extrapolation to the whole urban landscape. It is clear that a very high uncertainty is associated with this extrapolation method. However, the classification should embrace all the major land/vegetation cover types within the area of interest. It is also likely that local authorities will tend to use a fairly limited range of tree species for their planting programmes, and that individuals will tend to select plants for their gardens which local garden centres supply, and will also select plants that they admire in their neighbours' gardens. Thus, there may be a natural restriction on the extent of vegetation diversity within a neighbourhood, and a correspondingly lower degree of uncertainty in bottom-up extrapolations of urban vegetation.

15.3.4 Measuring Leaf- and Branch-Level Emission Rates

Once the canopy composition is determined, species-specific emission potentials can be determined either from literature surveys (e.g., Stewart et al. 2003; Keenan et al. 2009b) or by measurements specific for the canopy (e.g., Owen et al. 2003). Obtaining representative, meaningful and reliable data on the emission rates of BVOCs from trees, whether in their natural habitat, in the urban environment, or in a managed agroforestry plantation, poses both conceptual and technical challenges (Hewitt et al. 2011). These challenges arise because (1) different tree species emit different blends of BVOCs, each at different rates, (2) at the leaf level, emission rates vary with leaf age, leaf temperature, soil moisture and for some BVOCs with light intensity, (3) at the tree scale, size of tree (biomass or leaf area) influences BVOC emission rates, (4) BVOC emission potentials for an individual plant can change according to the conditions of light, temperature, stress in the days or weeks preceding the measurements, and (5) as discussed above, BVOC emission rates may be influenced (positively or negatively) by biotic and abiotic stresses. These issues are discussed in depth by Niinemets et al. (2010b, c).

There is already a large database of BVOC emission potentials for a range of plant species throughout the world (http://bai.acd.ucar.edu/Data/BVOC/index.shtml), in addition to a large source of research literature, but the existing resources must be used with caution. As well as the challenges listed above, sampling and analytical techniques can also be a source of uncertainty (Niinemets et al. 2011). Niinemets et al. (2011) propose standardization of experimental and calculation

protocols as well as critical examination of past reports to develop accurate emission potential databases in the future. In view of this, it is therefore better if BVOC emission measurements are made according to this protocol, from a sample of plant species within the area of interest. It would be even better if extended measurements can be made over 24 h, and during different portions of the growing season. Alongside the excellent overview of Niinemets et al. (2011), Ortega and Helmig (2008) and Ortega et al. (2008) also review enclosure methods of sampling and measuring BVOC emission rates. Here, we provide a brief summary of methodology and direct readers to these reviews for further details.

Estimates of leaf-level BVOC emission rates may be obtained by measuring the concentrations of emitted compounds in the head-space above leaf tissue, either in a static system without air flow (e.g., Hewitt and Street 1992), or in a dynamic system where air flows over the leaf and through the enclosure (e.g., Hayward et al. 2004). In static systems, it is possible that a compensation point is reached, as the concentration builds up over time, such that emission rates are suppressed or even reversed (Niinemets et al. 2011). In general, static systems should be avoided and a dynamic flow-through chamber or enclosure used, such that the analyte concentrations in both inflowing (C_{in}) and outflowing (C_{out}) air are measured. The differences between these, together with information on flow rate (v) and leaf area (A_{L}) or mass, are then used to calculate emission rates:

$$E = \frac{v\,(C_{\text{out}} - C_{\text{in}})}{A_{\text{L}}}. \tag{15.3}$$

In reality, the calculation is somewhat more complex due to changes in water vapour concentration (Niinemets et al. 2011). Dynamic enclosure systems have the additional advantage of ease of control and recording of leaf temperature and photosynthetically active photon flux, both of which may affect BVOC emission rates.

The preferred method for measuring leaf-level emission rates is therefore the use of portable photosynthesis systems with built in carbon dioxide and water vapour gas sensors with a compatible leaf cuvette or chamber with temperature and light control. The LI-Cor LI-6400 series of instruments is almost ideal, following modifications to reduce adsorption of volatiles on system component surfaces, but other suitable designs (including those made by PP Systems, ADC or Walz GmbH), are available. Plumbing modifications might be necessary to allow for sub-samples of the inflowing and outflowing chamber air to be diverted to a suitable BVOC detection system. This might be an on-line detection system such as a proton-transfer reaction mass spectrometer, or a sample preconcentrator, prior to transfer to an off-line detector such as a gas-chromatography system.

Some studies have estimated emission rates at the branch scale, by enclosing branches (or parts of branches) with leaves in a dynamic chamber (e.g., made of Teflon film; Owen and Hewitt 2000; Owen et al. 2001) with internal measurement of light intensity, and air or leaf temperature. However, these do not readily lend themselves to the control of light and temperature and have largely been superseded by the controllable leaf chambers described above.

15.3.5 Measuring Canopy-Scale Emission Rates

With the invention of fast-response sonic anemometers and fast-response and sensitive detectors for BVOCs, it is now possible to make tower-mounted, micrometeorologically-based, measurements of canopy-scale BVOC emission rates. This avoids the need to extrapolate from leaf- or branch-scale measurements to the canopy scale. However, these methods are only suitable in simple terrain with a large upwind fetch of 100–1,000 m over reasonably homogeneous canopies. They may be especially suitable for agroforestry plantations (e.g., Misztal et al. 2011) but not for small patches of woodland (e.g., small urban parks).

One of the technically simplest methods of measuring canopy-scale emission rates is relaxed eddy accumulation (REA) (e.g., Gallagher et al. 2000; Greenberg et al. 2003). In REA measurements, ambient air is sampled at an appropriate height above a uniform canopy at a constant flow rate. The vertical wind vector is measured at high frequency and the sample air stream switched such that it passes into separate sampling reservoirs for the upward and downward flowing air. Analysis of the contents of the reservoirs is then carried out off-line. The disadvantage of the method is that it gives a single flux estimate integrated over, say, one hour, and is generally very labour-intensive (Ciccioli et al. 2003).

More recently, eddy covariance (EC) and its variant, virtual disjunct eddy covariance (vDEC) have been developed and used for BVOC flux estimates. In EC, co-located high frequency measurements of the vertical wind velocity and BVOC mixing ratio are combined to calculate fluxes. Currently, only a few instruments are capable for EC of BVOCs. For single BVOCs, these instruments include the "fast isoprene sensor" (Guenther and Hills 1998) and the proton-transfer reaction mass spectrometer with quadrupole mass spectrometer (PTR MS) which can sample individual masses at 2 Hz (Karl et al. 2001). However, for simultaneous measurements of multiple BVOC fluxes, only the protontransfer reaction time-of-flight mass spectrometer (PTR TOF MS) (Jordan et al. 2009; Ruuskanen et al. 2011) has high enough temporal resolution for EC measurements (see e.g., Müller et al. 2010).

In vDEC, the requirement for very high frequency BVOC measurements is mathematically relaxed and the standard PTR MS can be used. The detector is used to scan over a small suite of predetermined masses, which generates a discontinuous or "disjunct" set of fast response data. These are then combined with the vertical wind velocities to calculate fluxes, normally averaged over one hour or half an hour. The method of vDEC has now been used extensively to measure BVOC fluxes from both natural and managed vegetation, including an oil palm (*Elaeis guineensis*) agroforest in Malaysia (Misztal et al. 2011). The next stage of development in landscape-scale flux measurements is likely to result from the application of vDEC using instruments mounted on low-flying slow aircraft.

Directly measuring canopy-scale emission rates from urban habitats is very difficult, except from the largest of urban parks because of the need to have a large upwind fetch over a homogeneous canopy. However, Langford et al. (2010)

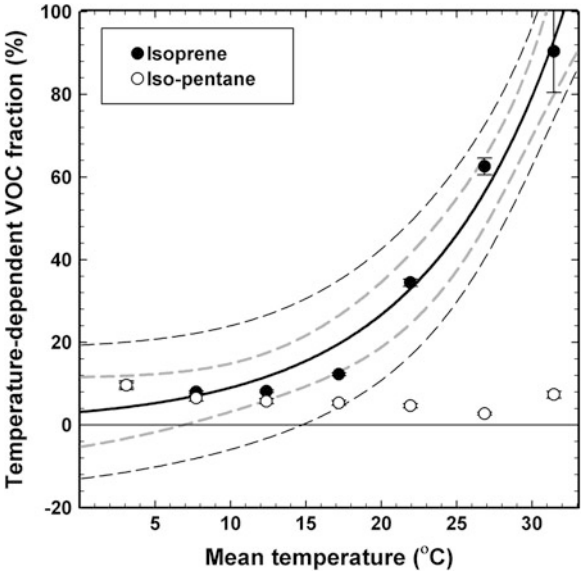

Fig. 15.1 Temperature responsiveness of isoprene (closed circles) and isopentane (open circles) concentrations at the Marylebone Road automatic monitoring station, London, UK. The data are based on 5 years of hydrocarbon measurements (modified from Langford et al. 2010). To estimate the percentage of temperature-dependent isoprene concentration, scatterplots of isoprene vs. benzene (marker of vehicle emissions) were derived for different temperatures, and the intercepts of these relationships were used for the background concentrations of isoprene not attributable to direct emissions from vehicles. The temperature-dependent percentage was calculated as the ratio of the intercept at given temperature to the total isoprene present. To assess the effect of temperature on evaporation, analogous relationships were derived for non-biogenic compound isopentane. Error bars indicate the uncertainty of intercept values for the temperature bands −5–0 °C, $n = 114$; 0–5 °C, $n = 3{,}405$; 5–10 °C, $n = 9{,}539$; 10–15 °C, $n = 12{,}176$; 15–20 °C, $n = 9{,}340$; 20–25 °C, $n = 3{,}171$; 25–30 °C, $n = 673$; 30–35 °C, $n = 73$. The regression line is given by $y = 1.8 + 3.0\exp(0.11x)$ ($r^2 = 0.97$, $P < 0.0001$)

used vDEC to measure the fluxes of a range of volatiles, including some emitted by vegetation, over the city of London. They correlated fluxes with traffic flow to estimate the vehicle- and non-vehicle-related components of the observed fluxes. They also applied a novel analysis, using the long-term measurements of benzene concentrations, to determine the temperature-dependent biogenic component of the isoprene flux, shown in Fig. 15.1. At an ambient temperature of 25 °C they estimated that biogenic emissions of isoprene account for about 50 % of the observed concentration of the compound in the middle of London. However, this percentage drops significantly as ambient temperature falls and non-biogenic sources become relatively more important.

15.4 Quantifying Emissions of BVOCs from Urban Regions and Agroforestry Plantations

15.4.1 Quantifying Urban Emissions

As indicated above, estimating the BVOC flux from urban vegetation poses a number of challenges. In the urban environment, vegetation is fragmented and trees from very many species are present, including both native and exotic species, as individual specimens in domestic gardens or common areas, as linear features along streets, canals and railways, as groups in squares, gardens, allotments, or other smaller open spaces, or in larger numbers in parks, community woodlands or other large non-built areas. In size, they vary from small shrubs and saplings in gardens or new plantings, to fully grown specimens which may reach 40 m or more in height. The number of different species present in an urban area varies with land-use type, sub-urban residential areas typically containing many more exotic species than, for example, dense inner-city areas.

15.4.1.1 A Case Study

A simple extrapolation, multiplying leaf area based emission factors by leaf area index (LAI), and applying scaling factors depending on light and temperature dependencies was used by Owen et al. (2003) to estimate BVOC flux from the West Midlands, UK urban area. To estimate canopy emission potential per unit ground area for a particular BVOC compound at standard conditions of temperature of 30 °C and light of 1,000 μmol m^{-2} s^{-1}, the following model was used:

$$F_{\text{pot}} = \sum_{i=1}^{i=k} E_{\text{psi}} M_{\text{i}} S_{\text{f,i}} \tag{15.4}$$

where k is the number of plant species within the canopy, F_{pot} is the habitat emission potential at standard conditions of temperature and light, E_{psi} is the BVOC emission potential for species i (μg g^{-1} dry mass h^{-1}), M_{i} is plant species biomass per ground area (g m^{-2} of ground) and $S_{\text{f,i}}$ is the fractional contribution of species i to total canopy area. As discussed in Sect. 15.3.1, and Guenther (2013), this approach provides a rough estimate of canopy emission as it neglects the within-canopy variation in emission potentials and environmental drivers, and does not consider shading of vegetation by neighbouring plants and buildings. Nevertheless, all this information may be very difficult to obtain in urban environments due to numerous unique radiative transfer situations (Osmond 2010).

As described in Sects. 15.3.2 and 15.3.3, simulation of urban emissions requires estimation of the species fractional composition in the study area. This can be done by remote sensing (Sect. 15.2.3) or ground survey method (Sect. 15.3.3), although

combined approaches are also possible. Owen et al. (2003) used a ground survey method (Sect. 15.3.3) for the 900 km^2 West Midland (UK) area, which is described in more detail by Owen et al. (2006) and Donovan et al. (2011).

15.4.1.2 Assigning Emission Factors to Species Contributing to the Canopy

Once the dominant species composition for each urban land-class is known, BVOC emission factors for each species can be extracted from databases or literature, taking care to note the environmental conditions for which the emission factors are defined, and the methods used to derive them. Where no literature exists, it is possible to use values from members of the same genus or family (Benjamin et al. 1996) although allocating emission factors in this way is likely to be prone to errors and large uncertainties. This can be demonstrated by the *Quercus* genus whose members either emit isoprene or monoterpenes, at different rates and with different chemical speciation. It is therefore better to sample as many species as possible to obtain missing emission factors.

15.4.1.3 Extrapolating to Canopy Flux Estimates

The relative contribution of each species to the urban land-class canopy, and the LAI of each species can be used to weight the relative contributions of species emissions to the urban land-class canopy to give canopy-averaged emission factors which can then be used with climate data in emission flux algorithms and models to predict average flux of each compound from the canopy. This approach does not account for different emission factor values throughout the canopy nor for extinction of light through the canopy, but it has also been used by Owen et al. (1997, 2001) for the Mediterranean regions, and Pierce and Waldruff (1991) for continental US. More advanced schemes with simplified canopy models (e.g., Sellers et al. 1992 big leaf approach) have been employed in Guenther et al. (1995, 2006, 2012) and Lavoir et al. (2011) to model regional and global BVOC fluxes. Using the simplified canopy model, Lavoir et al. (2011) obtained a simple relationship between canopy monoterpene emission and a remotely-sensed spectral vegetation index for spatially heterogeneous vegetation cover. MODIS satellite data were used to obtain the fraction of light absorbed by the canopy at 1 km^2 resolution. Nevertheless, simple big-leaf models tend to overestimate the flux (Dai et al. 2004) and for correct integration of fluxes, either layered models (Guenther et al. 2006, 2012; Niinemets et al. 2013) or two-big-leaf models (de Pury and Farquhar 1997; Dai et al. 2004) should be used.

15.4.1.4 Assessing the Biogenic Component to Total Urban Volatile Emissions

Hundreds of hydrocarbon compounds are detected in the air over towns and cities. Measurements of BVOC fluxes show mainly compounds of anthropogenic origin, from traffic, industrial and residential emissions. Isoprene is commonly detected in city air, but it is not necessarily emitted from the vegetation planted within and around the urban area. It has been shown that exhaust from vehicles is a source of isoprene (Borbon et al. 2001; Langford et al. 2010). Several methods are used to distinguish between anthropogenic and biogenic isoprene in cities. A primary indication of a biogenic source is an increase of concentrations/flux during the summer months. A diurnal peak of isoprene concentrations during the rush hours in winter indicates the anthropogenic contribution. If this is assumed to be constant, then the biogenic contribution in summer can be estimated by subtracting the winter concentrations (Borbon et al. 2001). A further quantification of biogenic isoprene can be made by calculating the isoprene/acetylene ratio. Traffic emissions are thought to be the major source, if not the only source, of acetylene in urban air. A constant ratio over time indicates the same origin for both compounds. Durana et al. (2006) adopt the same approach, using the ratio of isoprene/iso-butene concentrations to estimate the biogenic contribution to isoprene.

Different studies report different biogenic contributions to urban isoprene concentrations. von Schneidemesser et al. (2010) report that biogenic isoprene is not significant in London and Paris. Langford et al. (2010) separated the biogenic fraction of isoprene in London air using 5 years of hydrocarbon data collected between 2001 and 2006 by the Hydrocarbon Network monitoring station situated on Marylebone Road using regressions of isoprene against benzene concentrations (a marker of vehicle emissions) at different temperatures, and used the isoprene intercept of the regression as an estimate of biogenic emissions (Fig. 15.1). Langford et al. (2010) found only slight increases in iso-pentane concentrations relative to benzene at higher temperatures due to increased evaporative emissions, but the temperature-dependent fraction of isoprene was large and increased exponentially with temperature. Throughout year, 19 % of isoprene at the London site was estimated to originate from biogenic sources, but this percentage was much greater on warmer days (Fig. 15.1).

15.4.2 Quantifying Emissions of BVOCs from Agroforestry Plantations

Because agroforestry plantations generally are monospecific, it is more straightforward to quantify BVOC emissions from these canopies, compared with the urban canopies described in Sect. 15.4.1. Plantation canopies lend themselves more easily to direct canopy-scale flux measurements. For example, BVOC fluxes have been measured directly from oil palm (*Elaeis guineensis*) (Misztal et al. 2010,

2011) and from rubber tree (*Hevea brasiliensis*) plantations (Baker et al. 2005). The UN Food and Agriculture Organisation (FAO) publishes annual information on global crops and forestry plantations, including statistics and lists of globally important agroforestry plantation species (e.g., FAO 2006). The plantation species in Table 15.3 are extracted from FAO (2006), and represent the world's most important agroforestry plantation species. Three types of emissions and flux estimate methods are presented in Table 15.3: (i) canopy-scale flux measurements, (ii) leaf- or branch-scale species emission rate measurements, and (iii) leaf or branch-scale emissions extrapolated to canopy flux estimates. Table 15.3 shows a wide range of plantation species' BVOC emission rates, and canopy-scale flux measurements and extrapolated estimates. It is remarkable that many of the world's important agroforestry plantation species are high BVOC emitters. *Elaeis guineensis*, *Salix* spp., *Populus* spp. and *Eucalyptus* spp., for example, are all strong BVOC emitters. This has implications for regional air quality, and this is already being addressed in recent research to provide low BVOC-emitting *Populus* (Behnke et al. 2012; Rosenkranz and Schnitzler 2013). However, to our knowledge, there are no emission measurements to date for important plantation species such as *Cunninghamia* spp., *Tectona* spp. and *Ziziphus* spp.

15.5 Uncertainties and Challenges for Future Research

15.5.1 Uncertainties

As we have previously mentioned, extrapolations carry high uncertainty when they are derived from field survey data based on a stratified random sample using an urban land-use classification to estimate foliar and whole tree biomass, leaf area and leaf area index for individual urban land class kilometre squares. Further uncertainties arise when these estimates of urban forest attributes are used to characterize the typical BVOC emission and gaseous dry deposition potentials of each land class kilometre square and thus entire urban regions. Errors are therefore propagated, and should be estimated and presented with extrapolated results. A method for doing this is described in Donovan et al. (2011), and is based on simple first principals of uncertainty analysis. Donovan et al. report the following uncertainties associated with extrapolating their field measurements and survey data to urban regional scale in West Midlands conurbation of UK: leaf area per tree $\pm$ 35 %; foliar biomass per tree $\pm$ 60 %; foliar biomass per km^2 $\pm$ 70 %; foliar biomass for the West Midlands $\pm$ 120 %; projected crown area per tree $\pm$ 25 %; tree cover per km^2 $\pm$ 40 %; tree cover in the West Midlands $\pm$ 95 %; West Midlands BVOC emission estimate $\pm$ 240 %. Uncertainties associated with the top-down approach include uncertainties with the model assumptions made, and instrumentation uncertainties. Niinemets et al. (2011) give a comprehensive analysis of the uncertainties associated with extrapolating from leaf and branch measurements to

canopy-scale fluxes. In fact, comparisons between canopy-scale isoprene emissions based on leaf-level emission measurements extrapolated with a canopy environment model and above-canopy flux measurements suggest that discrepancies are less than the uncertainty associated with these two approaches (Guenther et al. 2006; Niinemets et al. 2013).

15.5.2 Future Challenges

With increasing urbanization, rapid land-use change driven by expansion of food and biofuel crop production, and changing climate, it will be increasingly important to monitor, estimate and predict the resulting changes in BVOC emissions, as these cause changes to atmospheric chemistry and climate. It is important that urban greening or de-greening and planting of biofuel crops is done in an environmentally responsible manner, which includes a consideration of the BVOC emission potential both at the time of planting and in the future. The research challenges include making better use of remote sensing data that are becoming available at higher spatial and spectral resolutions. Methods for interpreting these data using inverse modelling and by direct spectral analysis to estimate BVOC emissions are in their infancy, but are improving. Future progress in BVOC emissions and effects research depends on the development of more efficient methods for ground-truthing of biophysical parameters, and on simple, fast chlorophyll fluorescence techniques to indicate photosynthetic status and stress within individuals. Further progress also critically depends on and more sensitive and rapid analytical methods for determining BVOC concentrations and fluxes. Much has been achieved, but much more work is required to understand these complex processes in an increasingly urbanized world.

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Chapter 16
Global Modelling of Volatile Organic Compound Emissions

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Abstract The majority of volatile organic compounds emitted from the terrestrial biosphere (BVOCs) are highly reactive hydrocarbons that have been shown to affect atmospheric composition across the full range of temporal scales from fractions of seconds to centuries and spatial scales from μm to global. Furthermore, biogenic emissions are thought to account for around 90 % of the total quantity of non-methane hydrocarbons released into the atmosphere each year. As a result, BVOCs have substantial air quality and climate impacts, and there is an urgent need to quantify and map their emissions as precisely as possible. In this chapter we outline the use of computer models to estimate annual global emissions of BVOCs and the on-going efforts to validate and constrain the output from such models. The current generation of BVOC emission models generally includes only the constitutive emissions of a handful of compounds: chiefly isoprene, monoterpenes and methanol, which are thought to account for about 80 % of the total flux from the biosphere. At present, it is estimated by global models that total annual emission of isoprene amounts to around 500 Tg of carbon, with the emissions dominated by tropical ecosystems and by tree species. The emissions of monoterpenes are

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similarly distributed, although high levels of monoterpene emissions are also seen from the boreal forests. There is currently no consensus on the annual estimate of monoterpene emission, with estimates ranging from 30 to 150 Tg of carbon. Apart from these main compounds, the biosphere emits many hundreds of different compounds, some of which are produced as a short-lived, transient response to stress rather than as constitutive emissions. We discuss the role that biogenic emissions of reactive trace gases play in the Earth system as a whole, and consider the potential feedbacks that exist between BVOC emissions, atmospheric composition, air quality and climate, and the terrestrial biosphere, and how these can be studied with Earth system models. We finally suggest ways of improving and further developing the global models.

Abbreviations

ANN	Artificial Neural Networks
ATP	Adenosine triphosphate
BER	Basal emission rate
BVOCs	Biogenic volatile organic compounds
CCMs	Chemistry-climate models
CCN	Cloud condensation nuclei
CTMs	Chemistry-transport models
DMADP	Dimethylallyl diphosphate
ESMs	Earth system models
GCMs	General circulation models
NADPH	Nicotinamide adenine dinucleotide phosphate
PPFD	Photosynthetic quantum flux density
PFTs	Plant functional types
SOA	Secondary organic aerosols
VOCs	Volatile organic compounds

16.1 Introduction to Global Modelling

Biogenic volatile organic compounds (BVOCs) constitute a major sink for the OH radical, the atmosphere's most powerful oxidant, particularly over land. Emissions of BVOCs, thus, mediate the oxidative capacity of the atmosphere, affecting the atmospheric lifetime of other chemical species, such as methane. Biogenic volatiles released into the atmosphere react rapidly with atmospheric oxidants: O_3, and the OH and NO_3 radicals (see e.g., Atkinson 2000; Atkinson and Arey 2003). Although they may form peroxy radicals that go on to participate in ozone formation, the products of these initiation reactions are often oxygenated species of much

lower volatility than the parent BVOC (Griffin et al. 1999). Such products can partition into the particle or aerosol phase, either through direct nucleation or by condensing onto existing particles (see e.g., Hallquist et al. 2009 and references therein). Detailed analyses of the composition of aerosol particles, through the use of carbon isotopes for example, have shown that the majority of their mass is of biogenic origin, even in highly polluted regions (Zhang et al. 2007; Jimenez et al. 2009). This is not captured with current atmospheric chemistry and aerosol models, which are known to underpredict the concentration of biogenic secondary organic aerosol (SOA) almost everywhere (Heald et al. 2005). Biogenic volatiles, and particularly isoprene, also play a key role in the distribution of reactive nitrogen in the atmosphere through the formation of organic nitrates (von Kuhlmann et al. 2004; Ito et al. 2009), especially peroxyacetyl nitrate (PAN). PAN is a long-lived compound, with an atmospheric lifetime of a few months in the cold-free troposphere.

Modelling studies suggest that including isoprene emissions in atmospheric chemistry models increases the methane lifetime by over 20 % as compared to model simulations with no isoprene (Pike and Young 2009). There is currently considerable uncertainty over the role of isoprene as a sink for the OH radical in very low NO_X environments. Flux and concentration measurements taken during field campaigns in the Amazon suggest that the OH radical is somehow "recycled" during the chain of reactions involving isoprene (Lelieveld et al. 2008). Various novel chemical mechanisms have been proposed to account for this (see e.g., Peeters et al. 2009; Paulot et al. 2009), as has the concept of segregation, or lack of mixing, of isoprene and the OH radical (Butler et al. 2008). As yet, none of the existing mechanisms accounts fully for the observed ambient OH radical concentrations (see e.g., Pugh et al. 2010). Although the uncertainty in the mechanism is confined to low-NO_X regions, the impact is felt globally, with Archibald et al. (2010) reporting that inclusion of an "OH-recycling" mechanism in a global atmospheric chemistry model resulted in a 14 % reduction of the projected methane lifetime.

Biogenic volatiles are therefore a crucial component and have to be considered when investigating the evolution of the Earth system, especially in the context of globally changing climate, land use and landcover, and atmospheric composition. In this chapter, we first review the different modelling approaches used so far to estimate emissions of BVOCs such as isoprene, monoterpenes or other emitted species (Sect. 16.2), addressing particularly the question of model evaluation (Sect. 16.3). The different feedbacks involving BVOCs in the context of the Earth system study and modelling will be detailed in Sect. 16.4, with special attention to feedbacks with methane, ozone, secondary organic aerosols or changes in diffuse radiation, involved not only in the atmospheric composition but also in climate forcing. Finally, perspectives and challenges to take global modelling forward and find new approaches for BVOC emission estimates, and future experimental investigation needs are presented in Sect. 16.5.

16.2 Modelling the Emissions from the Terrestrial Biosphere

Ground-breaking work by Chameides et al. (1988) demonstrating the importance of BVOCs on local- to regional-scale air quality resulted in increasing efforts to quantify the emissions of these compounds. This work soon expanded in scale from regional to global and in approach from detailed inventories based on laboratory measured fluxes to parameterizations for use in computer-based simulation models (Pierce and Waldruff 1991; Guenther et al. 1995, 2006). Initially, these parameterizations for light and temperature responses were empirical fits to laboratory leaf-level emissions (see e.g., Guenther et al. 1991), based on observed photosynthesis-like response curves (Monson et al. 1992). More recently, biochemical techniques such as isotope labelling studies have improved understanding of the synthesis routes involved in the production of BVOCs within plants (see e.g., Fuentes et al. 2000), permitting a more process-based approaches to be adopted.

There are currently two main ways to modelling the emissions of BVOCs from vegetation: models that were developed to empirically represent the observed responses to environmental drivers – here referred to as empirical models – and models that were developed based on theoretical understanding of the underlying processes – here referred to as process-based models (Grote and Niinemets 2008). Although these two types of models are fundamentally different, they can be parameterized to describe a similar degree of variance in large-scale models (Keenan et al. 2009; Niinemets et al. 2013). So, there are often no objective criteria for choosing a model for present-day simulations (Guenther 2013; Niinemets et al. 2013). For predicting emissions under global change when the predictions necessarily need to be extrapolated beyond the existing data, process-based models might be more useful (Arneth et al. 2007).

Here we focus on isoprene and monoterpenes as only limited data are available on emission rates of other BVOCs. The fluxes of "other" BVOCs are usually estimated using the same exponential temperature relationship as that for monoterpenes, although the temperature dependence of the emissions of many BVOCs is not known with any certainty (Guenther et al. 1995, 2012).

16.2.1 Empirical Isoprene Emission Models

Laboratory studies show that leaf-level isoprene emissions are strongly dependent on leaf temperature and light levels. Guenther et al. (1991) demonstrated clearly that the light dependence of the emissions closely matched the light dependence of photosynthesis, which had been earlier linked to electron transport (Farquhar et al. 1980). Thus the photosynthesis algorithms were modified to simulate isoprene emissions.

These algorithms were further developed into an empirical model (Guenther et al. 1995) that could be applied worldwide with refined coefficients, but still

based on measurements on a limited number of plant species (Guenther 2013). Thus, it was acknowledged that in this early application, basal emission rates (BERs) for five basic plant functional types (PFTs), and derived ecosystem-level emission estimates, had a large uncertainty (Guenther et al. 1995; Guenther 2013). Appropriate foliage densities were also assigned to these ecosystems. In addition, a simple canopy model was incorporated to determine the fraction of sunlit and shaded leaves within the canopy in order to calculate leaf-level photosynthetically active radiation based on the radiation reaching the top of the canopy.

Currently, the two most widely used empirical models are MEGAN (Guenther et al. 2006, 2012) and BEIS (Pierce and Waldruff 1991). They are briefly described below. For further detail, we refer to the original publications and Guenther chapter in this volume (Guenther 2013).

The Model of Emissions of Gases and Aerosols from Nature (MEGAN) is an empirical emission model developed at the National Center for Atmospheric Research (NCAR) that can be used to simulate the fluxes of 19 different groups of BVOCs (Guenther et al. 2006, 2012). While the basic mathematical forms of the temperature and light responses for isoprene developed by Guenther et al. (1995) were retained, the emission algorithms were extensively modified in an attempt to better capture seasonality through the inclusion of an activity factor dependent on leaf age. In addition, studies indicated that plants acclimate to their growth environment, responding both to instantaneous changes in light and temperature, and to past light and temperature environment. This environmental response was divided between an initial rapid phase and longer-term phase that lasted for a period of ca. 10 days (Guenther et al. 2006), and the temperature and light response algorithms were adapted to incorporate these rapid and longer-lasting effects.

The Biogenic Emissions Inventory System (BEIS) (Pierce and Waldruff 1991) is a high-resolution regional-scale empirical emission model that uses the same light and temperature dependence algorithms as those formulated by Guenther et al. (1995). Developed by the scientists of the US Environmental Protection Agency (EPA) to generate an emission inventory for the USA, it used highly detailed land cover maps, and species- and location-specific values of base emission rates for 230 land-use types (Pierce et al. 1998). The canopy model incorporated in BEIS (Gay 1987) also differed from that in MEGAN (Guenther et al. 2006, 2012; Guenther 2013 for a comparison).

Despite the similarity in approach and the same algorithms employed by the two models, the emission estimates from BEIS and MEGAN often vary widely due to the differences in landcover, emission factors and canopy models (see e.g., Warneke et al. 2010; Carlton and Baker 2011; Hogrefe et al. 2011; Guenther 2013). In general, comparisons between the models, and between models and observations suggest that MEGAN tends to overestimate and BEIS underestimate BVOC emissions (Carlton and Baker 2011), with MEGAN isoprene emission estimates more than a factor of two higher than BEIS (Warneke et al. 2010; Carlton and Baker 2011; Hogrefe et al. 2011). Carlton and Baker (2011) also found MEGAN methanol emissions to be more than four times higher than the estimates by BEIS. To date, the models have not been found to outperform each other consistently when

coupled to an air chemistry model and compared with measurements of a range of atmospherically relevant chemical species (e.g., ozone, aerosols, formaldehyde) and across a series of sites (see e.g., Warneke et al. 2010; Carlton and Baker 2011; Hogrefe et al. 2011).

16.2.2 Use of Neural Networks for Biogenic Isoprene Emissions

Artificial neural networks (ANNs) constitute a special type of empirical models. They have been shown in numerous studies to describe complex sets of environmental interactions. The existing emission algorithms mainly focus on more rapid variations in isoprene emission and do not consider acclimation over more than 10 days (Guenther et al. 2006). Nevertheless, lower frequency variations, e.g., seasonal changes in tree capacity to release isoprene have been observed to be responsible for a significant, in some cases a major, part of the overall emission fluctuations, reaching up to three orders of magnitude (Monson et al. 1994; Geron et al. 2000; Boissard et al. 2001; Petron et al. 2001). If not correctly assessed, this low frequency variability can represent a major source of discrepancies in isoprene emission assessments (Guenther et al. 1995). Furthermore, the impacts of some environmental parameters, such as water availability, are still not taken into account in these models, due to the complexity of the processes involved.

ANN is a statistical approach to calculate non-linear regressions between the output data, here BVOC emission rates, and a set of relevant input data, environmental parameters (Fig. 16.1). Among the other available statistical methods, ANNs present the advantage of being the most parsimonious, and as with other non-linear regression methods, ANN is not overly sensitive to co-linearity among different explanatory variables (Bishop 1995; Dreyfus et al. 2002). In simulating BVOC emissions, ANNs were first employed by Lasseron (2001), Simon et al. (2005a) and Boissard et al. (2008). Boissard et al. (2008) assessed variations in isoprene emission rates using environmental parameters integrated over a few days to a few weeks prior to the measurements using the emission datasets specifically built for the study. A total of 9 high (instantaneous) to low (up to 3 weeks) frequency regressors were obtained, which together accounted for up to 91 % of the variability in isoprene emission rate (compared to 42 % when the G95 algorithm was employed for the same dataset). The obtained isoprene emission algorithm was mainly sensitive to air temperature cumulated over 3 weeks (*T21*) and to instantaneous light intensity *L0* and temperature *T0* variations. *T21*, *T0* and *L0* alone accounted for 76 % of the overall variability.

Using a similar approach Simon et al. (2005a, b) coupled isoprene and monoterpene emissions measured from Amazonian tree species with physiological and environmental regressors. ANNs have also been used to provide km-scale emission maps of European forest carbon fluxes (Papale and Valentini 2003), and to improve assessments of biogenic soil NO_X emission variations (Delon et al. 2007).

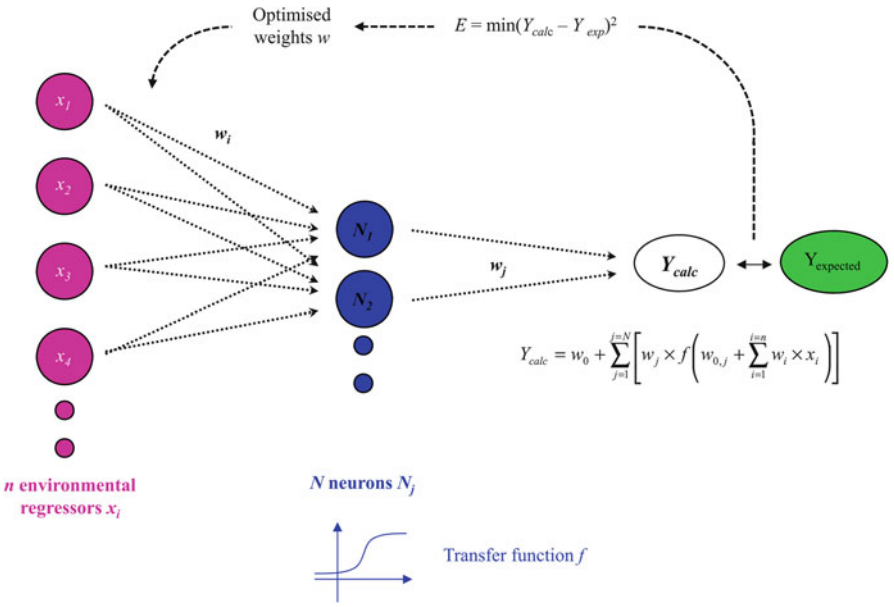

Fig. 16.1 Schematic representation of the structure and functioning principles of an artificial neural network (ANN) for isoprene emission rate purposes using a multi-layer perceptron (MLP) method (a feedforward artificial neural network model). Within an ANN, a different number of neurons N_j can be used and arranged in a network of different layers. Whatever the network (here based on two neurons), every input regressor x_i (for instance environmental parameters) is connected to each neuron N_j, all are connected together and the final output value, Y_{calc}, is calculated according to $Y_{calc} = w_0 + \sum_{j=1}^{j=N}\left[w_j f\left(w_{0,j} + \sum_{i=1}^{i=n} w_i x_i\right)\right]$, where w_0 is the initial connecting weight between the bias and the output, N the number of neurons N_j, f the transfer function (a parameterized bipolar, non-linear function), $w_{0,j}$ the initial connecting weight between the bias and the neuron N_j, w_i the connecting weight between the input and the neuron N_j, and x_i the input regressor. Optimised weights are assessed during a training phase, which consists, starting from random values of w_j, in minimising the difference E between the calculated and expected outputs (for instance measured isoprene emission rates). In this figure, E is calculated as follows: $E = \sum_{k=1}^{k=z}\left(Y_{calc} - Y_{exp}\right)^2$ where k is the number of the z output values

16.2.3 Process-Based Isoprene Emission Models

Process-based models for leaf-level isoprene emission are based on the biochemical processes underlying the synthesis of isoprene from the carbon assimilated during photosynthesis. As described in previous chapters (Li and Sharkey 2013; Monson 2013), isoprene is produced in plant chloroplasts where energy from sunlight is converted to chemical energy in the form of ATP and NADPH for use in carbon fixation and reduction. There are two different pathways for isoprenoid synthesis that both depend critically on the amount of energy available from ATP and NADPH

(Niinemets et al. 1999). The bulk of volatile isoprenoids released from plants are synthesized via the plastidic 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (Lichtenthaler 1999). Thus, the synthesis of these isoprenoids is strongly linked to chloroplastic electron transport rate (Rasulov et al. 2009), undergoing a series of catalytic conversions, via key intermediate compounds such as glyceraldehyde 3-phosphate and dimethylallyl diphosphate (DMADP), to produce the wide variety of hydrocarbons, including isoprene and other terpenes. The four process-based isoprene emission models currently in use assume that isoprene production is intrinsically and quantitatively linked to a single or several rate-limiting steps in precursor-forming reactions, and furthermore, that isoprene is released immediately, i.e., isoprene emission rate is equal to the rate of isoprene production. An overview of these models is provided by Arneth et al. (2007).

Due to the complexities of the biochemical pathways involved and the high degree of species-specificity of some of the processes and reactions, only one of the available process-based algorithms has been incorporated in global-scale emission models (Arneth et al. 2007; Pacifico et al. 2011). The Niinemets et al. (1999) model assumes that the rate-limiting step is the production of DMADP from ATP and NAPDH, which are in turn limited by the rate of electron transport within the chloroplast. The Niinemets et al. (1999) model assumes that the use of electron flow for isoprene production competes with the electron transport required for the assimilation of carbon during photosynthesis, and the model calculates the rate of synthesis, and therefore emission, of isoprene, assuming that a certain fraction of photosynthetic electron transport is diverted to the production of isoprene. The hypothesis has been recently revised in that it is not the competition between photosynthesis and isoprene emission per se, but low effective Michaelis-Menten constant for isoprenoid synthesis pathway leading to strong control of emissions by electron transport rate under physiological conditions (Rasulov et al. 2009, 2011). The emission flux of isoprene, I, is given by (Niinemets et al. 1999; Arneth et al. 2007):

$$I = \varepsilon J \alpha \tag{16.1}$$

where J is the photosynthetic electron transport rate, α is the electron cost for isoprene production, and ε is an empirically derived fraction of photosynthetic electron transport that is used for isoprene production. As the parameter ε represents the activity of the isoprene synthesis pathway in the chloroplasts, it is strongly dependent on temperature and the chloroplastic concentration of carbon dioxide. In this model, initially developed for strong isoprene emitters *Liquidambar styraciflua* and *Quercus* spp. (Niinemets et al. 1999), the electron fraction also depends on isoprene synthase activity, and thus, can vary with species as well as with leaf long-term environment (Monson et al. 2012; Grote et al. 2013 for more details).

As our understanding of the biochemical processes and reactions involved in the synthesis of isoprene and its intermediates improves, so does the prospect of a fully process-based model capable of reproducing the production and emission of isoprene and higher terpenoids without the need for species-specific parameters.

16.2.4 Monoterpene Emission Models

In most monoterpene emitting vegetation, monoterpenes are stored in specialized storage structures referred to as pools. These take a variety of forms, including leaf cavities (*Eucalyptus*), glandular cells (Lamiaceae), resin canals (*Pinus*) and ducts (*Abies*) in conifer needles (see e.g., Fuentes et al. 2000; Loreto and Schnitzler 2010). As a result, emissions of monoterpenes in species with storage structures are decoupled from their synthesis; the emission rate depends on the rate at which the monoterpene volatilizes from the pools and diffuses through the stomata (Fuentes et al. 2000; Grote and Niinemets 2008; Schurgers et al. 2009). Hence, monoterpene emissions are generally dependent only on temperature according to a simple exponential relationship (Guenther et al. 1995, 2012) in the form:

$$E = E_s e^{\beta(T-T_s)} \tag{16.2}$$

where E_s is a species-specific basal emission rate (BER), T_s is the temperature at standard conditions, typically taken 30 °C, and β is a scaling exponent linking the observed monoterpene emission rate to temperature.

Apart from evaporation from storage, several tree species emit monoterpenes entirely (e.g., *Quercus ilex*, Staudt and Seufert 1995; *Fagus sylvatica*, Dindorf et al. 2006) or partly (e.g., *Pinus sylvestris*, Taipale et al. 2011) light-dependently similarly to isoprene. To capture this, the empirical isoprene emission algorithm (Sect. 16.2.1) or the process-based isoprene model (Niinemets et al. 2002, 2013) have been employed to simulate monoterpene emissions. For global-scale studies, the MEGAN model version 2.1 (Guenther et al. 2012) applies varying fractions of light-dependent emissions for the different monoterpenes.

A more explicit approach to account for storage of monoterpenes within the plant has been developed by Schurgers et al. (2009), based on a detailed model by Bäck et al. (2005) that explicitly describes monoterpene synthesis and diffusion from the site of synthesis to ambient atmosphere (Niinemets and Reichstein 2002, 2003). The authors (Schurgers et al. 2009) assume that monoterpene synthesis and emission are decoupled. A fraction of the synthesized monoterpenes is emitted directly, and the remainder is stored in specific structures referred to as storage pools. Emissions from these storage pools are regulated by the size of the pool and a temperature-dependent residence time, such that:

$$E_{\text{emis}} = m/\tau \tag{16.3}$$

where E_{emis} is the monoterpene emission rate from the storage pool, m is the size of the storage pool (g m^{-2}) and τ is the average residence time (days), which also has a temperature dependence. The introduction of the storage pool into the emission algorithms improved the day-to-day pattern of emissions, leading to reduced variability as the pool acted as a buffer between production and release; the model also better described longer-term variations in the emissions as seasonality was much better represented (Schurgers et al. 2009).

16.2.5 Estimates of Global Fluxes

The first estimate of global biogenic isoprene flux was 250 Tg C year^{-1}, calculated by Müller (1992) for the IPCC 3rd assessment report. Guenther et al. (1995) revisited this, systematically assigning emission factors to global ecosystems and combining these with high resolution (0.5° by 0.5°) global vegetation distribution and meteorological data, and arrived at a total of 503 Tg C year^{-1}, but with a very large degree of uncertainty of a factor of three. The standard configuration of the MEGAN model (Guenther et al. 2006, 2012) estimates an average annual isoprene emission of ∼600 Tg isoprene year^{-1}, or ∼530 Tg C year^{-1} for different model parameterizations. Varying driving variables such as meteorology, vegetation distribution and leaf area index datasets yielded estimates ranging from 440 to 660 Tg C year^{-1} (Guenther et al. 2006).

In contrast, a review by Arneth et al. (2008a) on isoprene emission estimates for "present-day" (1990s) conditions generated by the Guenther et al. (1995) algorithms showed an apparent convergence to ∼520 Tg C year^{-1}, with a range of only 460–600 Tg C year^{-1}, in spite of a wide range of driving variables. Analogously, Guenther et al. (2006) found that total emissions varied between −11 and +29 % (from a "standard" run of 500 Tg C year^{-1}) when the MEGAN algorithms were driven with different meteorology, vegetation distributions or leaf area index data. However, Arneth et al. (2008a) argue that given the uncertainty in the basic driving variables, e.g., vegetation distribution and incoming radiation, as well as the assumption of the global validity of emission factors extrapolated from very few enclosure and field campaign measurements, such consensus is not warranted. In particular, comparisons of simulated emissions against measured fluxes (see e.g., Müller et al. 2008; Hewitt et al. 2009; Warneke et al. 2010) unanimously show large discrepancies in both the magnitude and temporal fluctuations (especially longer-term seasonal variations). Arneth et al. (2008a) suggest that there is an urgent requirement for the global biogenic emission modelling community to systematically tackle the sources of uncertainties, such as our understanding of the process of synthesis and emission of isoprene, as well as addressing unknowns, such as emission factors for some biomes. Even a full mechanistic understanding of factors controlling emissions at the leaf and stand level, however, will not eliminate all the uncertainties from calculations of global emissions.

Arneth et al. (2011) reported the findings of an emission model intercomparison study involving three of the most widely used and extensively evaluated global vegetation and emission models: MEGAN (Guenther et al. 2006), LPJ-GUESS (Arneth et al. 2007) and BVOCEM (Lathière et al. 2010). In their "standard" set-ups, average annual emissions for the period 1981–2002 were 378 Tg C year^{-1}, 463 Tg C year^{-1} and 496 Tg C year^{-1} respectively, slightly lower than those generated for the 1990s or 2000s from the respective models due to the lower global average temperature of the 1980s. However, when model inputs were exchanged (e.g., LPJ-GUESS driven with NCEP meteorology as used with MEGAN, etc.), not only did their estimates for total global emissions diverge, but the spatial

pattern of distributions was altered in ways that cannot be fully reconciled with the community understanding of emissions (Arneth et al. 2011). Furthermore, Ashworth et al. (2010) demonstrated that global emissions could vary by up to 32 % (and local emissions by up to 77 %) simply due to altering the time-resolution of the input meteorology. The introduction of a representation of the circadian control of base emission rate observed over a Borneo oil palm plantation (Hewitt et al. 2011) reduced total global isoprene emissions by as much as 21 % (but see Keenan and Niinemets 2012). These studies collectively not only highlight our incomplete understanding of the isoprene emission process, but also suggests that our confidence in a value of 500 Tg C $year^{-1}$ may be somewhat premature.

Differently from isoprene, there is no consensus about the total global flux of monoterpenes, with estimates ranging from ∼30 to ∼150 Tg C $year^{-1}$ (Arneth et al. 2008a; Guenther et al. 2012). It is unclear why monoterpene emission estimates should reflect the uncertainties in driving variables more closely than similar estimates for isoprene, although it has been suggested that greater range in monoterpene simulations reflects outlying observations using problematic parameterizations (Guenther 2013). Thus, convergence of isoprene models is likely to be a result of less variation in the parameter sets used in modelling isoprene fluxes.

The empirical and process-based methods for isoprene and monoterpene emissions explained above can be regarded as two alternatives for the same research questions, and they can be parameterized to result in similar fluxes. However, MEGAN and LPJ-GUESS have differences not only in the emission algorithm, but also in the way vegetation coverage is described. MEGAN, the "empirical" model, comes with a more detailed vegetation description than used in LPJ-GUESS, the "process-based" model, where the description of vegetation is limited to a relatively small number of plant functional types. However, LPJ-GUESS allows for dynamic changes in the vegetation distribution, and is therefore more representative than more detailed but static vegetation distributions in addressing climate change effects on BVOCs.

16.3 Model Evaluations

Measuring and analysing volatile organic compounds emitted by the terrestrial biosphere is essential to improve our understanding of the nature and quantity of chemical species emitted, together with the variability and sensitivity of their emissions to environmental parameters. All global simulation models share the use of basal emission rates (or emission capacities) as the basic way to parameterize vegetation emissions. Although the algorithms in use have developed additional parameterizations to consider effects resulting, for example, from meteorological history (Guenther et al. 2006), seasonal development (Arneth et al. 2008b) or leaf age (Guenther et al. 2006), a common and major drawback of all methods is the missing representation of the observed variability between different stands, individual trees or even branches (Niinemets et al. 2010a; Bäck et al. 2012). The

basal emission rates, as key parameters of the models, also bear large uncertainties, which directly affect the simulated emissions. In order to overcome this problem, large-scale (ecosystem-scale and upward) measurements of emission capacities would be needed. However, determination of the emission capacities at higher scale faces practical problems. Measurements in a controlled environment, as can be done with leaves or branches, are practically impossible, and within-canopy interactions, e.g., between vegetation, micrometeorology, and within-canopy chemistry, would need to be represented properly. Here the ways of conducting direct ecosystem and biome level emissions measurements and proxies for deriving emission estimates are analysed.

16.3.1 Field Measurements

Field campaigns conducted around the world such as BEMA (Seufert et al. 1997), BOREAS (Pattey et al. 1999), BIPHOREP (Laurila and Lindfors 1999), PROPHET (Westberg et al. 2001), ECHO (Spirig et al. 2005), ESCOMPTE (Simon et al. 2005a, b) or OP3 (Hewitt et al. 2010) provide fundamental qualitative and quantitative information improving our capability of testing, evaluating and constraining the BVOC emission models. Flux measurements, performed by different techniques such as gradient profile, relaxed eddy accumulation, disjunct eddy covariance and eddy covariance (Guenther et al. 1996; Ciccioli et al. 2003; Müller et al. 2010), are of particular importance for the direct evaluation of BVOC emission models. Field measurements therefore provide a unique opportunity to test, under different environmental and climatic conditions, the representativeness of BVOC emissions simulated by models for different ecosystems.

In order to evaluate the performance of global models on timescales of seasons to decades, long-term measurements that are run for several years are particularly needed, and currently scarce. Global models capture variability at timescales of hours to weeks well, but knowledge on both the short, episodic events (e.g., emissions related to insects or wind damage) and the long-term changes (seasonal developments and adaptation to changes in climate and CO_2 concentration) are currently poorly addressed in ecosystem-scale measurements and consequently poorly represented in global models.

16.3.2 Use of Satellite Data to Constrain the Emission Models

In parallel with the development of these so-called “bottom-up” methods of estimating and evaluating emissions of BVOCs, efforts have been made to use a “top-down” approach to constrain model estimates. The top-down approach is an indirect method that makes use of satellite measurements of relevant atmospheric constituents to back-calculate the flux of the parent BVOCs. It is not possible

to measure reactive BVOCs such as isoprene directly by satellite as atmospheric concentrations are too low and the spectral lines are broad and overlapping with other atmospheric constituents (see e.g., Palmer et al. 2003), so longer-lived short-chained reaction products that can accumulate at higher concentrations and have sharper spectral lines are used as a proxy. This work has not only led to constraints on total biogenic emissions, but has also highlighted sources of uncertainties in bottom-up flux estimates, advancing understanding of the operating processes (see e.g., Barkley et al. 2009).

Formaldehyde (HCHO) columns have been extensively used for the purpose of constraining isoprene emissions from satellite observations (see e.g., Palmer et al. 2003 , 2006; Barkley et al. 2008, 2009; Shim et al. 2005) and identifying seasonal and spatial variations (Abbot et al. 2003; Barkley et al. 2009; Harrison et al. 2013). HCHO is a useful proxy for isoprene as it is formed rapidly from both isoprene and its direct oxidation products, and has a sufficiently short atmospheric lifetime itself to ensure that it is not transported far from the original source of isoprene (Palmer et al. 2003). In addition, relatively little HCHO is formed from other BVOCs. Monoterpene oxidation products tend to partition to the aerosol phase, and while methanol does oxidize to form HCHO, it does so over a much longer timescale and forms part of the background signal, in theory allowing it to be distinguished from isoprene-derived HCHO (Shim et al. 2005). Satellite derived measurements of formaldehyde columns are, however, themselves subject to significant uncertainties on the order of $\sim$100 % (e.g., Palmer et al. 2006), and require an atmospheric chemistry model inversion or back-trajectory to deduce the required isoprene source strength. Thus, uncertainties in the knowledge and understanding of isoprene oxidation chemistry (see e.g., Prather et al. 2001; Butler et al. 2008; Lelieveld et al. 2008; Archibald et al. 2010) affect the ability of the "top-down" approach to accurately map HCHO to isoprene (Palmer et al. 2006).

Direct comparisons between "top-down" estimates of isoprene emission and those generated by the MEGAN algorithms (Guenther et al. 2006) show good agreement in North America (Abbot et al. 2003; Palmer et al. 2006), and South and East Asia, with the exception of China (Fu et al. 2007). These comparisons also suggest that MEGAN significantly overestimates isoprene emission in the tropics (Fu et al. 2007; Barkley et al. 2008). HCHO columns appear to show better seasonal agreement with direct measurements of both isoprene and HCHO over both North and South America (Palmer et al. 2003; Barkley et al. 2008) than do the MEGAN estimates, although it should be borne in mind that HCHO columns are subject to a great deal more spatial and temporal smearing than the "bottom-up" estimates by models. Shim et al. (2005) estimated global isoprene emission on the basis of HCHO columns, arriving at a value of 566 Tg C year^{-1}, i.e., an excellent agreement with estimates derived from the emission algorithms.

The use of glyoxal retrievals is being developed to further constrain emissions of isoprene and other BVOCs, as well as their atmospheric reactions (see e.g., Stavrakou et al. 2009). Work is on-going to elucidate links between other satellite retrievals, e.g., aerosol optical depth (Veefkind et al. 2011), CO columns (Jiang et al. 2011), and emissions of other BVOCs such as monoterpenes.

Apart from HCHO, other longer-lived volatiles such as methanol can also be measured by remote sensing. Stavrakou et al. (2011) used satellite derived observations of total methanol column to estimate the source strength of methanol from the terrestrial biosphere. Their calculation of 100 Tg year^{-1} was in close agreement to the estimate of 105 Tg year^{-1} generated by the MEGAN algorithm, although analysis of the spatial and temporal discrepancies between the two led to the development of improved MEGAN algorithm for methanol emission (Stavrakou et al. 2011).

16.4 Use of Global Models to Analyse the Feedbacks in the Earth System

16.4.1 Overview of Feedbacks with BVOC

A number of impacts and feedbacks among BVOC emission, vegetation activity and climate potentially occur (Fig. 16.2), and some of these feedbacks are currently being included in state-of-the-art Earth system models (ESMs, Kulmala et al. 2013 for detailed analyses of the impact of global feedbacks). These feedbacks link the terrestrial biosphere with the chemical composition of the atmosphere and, consequently, with climate. Central to these feedbacks are the production, emission and photochemical oxidation of BVOCs. The key processes that influence or are influenced by the production of BVOCs in plants, their emission to the atmosphere and their subsequent photochemical oxidation include the formation and removal of ozone, nitrogen compounds and secondary organic aerosols as well as the methane lifetime and burden in the troposphere. BVOC emissions are also impacted by drought and wildfires affecting the distribution and composition of vegetation, assimilation of CO_2 and evapotranspiration. Since BVOCs can directly and indirectly influence climate via various forcing processes (e.g., methane, ozone, SOA) these processes form multiple feedback cycles between the biosphere and the atmosphere. Arneth et al. (2010) have given a detailed review of the processes and feedbacks that are outlined in Fig. 16.2. Many of the processes outlined in Fig. 16.2 have been recognized only very recently and are still associated with very large uncertainties.

BVOCs emitted to the atmosphere interact with anthropogenic emissions to impact atmospheric composition. Formation of ozone and SOA directly affect climate by altering the atmospheric radiation budget (Myhre et al. 2013). BVOCs also compete with methane for OH radicals and thus can increase methane atmospheric lifetime, the methane burden in the troposphere and, ultimately, the climate because methane is the third most important atmospheric greenhouse gas (Voulgarakis et al. 2013). The resulting changes in surface temperature directly affect BVOC emissions closing the feedback cycle (Kulmala et al. 2013).

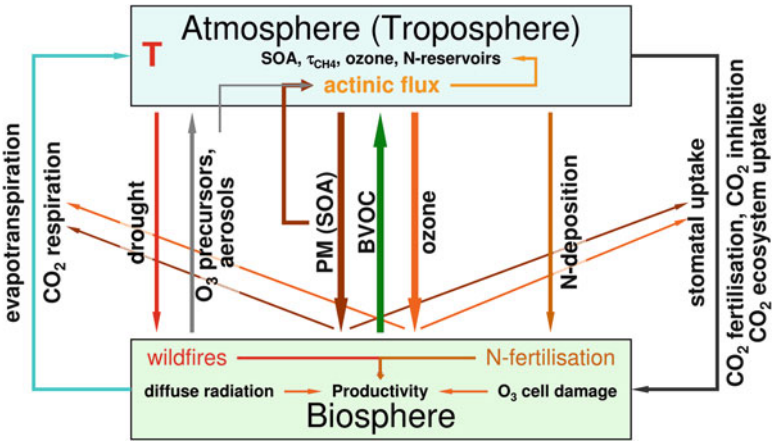

Fig. 16.2 Biogeochemical feedbacks linking biosphere and atmosphere via emission of biogenic volatile organic compounds (BVOC). Ozone and particulate matter (PM) – especially secondary organic aerosols (SOA) – that are produced through atmospheric oxidation influence plant productivity (diffuse radiation, ozone damage to plants). BVOC emissions also significantly influence atmospheric composition and climate, for instance, the lifetime of greenhouse gases such as methane (τ_{CH_4}). On a broader scale, climate and air composition have an impact on the biosphere via deposition of chemical species (e.g., nitrogen species) and growing conditions such a temperature, solar radiation intensity and water availability. Extreme events such as heat waves and droughts promote wildfires which in turn impact on plant productivity and atmospheric composition by emission of ozone precursor species and aerosols. Changes in plant productivity due to biosphere-atmosphere feedbacks also affect exchange processes between the biosphere and the atmosphere such as evapotranspiration and assimilation of CO_2, ozone and other atmospheric species that either promote or hamper plant productivity. From this picture it becomes clear that biogeochemical feedbacks represent a strong link between the biosphere and the atmosphere with important implications for atmospheric composition and climate

A second and potentially equally significant pathway in this feedback cycle is via the interaction of ozone with plant productivity and BVOC formation. Ozone is a potent oxidant and is harmful to plant tissue. Ozone enters the plants via the stomata, and plants react to elevated ozone concentrations by reducing stomatal conductance (Sitch et al. 2007). This has major consequences for CO_2 and water vapour exchange, ultimately affecting carbon assimilation and evapotranspiration, but also for most of the trace gases (Calfapietra et al. 2013 for detailed discussion; Harley 2013).

Such feedback cycles centered around the production and emission of BVOCs affect a number of other processes which could result in multiple feedbacks. Photochemistry of BVOCs, for instance, leads to the formation of SOA that has the potential to change the radiation flux in the atmosphere. Secondary organic aerosols influence the actinic flux and scatter incoming radiation, increasing the diffuse fraction of short-wave radiation, and this is expected to increase plant

productivity (Mercado et al. 2009). SOA also participate in formation of cloud condensation nuclei (CCN) affecting cloud droplet number density, cloud lifetime and precipitation (Carslaw et al. 2010).

Earth system models (ESM) are used to understand these feedbacks and their implications for climate and air quality on both regional and global scales. Many processes are still beyond the grasp of ESMs due to limited understanding. Furthermore, even for processes that are currently included, estimates of their importance in terms of impact and feedback strength vary largely with systematic studies only just beginning (see e.g., Lee et al. 2011). Here, we will make no attempt to present further details on the uncertainties connected with feedbacks involving BVOCs, but will focus instead on recent studies that have looked at some of the individual processes.

16.4.2 Impacts of BVOCs on Methane

Methane is an important greenhouse gas, atmospheric concentration of which has increased from 380 ppbv (Monnin et al. 2001) at the last glacial maximum to 715 ppbv in 1750 (Etheridge et al. 1998) and 1,787 ppbv in 2008 (Dlugokencky et al. 2009). The global mean atmospheric abundance of CH_4 is determined by the interplay between emissions and sinks. CH_4 emissions are very diverse, covering a wide range of natural (wetlands, termites, oceans, marine hydrates, geological sources, wild animals, and wildfires) and anthropogenic (energy, mining, landfills and waste treatment, ruminants, rice agriculture, and biomass burning) sources (Denman et al. 2007).

16.4.2.1 Competition Among Methane and BVOC for OH Radicals

Oxidation of CH_4 by OH radicals represents the main atmospheric sink for methane. However, BVOCs of both natural and anthropogenic origin other than methane (also called non-methane volatile organic compounds, NMVOCs) compete for the available OH radicals, thereby altering the oxidizing capacity of the atmosphere (Levy 1971; Hauglustaine et al. 1998; Bey et al. 2001; Collins et al. 2002; Folberth et al. 2006). Through this interaction, NMOVCs have a significant impact on the chemical lifetime of CH_4 in the atmosphere. Globally, BVOCs dominate by far the total amount of NMVOCs emitted into the atmosphere (Guenther et al. 1995)

Generally, it is expected that volatile compounds deplete OH in an unpolluted environment, thereby increasing the chemical lifetime of CH_4 (Granier et al. 2000; Lelieveld et al. 2002; von Kuhlmann et al. 2004). This perturbation to the CH_4 lifetime will be amplified by the CH_4 feedback on its own lifetime (Prather et al. 2001). However, in a polluted environment, where NMVOC emissions are generally collocated with pronounced emissions of NO_X, NMVOC oxidation leads to a

buildup of ozone and, consequently, OH formation by the catalytic action of NO_X. This widely accepted picture has recently been challenged by Lelieveld et al. (2008), who suggested that oxidation of natural NMVOCs, notably isoprene, might provide a mechanism for OH recycling even in a pristine, low-NO_X environment. This proposed mechanism could effectively limit the impact of primary NMVOCs on the CH_4 lifetime. Thus, it could have significant implications for the future evolution of the CH_4 atmospheric burden, but the mechanism of OH recycling remains largely unclear.

16.4.2.2 Climate Change and Methane-BVOC Interactions

Recent modelling studies have shown that BVOC emissions could increase substantially due to climate change (Sanderson et al. 2003; Lathière et al. 2005). Lathière et al. (2005) calculated an increase in total annual BVOC emissions by 75 % from 725 Tg C $year^{-1}$ at present-day conditions to 1,250 Tg C $year^{-1}$ at the end of the twenty-first century (Fig. 16.3). An increase in BVOC emissions of this magnitude has the potential to significantly affect the methane lifetime, and consequently the methane burden. O'Connor et al. (2010) gave a very rough estimate of the impact of a 75 % increase in BVOC emissions on the methane lifetime and compared this impact to the change in methane lifetime due to climate warming. They came to the conclusion that an increase in BVOC emissions of this order and the chemical kinetic effect on the methane lifetime are of similar magnitude, but opposite in sign and could potentially cancel each other out.

These studies did not take into account the effect of increased atmospheric concentration of CO_2 on the emissions of BVOC. Monson et al. (2007) showed that higher than present-day levels of atmospheric CO_2 can significantly reduce or even completely abolish the effect of climate warming on emissions of isoprene. Modelling studies by Arneth et al. (2007) and Heald et al. (2009) have found little or no impact on future emissions of isoprene in relation to climate warming due to the CO_2 inhibition effect. Pacifico et al. (2012), including the effect of CO_2 inhibition on isoprene emission, calculated the change in methane lifetimes due to changes in isoprene emissions between the present and the end of the twenty-first century. They found a very small decrease in the methane lifetime of only 2 %. However, other studies demonstrate that depending on growth conditions, elevated CO_2 actually might enhance the emissions, especially if this is coupled to enhanced leaf area index (Sharkey et al. 1991; Sun et al. 2012), suggesting that any global modelling of elevated CO_2 effects on isoprene emission is bound to large uncertainty.

It is currently not fully clear whether the emissions of other BVOCs such as, for instance, terpenes are also sensitive to elevated levels of atmospheric CO_2 concentration. Increasing, decreasing or constant emissions in response to elevated CO_2 have been observed, and the effects apparently differ for different monoterpenes (Loreto et al. 2001; Staudt et al. 2001).

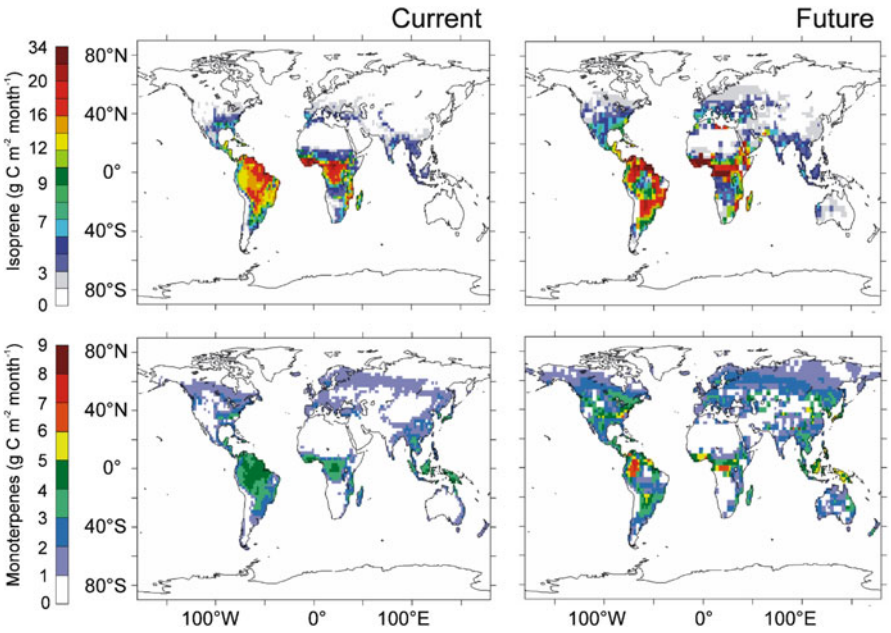

Fig. 16.3 Distribution of annual emissions (g C m^{-2} $month^{-1}$) of isoprene (*upper panels*) and monoterpenes (*lower panels*) for present-day (*left*) and future (*right*) scenarios according to Lathière et al. (2005). BVOC emissions are calculated using the ORCHIDEE global vegetation model including parameterizations from Guenther et al. (1995). The present-day simulation uses 1990s climate forcing from CRU (Climate Research Unit, UK). The future simulation employes the climate reconstructed by the LMDz general circulation model for 2100s for an atmospheric CO_2 concentration of 560 μmol mol^{-1}. The inhibition effect of increasing atmospheric CO_2 concentration on isoprene emissions is not taken into account in this work (Wilkinson et al. 2009; Sun et al. 2012)

16.4.2.3 Methane-BVOC Interactions: Lessons from the Past

In addition to model simulations, we may learn about the future evolution of the interactions between BVOC emissions and atmospheric methane concentrations by turning to the past. Studying past atmospheric CH_4 fluctuations from ice cores allows for some of the vast uncertainties around the magnitude of the CH_4-NMVOC feedback in future climate to be constrained. Atmospheric CH_4 concentrations have increased by more than 65 % from the last glacial maximum (LGM), 21,000 years before present, and to the preindustrial era (Chappellaz et al. 1993, 1997; Brook et al. 2000; Valdes et al. 2005; Kaplan et al. 2006; Harder et al. 2007). This has been attributed to a substantial increase in BVOC emissions from a more productive tropical forests and the developing boreal forests as a consequence of the shrinking of the continental ice sheets (Valdes et al. 2005; Kaplan et al. 2006; Harder et al. 2007).

Valdes et al. (2005) and Kaplan et al. (2006) have calculated an increase of the CH_4 lifetime from the last glacial maximum to the preindustrial holocene due to the significant increase in BVOC emissions over the same time period. They found an increase in the methane lifetime by approximately 1.3 years (19 %) and 2.1 years (29 %) from 7.1 (Valdes et al. 2005) and 7.3 (Kaplan et al. 2006) years at last glacial maximum to 8.4 (Valdes et al. 2005) and 9.4 (Kaplan et al. 2006) years, respectively, at the preindustrial Holocene (PIH) as a consequence of increasing BVOC emissions. The estimates of the two groups are based on different chemistry-climate models and BVOC emission models. In those two studies, BVOC emissions were calculated to increase by 100 % (Valdes et al. 2005) and 57 % (Kaplan et al. 2006) over the same time period. This increase in CH_4 atmospheric lifetime would account for between 55 and 88 % of the increase in the atmospheric CH_4 concentration from LGM to PIH (Valdes et al. 2005; Kaplan et al. 2006). However, these model studies have large uncertainties since many effects cannot be taken into account at the current stage of model development. Nevertheless, these modelling studies have found some experimental support recently (Loulergue et al. 2008). Because of its inherently non-linear nature, it is not possible to extrapolate the past feedback directly into a future atmosphere. Nevertheless, it seems legitimate to assume that a similar feedback process as in the past will result in a feedback of the same sign and possibly similar magnitude in the future.

16.4.3 Impacts and Feedbacks of BVOCs, SOA and Diffuse Radiation

16.4.3.1 Role of BVOCs in SOA Formation and in Cloudiness

Photochemical oxidation of BVOCs, in particular isoprene and terpenes, leads to semivolatile organic compounds that can then partition into the particulate phase to form SOA. Observations indicate that in many places around the world, more than half of the submicron aerosol mass is actually organic (Zhang et al. 2007). The organic material in these aerosols seems to be dominated by compounds of biogenic origin (Hallquist et al. 2009). For example, particle growth rates in boreal forests correlate with seasonal variation in primary productivity (Kulmala et al. 2004). However, the global budget of secondary organic aerosols remains very uncertain. Current best estimates of 12–70 Tg $year^{-1}$ (Kanakidou et al. 2005) may even be too small by up to an order of magnitude (Hallquist et al. 2009). A limited understanding of the relative contribution of biogenic SOA precursors and their atmospheric photo-oxidation mechanisms as well as the magnitude of their emissions from the terrestrial biosphere represents the dominant contributor to the uncertainties around formation of secondary organic aerosols. Other important factors include condensation, heterogeneous chemistry and evaporation of semivolatile oxidation

products of BVOCs on aerosol surfaces and even polymerization of these chemical species (Fuzzi et al. 2006; Hallquist et al. 2009).

Emissions of SOA precursors such as isoprene and many terpene species are very sensitive to temperature but also to levels of solar radiation, soil moisture, foliar biomass and to varying degrees to several other environmental factors (Guenther et al. 1995, 2006). Many of these environmental factors are likely to change in response to global alterations in climate. Furthermore, the abundance of secondary organic aerosols and their properties can also depend on the composition of the atmosphere. Atmospheric composition, in turn, is sensitive to changes in climate but also to the amount of anthropogenic volatile species and BVOCs. For example, key environmental factors that determine the concentration of the OH radicals in the atmosphere are temperature, water vapour content and solar irradiance. The latter itself is affected significantly by the amount, structure and geographic distribution of clouds on the global scale.

Oxidized organic aerosols dominate the submicron aerosol mass over a wide range of continental environments (Kanakidou et al. 2005; Zhang et al. 2007). Consequently, these organic aerosols, in fact, of both primary and secondary origin, have a significant direct impact on the atmospheric radiation budget by absorbing and backscattering of solar radiation. Furthermore, SOA have been found to represent an important factor in the growth of particles up to the size where they can act as cloud condensation nuclei (CCN) at tens of nanometres (Allan et al. 2006; Laaksonen et al. 2008). Via this route, SOA significantly contribute to indirect aerosol effects on climate. Particle formation via conversion of gas-phase species into the aerosol phase contributes between 5 and 50 % to the global mean CCN concentration in the boundary layer (Spracklen et al. 2008). Recently, it has been shown that emission of BVOCs may control particle growth (Bonn et al. 2009) but they can also suppress particle formation (Kiendler-Scharr et al. 2009).

Models of aerosol microphysics and chemistry have become fairly complex in recent years (e.g., Tsigaridis and Kanakidou 2003; Vignati et al. 2004; Mann et al. 2010; Bellouin et al. 2011). These models have been integrated into chemistry-climate models (CCMs) and ESMs to investigate the impacts of SOA and resulting feedbacks. In particular, aerosol models have been coupled interactively to BVOC emission models and atmospheric chemistry models (e.g., Tsigaridis et al. 2006; Bauer et al. 2010; Kulmala et al. 2013).

16.4.3.2 Influences of BVOCs on Diffuse Radiation Fraction and Implications for Productivity

Another mechanism that has the potential to impact the production of BVOCs by the terrestrial vegetation is the effect of changes in the fraction of diffuse radiation on plant photosynthesis. Changes in cloud cover or atmospheric aerosol, the latter arising from natural sources such as volcanoes or anthropogenic sources such as fossil fuel use and biomass burning, can alter both the total photosynthetic quantum flux density (PPFD) and its diffuse fraction. Aerosols emitted from wildfires or

secondary organic aerosols (SOA) produced in situ from oxidation of BVOCs can also increase the diffuse PPFD fraction.

Changes in diffuse PPFD fraction can have major effects on plant photosynthesis. In general, plant photosynthesis increases non-linearly with incident photosynthetic quantum flux density (PPFD). A saturation point is often reached at light levels on bright days during the growing season. Under clear-sky conditions only a fraction of the canopy is directly exposed to sunlight while the rest of the canopy remains in the shade. Under cloudy or hazy conditions, the incoming sunlight is subject to a higher degree of scattering, and so the PPFD is more uniformly distributed. In fact, both theoretical and observational studies have demonstrated recently that photosynthesis can be more efficient under diffuse light conditions (e.g., Gu et al. 2003; Niyogi et al. 2004; Oliveira et al. 2007). In a modelling study, Mercado et al. (2009) have investigated the impact of changes in diffuse PPFD on the global land carbon sink and estimated that "global dimming" between 1960s and 1999 has increased the carbon sink by about 25 %.

The effect of changes of diffuse PPFD on BVOC production and emission through increased photosynthetic activity is likely to lead to another globally active feedback cycle between plant productivity, BVOC emission fluxes, atmospheric photochemistry and (secondary) organic aerosols (Kulmala et al. 2013). Studies coupling aerosol models interactively to biogeochemical cycles, cloud formation and radiation fluxes have started to increase our understanding of how natural aerosols respond to changes in climate and atmospheric composition (Kulmala et al. 2013). However, the Earth system models that are required for these studies are still in their early phase. In addition, much of the immediate connections among different processes are still so poorly understood that process-level description in models is not yet possible. Nevertheless, models including these feedbacks at the level of best understanding provide encouraging evidence of the major significance of the feedback loops between global change, BVOCs and SOA (Kulmala et al. 2013).

16.4.4 Impacts and Feedbacks of Ozone Leaf Damage

Further environmental impacts on BVOC emissions currently under investigation are ozone leaf damage due to exposure to surface ozone and reduced isoprene emission as a consequence of severe drought (Pegoraro et al. 2004). Ozone affects adversely both human health and vegetation with the adverse effects on plants first identified in the 1950s. Ozone causes cellular damage inside the foliage, thereby reducing plant photosynthetic rate, accelerating leaf senescence and requiring greater resource allocation to detoxification and repair (Ashmore 2005). At present, many regions worldwide already experience near-surface ozone concentrations that are persistently higher than 40 ppbv. At these levels, ozone may already cause visible damage such as leaf reddening and necrosis, and reduction in crop yields (Ashmore

2005). A further increase in the tropospheric ozone concentration is expected over the twenty-first century (e.g., Hauglustaine et al. 2005).

Tropospheric ozone enters plants primarily through the stomata. Its potential effect on the emission of BVOCs is through its impact indirectly on plant primary productivity and directly on stomatal openness which is expected to temporally alter water-soluble BVOC emissions (Niinemets and Reichstein 2003; Harley 2013). However, an increase in the production of BVOCs as a protection mechanism against ozone damage has also been suggested (e.g., Sharkey et al. 2008; Vickers et al. 2009). In the NO_X-depleted leaf interior, BVOCs react with ozone leading to depletion in ozone concentration thereby reducing the damage.

The uptake of ozone by plants via the stomata is in itself an important ozone removal process (e.g., Folberth et al. 2006; Fowler et al. 2009). Hence, the emission of BVOCs by plants, the subsequent formation of ozone through photochemical oxidation of BVOCs initiated by OH or even ozone itself, the removal of ozone through stomatal exchange fluxes and the subsequent damage to leaf cells by ozone altogether form a closed feedback cycle that can influence both ozone concentrations, at least close to the surface, and BVOC emission. In addition, the ozone-BVOC feedback cycle has the potential to affect other important atmospheric constituents, in particular CO_2 and water vapour that are subject to stomatal regulation. Thus, the ozone-BVOC feedback cycle could potentially have a significant impact on climate by affecting CO_2 assimilation by terrestrial vegetation (Sitch et al. 2007) and even evapotranspiration.

Currently, the mechanisms that contribute to, and the strength of the ozone-BVOC feedback cycle are poorly understood. Only one model study has incorporated the effect of ozone on plant productivity to assess the impact of ozone on the carbon cycle (Sitch et al. 2007). To date, the link between ozone and BVOC emissions is not well understood, although it potentially can have a major significance at local and global scales (Lerdau 2007).

16.5 Perspectives and Challenges

While the global- and regional-scale emission models discussed above have the capability of calculating emission rates of a wide range of BVOCs, most simulations are confined to estimates of so-called constitutive emissions of the trace gases believed to account for the majority of the reactive carbon flux from vegetation. Although it has been suggested that many thousands of different BVOCs are released from the biosphere (Goldstein and Galbally 2007), a recent highly detailed inventory of BVOC emissions (Guenther et al. 2012) demonstrated that to the best of our understanding, and in line with observations, a mere 11 compounds account for over 80 % of the flux to the atmosphere each year. Four compounds are routinely included in global emission estimates. These are isoprene, a generic monoterpene (typically α-pinene), methanol and acetone, accounting for nearly 75 % of the estimated total flux (Guenther et al. 2012).

In order to have confidence that our models are producing realistic estimates for BVOC emissions under present-day conditions that are accurate across a range of temporal and spatial scales, and for the right reasons, requires not only a thorough understanding of the physiological and phenological drivers of the synthesis and emissions of these compounds but also an appreciation of the level of detail necessary to achieve a suitable precision in model output. As a community, global emission and atmospheric chemistry modelers are constantly confronted by the question of how much detail is sufficient detail. While this is obviously governed to a certain extent by the scientific question being addressed by the model, and its spatial and temporal extent, we do not have the capability at present to include the emissions of all compounds under all conditions. Nor should we strive to. However, we must be certain that we have sufficient understanding of the compounds emitted and their subsequent impacts on atmospheric composition, air quality and climate to ensure that correct decisions can be made regarding the level of detail required to answer the research question being addressed.

An estimate of the total annual global emission of a handful of BVOCs may be sufficient for a global study focusing on long-term climate impacts on terrestrial emissions. On the other hand, model simulations intended to provide air quality forecasts for a limited area require a much more spatially and temporally explicit representation of both emissions and atmospheric reactions. If we are to accurately capture rapid fluctuations in atmospheric concentrations of critical pollutants, a similarly precise level of detail is required from biogenic emission models. So, what do we currently miss in our global models, and what do we need?

16.5.1 Facing the Uncertainties

The main challenge facing global modelling of BVOC emissions is to reduce uncertainties and produce emission estimates that are robust enough to lend confidence to calculations of future projections and hindcasts. Uncertainties in global modelling arise from three main sources: internal variability (stability, resilience to random perturbations and errors), model uncertainty and, for all but present-day simulations, scenario uncertainties (Hawkins and Sutton 2009). Ultimately, the greatest confidence in modelled emission estimates must be associated with a model that is grounded on the fundamental processes governing the synthesis and emission of these compounds, rather than on empirical fits to data. As discussed earlier in this chapter, the best-developed and most widely used BVOC emission model represents the latter, although it is based on the temperature and light responses similar to photosynthesis (Guenther et al. 1995, 2006, 2012), while the process-based models still require some empirical fitting to data (Grote et al. 2013). While both approaches are able to a similar degree capture present-day emissions (Niinemets et al. 2013), their robustness under past and future climate trajectories is uncertain.

In the case of BVOC emissions, internal variability may simply reflect our lack of fundamental understanding of the processes governing the synthesis and emission of

these compounds (Monson 2013). As discussed in the previous chapters (Fineschi et al. 2013; Li and Sharkey 2013; Monson 2013), even the base emission rates for isoprene, the most studied among the biogenic trace gases, and for monoterpenes, the second most widely studies class of compounds, appear to fluctuate, even between neighboring trees of the same species, for reasons that are currently beyond our ability to explain (see e.g., Niinemets et al. 2010b; Bäck et al. 2012). Comparisons between modelled and measured isoprene fluxes appear to show emission factors that change due to time of day (Funk et al. 2003; Hewitt et al. 2011), season (Müller et al. 2008; Grote et al. 2010), geographical location and position within the canopy (Niinemets et al. 2010b, 2011). These changes can be partly explained by our current knowledge of the drivers of isoprene synthesis, but full mechanistic understanding has not yet been reached.

Model uncertainties are a result of simplifications in the representations of BVOC emission mechanisms, as well as other simplifications within the model itself. Each of the process-based models that has been developed for simulating isoprene and monoterpene emissions is based on a single process within the isoprene synthesis pathway, e.g., electron transport (see Arneth et al. 2007 for an overview), rather than integrating all the processes known to occur (Grote et al. 2013). In addition to this simplification of the overall process description, the genuine unknowns, such as the fraction of electrons used for isoprene synthase, are further parameterized through empirical fits to data (Arneth et al. 2007). Furthermore, in order to apply these models, developed from observations at the leaf level, to the ecosystem and then to the regional and global scales, a limited number of plant functional types is used, in spite of the known variation between synthesis and emission rates between species (see e.g., Schurgers et al. 2011; Guenther et al. 2006). At the regional and global scales, these uncertainties are further compounded by uncertainties in the input data, such as global vegetation distribution, leaf area, and meteorology (Guenther et al. 2006; Arneth et al. 2011).

Quantification of these uncertainties is difficult, but as Sect. 16.2.5 demonstrates, the effects can be very large in some cases, albeit the isoprene models tend to converge to a "right" value. This remarkable agreement in total global emissions within and between models does not extend to comparisons between modelled and measured fluxes. For example, Müller et al. (2008) found that modelled hourly fluxes at a site in the Northern mid-latitudes were, on average, 35 % lower than the measured fluxes, while the emissions estimated in the Amazon were between a factor of two and five times too high in the wet season. Langford et al. (2010) reported emissions estimated with the MEGAN algorithms (Guenther et al. 2006) over South-East Asian rainforest that were four times higher than observed fluxes, but a factor of two lower than the peak midday flux of isoprene from a nearby oil palm plantation (Misztal et al. 2011). Hewitt et al. (2010) showed that, in spite of large discrepancies in emission rates and landcover data, two estimates of total isoprene emission for the whole Borneo agree to within 5 %. However, there were significant differences in the spatial distribution of the emissions (Hewitt et al. 2010), suggesting that global emission estimates may converge to similar values due to compensation of errors.

Obviously, the predictions of future NMVOC emissions from the biosphere are surrounded by extremely large uncertainties related to both the magnitude and sign of the emission change in the future but also to ecosystem adaptation and distribution in the changing climate. Current knowledge only gives a wide range of possible scenarios of BVOC emissions under future climate conditions spanning from no change to a large increase at the end of the twenty-first century. These predictions also depend on the climate scenarios used to extrapolate future climate conditions.

16.5.2 The Crucial Need for Observations

The large number of studies carried out over the last few years, including field campaigns, laboratory experiments, satellite data integration and model development, have significantly improved our knowledge regarding BVOCs. However, there are still large gaps in data regarding the diversity, emission rate and variability, and chemical reactivity of BVOCs emitted by the terrestrial biosphere, underlining the crucial need for observations. The validation of regional and global models developed for BVOC emission is essential, but also highly complex, because the emissions are characterized by a strong spatial and temporal variability and a high sensitivity to environmental conditions (including climate, ecosystem, and atmospheric concentrations of trace gases such as CO_2). Observations, both field- and laboratory-based, under a range of conditions, are therefore highly valuable to elucidate the major emission drivers and improve BVOC emission schemes, with flux measurements being the only way to evaluate emission model performance directly. However, the sparse data we do have are, in many cases, corrected to "standard conditions" using the very same emission models we are trying to use for evaluation of the measurements, thereby introducing further bias and uncertainty into the process (Niinemets et al. 2010a, b for a discussion).

To perform a consistent evaluation, a full set of data acquired over long time periods and in a large variety of ecosystems and meteorological conditions, throughout the tropics, and mid- and high-latitudes, are of special interest. Such data would facilitate testing the model capacity to represent the spatial and temporal variabilities in BVOC emissions. On top of the emission flux data, complementary information and observations regarding vegetation characteristics (ecosystems considered, leaf area, canopy height) and meteorology are essential to constrain the model with the field site specifics. These evaluations are typically performed by comparison of simulated emissions with observations from a number of different field sites. Even when showing that model results lie in the range of field data, it should be acknowledged that such model evaluation often leaves the modeler with the feeling of unfinished and unsatisfying work. To be fully robust, the evaluation of large-scale regional or global emission models should include the use of long-term, preferably permanent, quality controlled, fully documented flux measurements, conducted with standardized evaluation tools, for example, in line with the procedures developed for

data from the FLUXNET network (a network integrating worldwide CO_2, water and energy flux measurements, http://fluxnet.ornl.gov/).

There is also a need to bridge the gap in both measurements and models between the instantaneous leaf-level processes and fluxes, and the canopy- or ecosystem-scale fluxes. This gap is, in reality, bridged by a plant canopy, a complex structure with a high level of heterogeneity of temperature, light and chemical composition (e.g., Guenther 2013; Noe et al. 2012). Such heterogeneity drives rapid dynamic chemical processes that are at best highly simplified in models. To what extent should processes acting on short timescales be included? Again, this is likely to depend on the location, time, and precise nature of the scientific question being addressed, and requires systematic sensitivity studies and assessments coupled to high-quality flux data.

Data for BVOCs or their oxidation product concentrations from field studies or from satellite products offer an indirect but complementary approach to evaluate BVOC emissions and atmospheric chemistry and transport models. Satellite data, in particular, enable the evaluation of model performance on large spatial and temporal scales. The main issue in the use of concentrations, rather than fluxes, is that concentrations provide a proxy for integrated fluxes rather than instantaneous fluxes, and reflect the balance between compound production and destruction. Thus, use of concentrations requires inclusion of atmospheric chemistry schemes, thus, adding a layer of uncertainty and complexity.

We conclude that in order to gain an understanding of the fundamental processes behind the synthesis and emissions of BVOCs, and to address the question of how much detail is indeed enough detail, we require more observations across larger spatial and longer timescales.

16.5.3 Taking Global Modelling Forward

As outlined above, global models currently confine BVOC emission estimates to so-called constitutive emissions, i.e., those compounds that are continuously emitted under plant's normal growing conditions. In addition to these compounds, vegetation is known to produce a wide range of induced BVOCs, i.e., compounds that are synthesized and emitted in response to both biotic and abiotic stresses, and during particular phenological periods, for example during bud-burst or flowering. Emissions under such conditions differ from constitutive emissions in both quantity of carbon emitted and precise compound mix (Loreto and Schnitzler 2010; Niinemets 2010). In some cases, a large number of different trace gases are produced in small quantities. In others, a few compounds are released in very large quantities (Loreto and Schnitzler 2010). In all cases, the importance of such emissions depends on the precise compound mix, the size of the flux to the atmosphere, the timing of the event (both in terms of time of day and time of year), the location of the flux and the reactivity of the compounds involved (Arneth and Niinemets 2010; Niinemets et al. 2010a, b). While regular, well-documented events such as the emissions that occur

in response to mechanical wounding during forest or meadow harvesting periods could be included in a model, sporadic, irregular events, such as herbivory would be significantly more difficult to incorporate, although ways on incorporating such emissions have been proposed (Arneth and Niinemets 2010).

Furthermore, can we be certain that the responses of vegetation to environmental stresses observed now will continue in the future, or are the plants likely to adapt to future changes? It has been observed that vegetation growing in hotter regions, for example, displays a different temperature response than vegetation that naturally grows in cooler areas, with a higher temperature optimum for BVOC emissions (see e.g., Geron et al. 2006). It could perhaps be expected that, as temperature rises in the future, vegetation will acclimate to this, with a slow shift in temperature response. Similar changes could occur in responses to increasing atmospheric concentrations of carbon dioxide or to changing patterns of precipitation or radiation, and clearly again emphasize the need for more experimental data to bring modelling global processes forward.

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Chapter 17
Climate Feedbacks Linking the Increasing Atmospheric CO_2 Concentration, BVOC Emissions, Aerosols and Clouds in Forest Ecosystems

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Abstract Biogenic volatile organic compounds (BVOCs) play a central role in atmospheric chemistry via their high reactivity in the gas phase and via their participation in atmospheric new particle formation and secondary organic aerosol formation. The emissions of BVOC to the atmosphere depend on several climate-related variables, making these compounds part of complex, yet potentially very important, climate feedback mechanisms. Here we illustrated the role of BVOCs in enhancing gross primary production (GPP) and cloud droplet number concentrations. The first of these phenomena forms a positive feedback loop for the terrestrial carbon sink (GPP feedback), whereas the second one forms a negative feedback loop for the ambient temperature increase (temperature feedback).

17.1 Introduction

The atmosphere forms a major part of the environment that strongly impacts life on the Earth. The atmosphere closely interacts with the biosphere, hydrosphere, cryosphere and lithosphere on timescales from seconds to millennia. Changes in any of these components are directly or indirectly communicated to others via intricately linked processes, feedbacks, and interactions.

Recently, the importance of atmospheric aerosols to global radiation budget, cloud formation, and alleged human health effects has motivated several investigations. Reactive gases, greenhouse gases and atmospheric aerosols are tightly

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connected with each other not only via physical, chemical and meteorological, but also via biological processes occurring in the atmosphere and at the atmosphere-biosphere interface (Arneth et al. 2010; Carslaw et al. 2010; Mahowald 2011; Quinn and Bates 2011). Human actions, such as emission policy, forest management and land-use change, as well as various natural feedback mechanisms involving the biosphere and atmosphere, have substantial impacts on the complicated couplings between atmospheric aerosols, trace gases, greenhouse gases, air quality and climate (Arneth et al. 2009; Raes et al. 2010; Shindell et al. 2012).

Anthropogenic emissions of greenhouse gases have increased substantially during the past century. Elevated concentrations of CO_2 and methane have been pointed out as the most important forcing agents on climate during recent decades (IPCC 2007). However, it is not straightforward to describe the climate change in sufficient detail, since there are several feedback mechanisms that are hard to understand quantitatively. It has been recognized for decades that the biosphere plays an important role in climate. It has also been suggested that the biosphere tends to regulate and stabilise climate in order to keep it optimal for living organisms, as described by the Gaia hypothesis (Lovelock 1979). Charlson et al. (1987) presented the so-called CLAW-hypothesis, supporting the Gaia hypothesis. The CLAW hypothesis connects ocean biochemistry and climate via a negative feedback loop involving the ambient temperature, plankton activity, natural sulphur emissions from the ocean to the atmosphere, cloud condensation nuclei production and cloud albedo change.

17.1.1 Setting the Scene: The Terrestrial CLAW Hypothesis

In terrestrial ecosystems, a number of potential climate feedback mechanisms have been identified (Arneth et al. 2010) (see also chapter by Ashworth et al. 2013). Here we focus on the continental CLAW hypothesis related to forest-atmosphere-climate interactions proposed by Kulmala et al. (2004). We will investigate two feedback loops associated with the continental CLAW summarized in Fig. 17.1. Both these loops are initiated by increased CO_2 concentrations, but are then separated by two partly interacting branches. The central points of these two loops are (i) the plant gross primary production (GPP) driven by photosynthesis connected with the atmospheric CO_2 concentration, and (ii) the ambient temperature (T) which, on average, is higher in the world with larger CO_2 concentrations. Increased GPP and T are hypothesized to cause higher emissions of biogenic volatile organic compounds (BVOC) which, as a result of atmospheric chemistry, lead to increased secondary organic aerosol (SOA) concentrations. An increased SOA concentration is expected to increase (i) the ratio between diffuse and global solar radiation via enhanced scattering by aerosol particles, and (ii) cloud droplet number concentrations (CDNC) via increased concentrations of cloud condensation nuclei (CCN). Increased diffuse radiation fraction closes the GPP-loop via a positive feedback for the carbon sink, while increased CDNC in turn closes the T-loop via a negative feedback for the ambient temperature increase. While plausible, neither of these two loops has been quantified, nor firmly proved, so far.

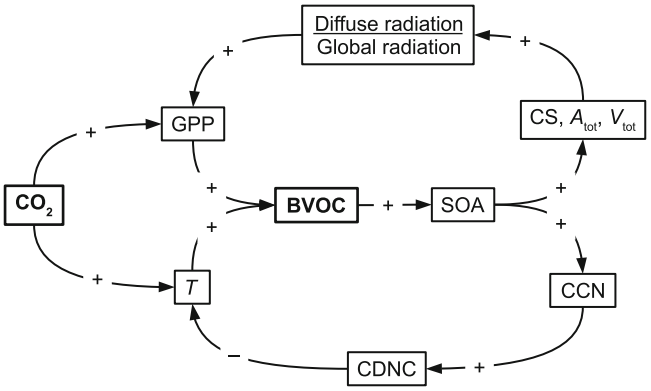

Fig. 17.1 Two feedback loops associated with the continental CLAW hypothesis driven by increased atmospheric CO_2 concentration. The loop *above* is the clear-sky loop and the one below is the cloudy-sky loop. Here T is the ambient temperature, GPP is the gross primary production, BVOC refers to the biogenic volatile organic compounds, SOA to the secondary organic aerosol, CS is the condensation sink (Eq. 17.1), A_{tot} is the total aerosol surface area and V_{tot} is the total aerosol volume, CCN refers to the cloud condensation nuclei, and CDNC is the cloud droplet number concentration

17.1.2 Components of the Terrestrial CLAW Hypothesis

The plant gross primary production (GPP) is the difference between the net ecosystem exchange of CO_2 (NEE) and the total ecosystem respiration (TER). In the boreal forest zone, photosynthesis occurs predominantly in sunlight during the growing season (Hari and Mäkelä 2003) and is inhibited in winter. Forest ecosystems are usually sinks of CO_2, and a direct negative feedback (the higher the CO_2 concentration, the higher the rate of photosynthesis, reducing CO_2 in the air) exists between increasing atmospheric CO_2 concentrations and photosynthesis. On the other hand, a positive feedback exists between ecosystem respiration and temperature. At higher temperatures, water becomes a more important factor influencing both GPP and TER.

Terrestrial vegetation contributes substantially to emissions of BVOCs, and these emissions are closely related to photosynthesis (Fuentes et al. 2000). The ratio of BVOC emission to carbon assimilation is generally a few percent (Guenther et al. 1995; Grace and Rayment 2000). These emissions serve many functions in plants, and may vary between plant species (Niinemets et al. 2010). BVOCs are emitted as mixtures of a variety of compounds depending on the seasonal and diurnal metabolic activity of the plants. The most important compounds with respect to atmospheric chemistry are terpenoids, i.e., isoprene, monoterpenes and sesquiterpenes. These compounds can be synthesized in both aerial and belowground plant parts, and large storage pools of some compounds are found in e.g., conifer foliage, trunks and roots. The turnover rates of the terpenoid pools depend on both prevailing synthesis level and factors controlling their evaporation. The biosynthesis of terpenoids is regulated either by the supply of substrates, by the availability of energy, or by

enzyme activities in the metabolic branching points or end-points of the biosynthetic pathways (Bohlmann et al. 1998; Fischbach et al. 2002; Dudareva et al. 2004) (Li and Sharkey 2013; Rajabi Memari et al. 2013; Monson 2013). Diffusion of compounds from the site of their synthesis and storage to plant surfaces and further to atmosphere is proportional to the concentration differences between the components of the pathway and conductance along the diffusion pathway. Emission of many BVOCs (including many non-oxygenated mono- and sesquiterpenes) is not controlled by stomatal opening, and therefore they evaporate from the surfaces at a compound-specific rate, as a function of temperature (e.g., Copolovici and Niinemets 2005; Grote and Niinemets 2008; Grote et al. 2013; Harley 2013).

In the short term, both isoprene and monoterpene emissions show a clear temperature dependence owing to the exponential relationship between their volatility and temperature. Isoprene emissions are connected to irradiance as well because of the close link between the isoprene emission and electrons derived from photosynthetic light capture (Niinemets et al. 1999). Furthermore, the observed *de novo* synthesis of monoterpenes indicates that prevailing light conditions can also be important for their biosynthesis even in terpene-storing species such as several conifers including pine (*Pinus* spp.) and spruce (*Picea* spp.) species (Shao et al. 2001; Ghirardo et al. 2010), but especially in non-storing species such as Mediterranean evergreen oaks (e.g., *Quercus ilex*, *Q. coccifera*, and *Q. suber*) (Staudt and Seufert 1995; Loreto et al. 1996) and some temperate broadleaf trees such as European beech (*Fagus sylvatica*) (Dindorf et al. 2006). In addition to the short-term drivers, emissions are responsive to medium-term changes in growth conditions, which may affect the emission capacity, the shape of the light response as well as the temperature optimum of emission (Sharkey and Loreto 1993; Lerdau and Throop 2000; Wilkinson et al. 2009; Heald et al. 2009; Grote et al. 2013; Monson 2013).

Effects of climate change on terpenoid emissions, and especially the effect of elevated atmospheric CO_2 concentration, have obtained increasing attention in recent years. Abundant literature on the direct effect of elevated CO_2 concentration shows that, in general, isoprene emissions tend to decline with the rise of CO_2 concentration (e.g., Wilkinson et al. 2009; Possell and Hewitt 2011, but see also Sun et al. 2012). In contrast, studies of mono- and sesquiterpene emission responses under elevated CO_2 are rather scarce. Based on the available evidence, it is likely that monoterpene emissions may not be as responsive to changes in CO_2 concentration as isoprene emission (e.g., Constable et al. 1999; Loreto et al. 2001). Also, increases in monoterpene emissions due to a simultaneous exposure to elevated CO_2 and temperature have been reported (Staudt et al. 2001; Räisänen et al. 2008).

In the longer term, emissions of isoprene and monoterpenes may increase significantly when the projected changes in plant species composition, vegetation productivity and leaf area index (LAI) or density (LAD) associated with the climate change are taken into account (Heald et al. 2009). The CO_2-driven increase in the photosynthetic rate leads consistently to an increase in the tree productivity in terrestrial ecosystems, although in the long term, down-regulation associated with the nutrient or water availability may influence the magnitude considerably (for a review, see Medlyn et al. 2011). If no down-regulation is considered, model

estimates predict consistent increases in net primary production (NPP), and even more so if the interaction with temperature and prolonged growing season is taken into account. These predictions are supported by the results of free-air CO_2 enrichment (FACE) studies (Norby et al. 2005). Thus, although elevated CO_2 concentrations may or may not affect the rate of monoterpene emission per unit foliage mass, the simultaneous effects on the LAI and productivity (in this paper we use gross primary production, GPP), combined with the effects of increasing temperature on biosynthesis and volatilization in the short term, and on growing season length in the long term, are predicted to increase isoprene and monoterpene emission rates in large regions over the Northern Hemisphere.

Once emitted to the atmosphere, BVOCs participate in atmospheric oxidation processes initiated by OH and NO_3 radicals, ozone and by some yet poorly quantified oxidants such as stabilised Criegee intermediates (Mauldin et al. 2012). Some of the resulting oxidation products condense on the pre-existing aerosol particles increasing their size, and some are able to form the very smallest aerosol particles formed by atmospheric nucleation. This process, due to the strong size-dependence of the aerosol scattering coefficient, is expected to lead to an increased fraction of diffuse solar radiation. Increased diffuse radiation in turn enhances the plant photosynthesis by providing more light into shaded areas in forest canopies. This effect of the increased fraction of diffuse to total solar radiation on photosynthesis has been studied by Mercado et al. (2009) on a global scale using a global climate and land-use model. This will cause a positive feedback mechanism between increasing CO_2 concentrations and biogenic activity.

Over the boreal forest environment, secondary organic aerosols formed from BVOCs frequently dominate aerosol particle number concentrations (Tunved et al. 2006), affecting notably aerosol light scattering and even more so CCN concentrations (Lihavainen et al. 2009). Globally, atmospheric new-particle formation and growth associated with sulphur and BVOC emissions has been suggested to give a large contribution to the CCN budget (Spracklen et al. 2008; Merikanto et al. 2009; Pierce and Adams 2009; Yu and Luo 2009; Kerminen et al. 2012), which causes a major uncertainty in the present-day indirect climate forcing estimates (Wang and Penner 2009; Kazil et al. 2010; Makkonen et al. 2012a, b). In the future, when primary aerosol particle concentrations associated with anthropogenic activities are projected to decline, the climatic role of natural aerosols associated with BVOC emissions is probably even larger than today (Makkonen et al. 2012a).

17.2 Methods to Analyse the Significance of Feedback Loops

17.2.1 GPP Loop in Boreal Forests

The field data used in this study have been measured during the years 1996–2011 at the University of Helsinki SMEAR II station in Hyytiälä, southern Finland (61°51′N, 24°17′E, 181 m above sea level) (see Fig. 17.2 for a view of the

Fig. 17.2 A view from the measurement mast of the Hyytiälä SMEAR II station. The station is located in a Scots pine (*Pinus sylvestris*) dominated 50-year-old forest. Basic meteorological parameters such as temperature, relative humidity and wind speed are continuously measured at six levels of the station's 74 m tall mast, as well as concentrations of trace gases such as SO_2, CO_2, CO and O_3. Solar radiation is measured with pyranometers (Middleton Solar and Delta-T Devices Ltd). Aerosol size distributions are measured in the mobility size range of 3–1,000 nm (3–500 nm until December 2004) using a twin-DMPS setup (Differential Mobility Particle Sizer; Aalto et al. 2001). Larger particles are measured with an Aerodynamic Particle Sizer (APS). Enclosure measurements are performed for gas-exchange (photosynthesis, transpiration and BVOC emissions) at shoot level (For additional details see Hari and Kulmala 2005)

measurement site). At the SMEAR II station, continuous and comprehensive measurements on the exchange processes between the atmosphere and land-ecosystem are performed. The SMEAR II station environment represents a typical boreal coniferous Scots pine (*Pinus sylvestris*) dominated forest. Further details of the station and the measurements are given by Hari and Kulmala (2005).

In order to characterize the effect of secondary organic aerosol production on the number concentration and size distribution of the aerosol population, we calculated from the Differential Mobility Particle Sizer (DMPS, Fig. 17.2 for the description of experimental setup) data the condensation sink (CS). The condensation sink describes the aerosol particles' ability to remove vapour molecules from air, and it is closely linked to the total surface area of the particles and can therefore be used as a proxy for the light scattering properties of the aerosol population. The value of CS is calculated from the aerosol number size distributions according to (see e.g., Kulmala et al. 2012):

$$\mathrm{CS} = 4\pi D \sum \beta_m \left(d_p\right) d_p N \left(d_p\right), \tag{17.1}$$

where D is the vapour diffusion coefficient of the condensing vapour (typically assumed to be sulphuric acid when calculating the value of CS), d_p is the particle diameter, $\beta_m(d_p)$ is the Fuchs-Sutugin transition-regime correction factor for particles of given size, and $N(d_p)$ is the number concentration of particles of size d_p. The summation in Eq. 17.1 is carried over all the size bins of the measured aerosol size distribution.

To quantify the GPP feedback loop in a consistent way, it is crucial to look at all components of the loop at a certain temperature and radiation window. Without this approach, it would be impossible to separate the effects of CO_2 increase from the effects of solar radiation and temperature. Actually, the GPP feedback loop could also be called clear-sky feedback loop. Here all the calculation steps are based on measured data.

The initial driving force in the feedback loop is the increase in atmospheric CO_2 concentration, which enhances photosynthesis, i.e., the forest gross primary production, GPP. The value of GPP is obtained as the difference between the total ecosystem respiration (TER) and net ecosystem exchange (NEE) of CO_2:

$$\mathrm{GPP} = \mathrm{TER} - \mathrm{NEE}. \tag{17.2}$$

Here, NEE was measured with the eddy covariance technique, i.e., has a negative sign for net carbon uptake by ecosystem (Markkanen et al. 2001; Suni et al. 2003), whereas TER was modelled on the basis of nighttime NEE measurements (Suni et al. 2003; Kulmala et al. 2004). This gives the amount of chemical energy fixed by the vegetation.

In order to obtain as reliable trend in the atmospheric CO_2 concentration as possible, we utilised the measurements made at the Global Atmosphere Watch station at Mauna Loa, Hawaii. These measurements should represent the global CO_2 values in the northern hemisphere as the Mauna Loa station is situated far away from any local sources of CO_2.

Since BVOC emissions depend on both GPP and temperature, we divided all the studied data into 5-degree temperature bins and considered each season separately. The diffuse radiation fraction is also one component in our feedback loop, and to separate out the effect of cloudiness in the other steps, we divided the measurement days into cloudy and cloud-free conditions according to the brightness parameter, P, defined as the daily ratio of the summed global radiation to the theoretical radiation sum. The theoretical radiation is calculated based on the elevation angle of the sun and the latitude of the measurement site, and it describes the maximum amount of solar radiation that can be received in totally cloud-free conditions. The calculation of the brightness parameter has been explained in more detail by Kulmala et al. (2010). As a threshold value for a day to be classified as cloudy we used $P < 0.3$ and for cloud-free days we used $P > 0.6$. These values are derived from comparisons of the brightness parameter to cloudiness estimated from satellite images (Sogacheva et al. 2008).

Here, the following five connections in the GPP loop (Fig. 17.1) will be investigated based on the field measurements: (1) the effect of increased CO_2

concentration on the forests' photosynthesis and gross primary production (GPP) (the measurement data were taken from the period 1996–2011), (2) the effect of the changing GPP on the BVOC emissions, more specifically, on monoterpene concentrations (period 2006–2011), (3) the effect of the changes in monoterpene concentrations on the SOA concentration in terms of the increase in the value of CS (period 2006–2011), (4) the connection between CS and diffuse radiation fraction of the global radiation (period 2000–2009), and (5) the connection between the diffuse radiation fraction and GPP (period 2000–2009).

17.2.2 Analysing the Temperature-Related Loop with Modelling

Modelling the feedback loop associated with the ambient temperature increase relies on several poorly quantified and often very crudely parameterized relationships.

Generally, the BVOC source is prescribed in global aerosol models. With a few exceptions, global models include the emission data from Guenther et al. (1995, 2006, 2012; Guenther 2013), although optional algorithms and emission inventories are available (Ashworth et al. 2013; Grote et al. 2013; Niinemets et al. 2013). However, the simulated aerosol distribution could be rather sensitive to spatial and temporal differences in BVOC emission fields. When using a climate model, the simulated climate most likely differs from the climate data used to obtain the results by Guenther et al. (1995). Recently, the BVOC emission parameterizations have been implemented to global aerosol models, allowing one to simulate the BVOC-aerosol-climate feedback interactively (e.g., O'Donnell et al. 2011).

In most global aerosol models, secondary organic aerosol formation is treated simply as an emission of primary particles. This "primary-SOA" approach disregards all the dynamics and chemistry related to SOA formation, and assumes that a certain amount of biogenic precursor immediately forms a certain number of particles, (e.g., Stier et al. 2005). When taking into account the dynamics of SOA formation, two approaches are generally used: the kinetic one and the thermodynamic one (Riipinen et al. 2011). The kinetic approach assumes that low-volatile organic vapours condense irreversibly on the existing particle population, usually according to the available aerosol surface area, whereas the thermodynamic approaches assumes equilibrium in the organic vapour concentration between the gas and aerosol phases. Despite the huge number of different organic compounds in the atmosphere, their properties are usually lumped together and described with one or two components. Recently, implementations of the volatility basis set (e.g., Donahue et al. 2011) capable of dealing with a larger number of organic vapours have been introduced into global aerosol modelling frameworks, yet this approach is computationally too expensive for climate simulations.

The connections between BVOC emissions, SOA formation and CCN concentrations are extremely sensitive to the assumptions made on new-particle formation, primary aerosol size distribution and SOA formation (e.g., Pierce and Adams 2009;

Spracklen et al. 2010). In the "primary-SOA" approach, the BVOC oxidation products do not provide growth for sub-CCN particles, and the CCN-increasing ability of BVOC emission depends on the assumed size distribution of the primary-SOA particles and other vapours available for particle growth (e.g., sulphuric acid). With the kinetic or thermodynamic approaches, the effect of BVOCs on the particle size distribution depends on the assumptions made on gas-particle partitioning. Pure thermodynamic partitioning might lead to an underestimation of the condensational flux to the smallest particles, leading to less growth for newly formed particles and increased particle sink.

Here, we used the global climate model ECHAM5-HAM (Stier et al. 2005) to simulate aerosol concentrations, cloud properties and total aerosol forcing with present-day (year 2000) and future (year 2100) emissions. A detailed description of the model implementation applied here, including the treatment of BVOC emissions, new particle formation, SOA formation and aerosol-cloud interaction, can be found in Makkonen et al. (2012a) and will not be repeated here.

Similar to most other global models of aerosol formation, particulate and gaseous emissions are implemented in the lowest model level, close to vegetation surface, in ECHAM5-HAM. The exception is wildfire emissions, which are assumed to rise higher up in the atmosphere. Sulphate emissions from industry and power generation are assumed to be injected a bit higher in the atmosphere, around 100–300 m. This means that, on average, the main sinks of condensable vapours (CS) and newly-formed particles (coagulation sink) are highest in the lowest model level with a sharp decrease with rising altitude. Following the decrease in condensation sink, the highest nucleation rates are usually found in the second-lowest model level. Near the surface, a larger fraction of the organic vapours formed by BVOC oxidation condense on pre-existing particles, increasing the condensation and coagulation sinks and decreasing new-particle formation rates. As a result, increased BVOC emissions may lead to decreased total particle number concentrations in the near-surface air. However, higher up in the atmosphere, where the influences of BVOCs on condensation and coagulation sinks are less effective, the increased particle growth rates caused by BVOCs act to increase the survival probability of nucleated particles.

17.3 Significance of the Feedback Loops

17.3.1 Quantitative Experimental Evidence on the Feedback Loop Associated with GPP

In the first step, which initiates the feedback loop, we studied the effect of increasing atmospheric concentration of CO_2 on the biogenic activity of the forest at the Hyytiälä SMEAR II station. This effect is called CO_2 fertilization. As a direct indicator of this biogenic activity we use the gross primary production (GPP)

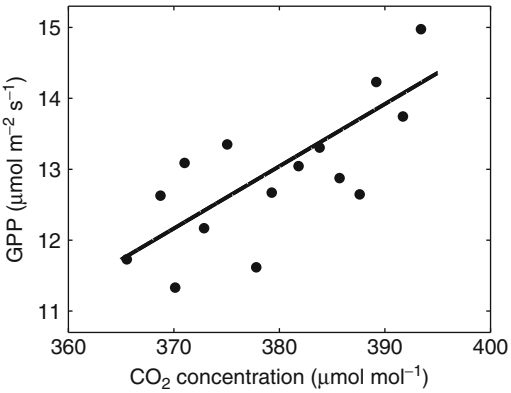

Fig. 17.3 Annual average gross primary production as a function of air CO_2 mole fraction in the Hyytiälä *P. sylvestris* dominated boreal forest (Fig. 17.2 for the site). The data are taken on clear days (brightness parameter larger than 0.6) from months May-August in the temperature range 18–23 °C. The *solid line* shows the linear least-squares fit to the data ($r = 0.74$, $P < 0.002$)

which describes the carbon uptake from the atmosphere into biosphere by the photosynthetic activity of the plants. The annual average GPP measured at the SMEAR II station by eddy covariance technique correlated positively with the atmospheric CO_2 concentration (we used the CO_2 measurement data from Mauna Loa representing the northern hemisphere average conditions). The correlation of annual average GPP with the atmospheric CO_2 concentration during the months May-August is shown in Fig. 17.3. The increase rate is on the order of 1 % per ppm of CO_2 concentration increase.

A significant correlation of the volatile organic vapour concentrations with the gross primary production was observed in the Hyytiälä Scots pine (*P. sylvestris*) dominated forest across the whole study period of 15 years (Fig. 17.4a). We used here the measured monoterpene concentrations, which are known precursors for condensing organic vapours. The highest observed monoterpene concentrations seemed to increase approximately linearly with increasing GPP, whereas at high GPP levels, also low monoterpene concentrations could be observed. Increasing monoterpene concentrations led to increased values of condensation sink, as shown in Fig. 17.4b. This is caused by the monoterpene oxidation products condensing on pre-existing particles and increasing their size, and hence their surface area which is related to the CS. Increased concentrations of oxidized BVOC can also enhance the growth of particles from nucleation events, which leads to increase in the number concentration of particles in the Aitken and even in accumulation modes. Especially, the accumulation mode particles larger than 100 nm in diameter contribute strongly to the condensation sink. The accumulation mode particles are also large enough to scatter sunlight. This was seen in our observations as positive correlation between the CS and the diffuse fraction of global radiation (Fig. 17.5a). Finally, to close the observational feedback loop, we plotted the daily average GPP against the diffuse radiation fraction in Fig. 17.5b. Although there were also high values of GPP at low values of R_{diff}/R_{glob}, the highest values of GPP and no low values of GPP were observed when the ratio R_{diff}/R_{glob} was high. In all the steps of the feedback loop, the results shown in Figs. 17.4 and 17.5 indicate a statistically significant positive correlation.

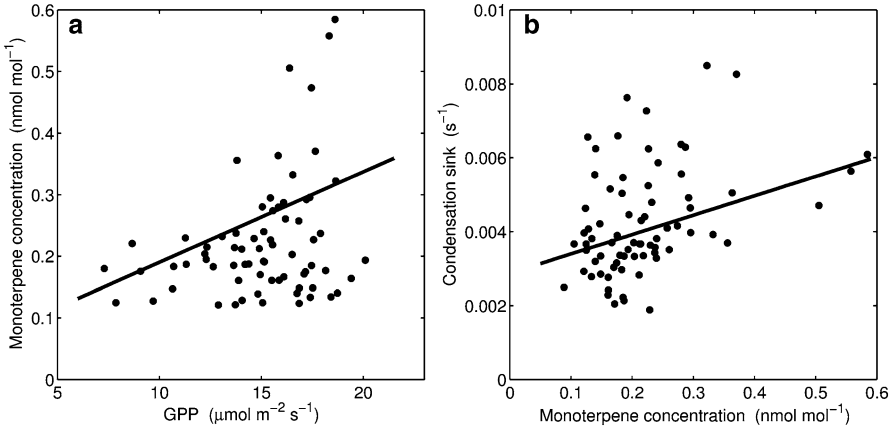

Fig. 17.4 Monoterpene ambient air concentration as a function of gross primary production (**a**) and the condensation sink (Eq. 17.1) as a function of the monoterpene concentration (**b**) in the Hyytiälä *P. sylvestris* dominated boreal forest. The number of data points (daily averages during months May–August in the years 2006–2011) is 78 for (**a**) and 76 for (**b**). The data were selected such that the temperature was in the range 18–23 °C and the brightness parameter larger than 0.6 (clear days). Monoterpene concentration was measured with a proton-transfer reaction mass spectrometer (PTR MS). The *solid lines* show the linear least-squares fits to the data ($r = 0.28$, $P < 0.02$ for (**a**) and $r = 0.27$, $P < 0.02$ for (**b**))

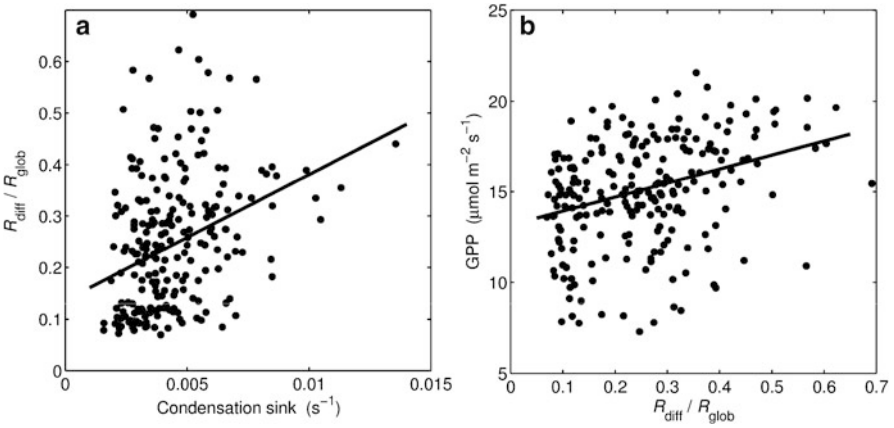

Fig. 17.5 Diffuse radiation fraction of the observed global radiation (R_{diff}/R_{glob}) as a function of the condensation sink (**a**) and gross primary production as a function of R_{diff}/R_{glob} (**b**) in the Hyytiälä *P. sylvestris* dominated boreal forest. The number of data points (daily averages during months May–August in the years 2000–2009) is 224 for (**a**) and 230 for (**b**). The data selection as in Fig. 17.4. The solid lines provide the linear least-squares fits to the data ($r = 0.39$, $P < 0.001$ for (**a**) and $r = 0.28$, $P < 0.001$ for (**b**))

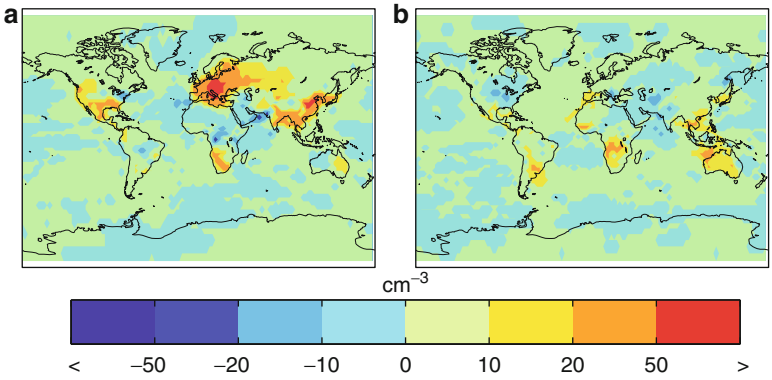

Fig. 17.6 Simulated global absolute increases in the concentration (cm^{-3}) of cloud condensation nuclei at 0.2 % supersaturation, CCN(0.2 %), due to doubling of BVOC emissions and with anthropogenic emissions of the year 2000 (**a**) and 2100 (**b**)

Short-term drivers may decouple the link between CO_2 and BVOC emissions, especially isoprene (see e.g., Arneth et al. 2007, 2011), but these processes are not taken into account here. Water limitation can further override the inhibitory effect of elevated CO_2, leading to increased isoprenoid emissions in a climate change scenario with warmer and drier climate (Pegoraro et al. 2005; Sun et al. 2012). As discussed by Arneth et al. (2011), the overall direction and magnitude of emission change will strongly depend on the vegetation changes, relative importance of the short-term and longer-term factors, and the potential physiological acclimation responses resulting in gradual and continuous increase in CO_2.

17.3.2 The Feedback Loop Associated with Temperature

As a demonstration of the potential strength of the feedback loop associated with future temperature increases, we made model simulations with standard and doubled global BVOC emissions. Since the connection between BVOC emissions and resulting changes in CCN concentrations and cloud properties are expected to depend strongly on anthropogenic emissions, the simulations were made with both present-day (year 2000) and anticipated future (year 2100) anthropogenic aerosol and their precursor emissions. Yet another simulation was carried out with the year 1750 emissions in order to calculate the radiative forcing. In our model implementation, doubling monoterpene emissions from 127 to 254 Tg per year increased the annual SOA formation from 19 to 38 Tg of SOA, which is still in the low end of SOA formation estimates (Carslaw et al. 2010). Maps of CCN concentration changes in different model runs are shown in Figs. 17.6 and 17.7 and summarized in Table 17.1 and Fig. 17.8.

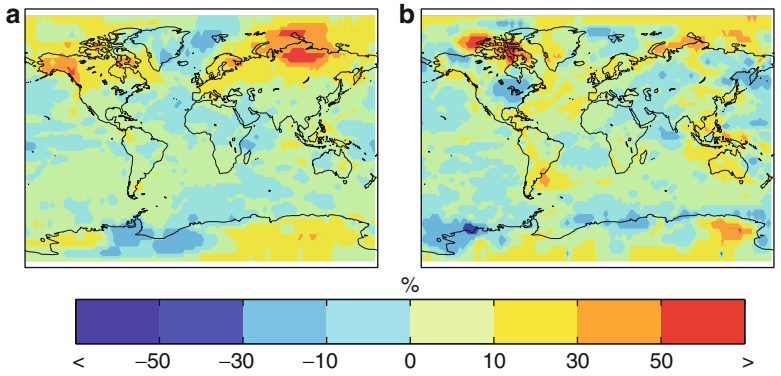

Fig. 17.7 Modelled relative increases (%) in CCN(0.2 %) concentrations due to doubling of BVOC emissions and with anthropogenic emissions of the year 2000 (**a**) and 2100 (**b**)

Table 17.1 Global average surface-level concentration (cm^{-3}) of cloud condensation nuclei at 0.2 % supersaturation, CCN(0.2 %), over land and ocean areas in years 2000 and 2100

	Year 2000		Year 2100		Decrease between years 2000 and 2100	
	Land	Ocean	Land	Ocean	Land	Ocean
1 × BVOC	217	60	89	38	−60 %	−36 %
2 × BVOC	226	62	93	41	−59 %	−35 %
Increase due to doubled BVOC emission	+4 %	+3 %	+5 %	+6 %		

Bottom row shows the increase in CCN(0.2 %) concentration due to doubling of BVOC emissions. The *right columns* show the decrease in CCN(0.2 %) concentration due to changes in anthropogenic emissions between years 2000 and 2100

With the exception of Central and Northern Africa, doubling of BVOC emission with present-day (year 2000) anthropogenic emissions increased CCN concentrations above the continental areas (Fig. 17.6a). The strength of this increase was related to the number concentration of sub-CCN sized particles in each location: local hotspots of CCN increases can be seen in Europe, North America and South Africa, which all have larger numbers of sub-CCN size anthropogenic particles available for growth. To the contrary, tropical regions in Africa and South America showed smaller concentrations of sub-CCN particles. Although BVOC emissions are high in the tropics, absolute increases in CCN concentrations were modest there compared with regions affected by anthropogenic emissions. Over areas dominated by sea-salt or dust particles, a major portion of condensable organic vapours was taken up by these relatively large particles, leading to a slower growth of newly-formed particles. AeroCom recommendations (Dentener et al. 2006) followed by many global aerosol models suggest a rather large primary particle emission size

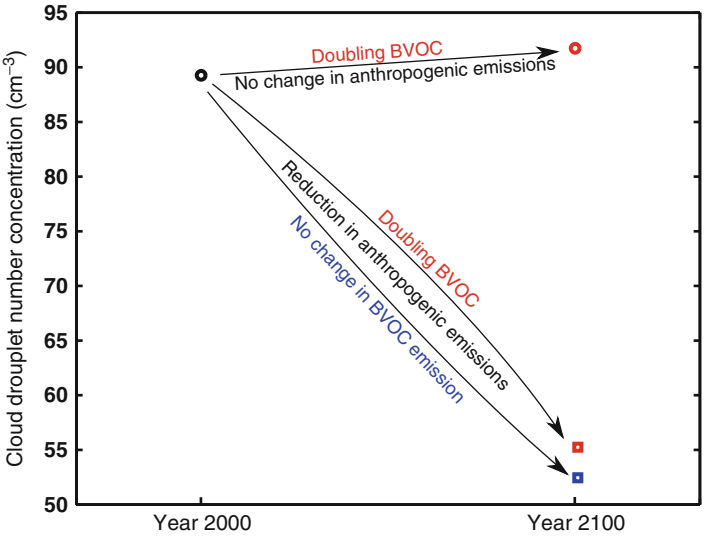

Fig. 17.8 Predicted change in global cloud droplet number concentration (CDNC, cm^{-3}) due to changes in anthropogenic and biogenic emissions

(median diameter 80 nm) from wild-fires. These particles are already near cloud droplet activation sizes at the time of emission, which reduces the sensitivity to BVOC emission in areas dominated by wild-fires.

The cooling effect of an increased cloud albedo is higher when the contrast between land and cloud albedo is higher. Above a snow-covered landscape, a large portion of radiation would be reflected back to space even without clouds, leaving less room for the first aerosol indirect effect. On the other hand, above a dark forests or ocean, the difference between cloud and land albedo can be very large. Even when assuming the SOA formation from BVOCs to take place over the continents only, some fraction of particles affected by the SOA formation will be transported to atmosphere over oceans. In our simulations, doubling of continental BVOC emissions increased CCN concentrations by 4 % over land and 3 % over ocean areas (Table 17.1). The doubling of BVOC emissions with present-day anthropogenic emission levels leads to a 3 % increase in global CDNC in our simulations, which modified both cloud albedo and lifetime causing the global cloud radiative effect of about -0.2 W m^{-2}. This suggests that the cooling potential associated with the feedback loop connecting BVOC emissions to increased ambient temperatures is definitely non-negligible.

As discussed before, the indirect effects of BVOCs depend on the existing population of sub-CCN particles. In many models, the continental sub-CCN sized particles are mainly of anthropogenic origin. Strong emission reductions of anthropogenic primary particles and aerosol precursors are predicted throughout the twenty-first century (Lamarque et al. 2011). The reductions in particle number concentration will hence decrease the CCN formation from BVOCs. However, the

overall decrease in the CCN concentrations might lead to an increased susceptibility of cloud albedo to CCN concentrations. Figure 17.6b shows increases in CCN concentrations due to the doubling of BVOCs when using anthropogenic emissions for the year 2100. While the absolute global CCN concentration increase of 3 cm^{-3} with the year 2100 anthropogenic emissions is slightly less than with present-day emissions (4 cm^{-3}), the relative increase is higher due to overall lower aerosol concentrations (Fig. 17.7). Also, the land/ocean contrast is changed with present-day and future anthropogenic emissions (Table 17.1). Due to the reductions in anthropogenic emissions, CCN concentrations over the oceans are decreased by 36 %, and the relative effect of doubling BVOC emission is increased from 3 to 6 %. This could indicate potential modification in marine cloud properties. Relative increases in CCN concentrations due to doubling of BVOC emissions are emphasized at the high latitudes of the Northern Hemisphere (Fig. 17.7).

It is clear that the magnitude of the possible climate feedback via BVOC emissions is strongly dependent on the evolution of anthropogenic emissions (Fig. 17.8). In addition to providing seed particles for further growth, human activities can influence the oxidative capacity of the atmosphere. The SOA formed from BVOCs with the aid of anthropogenic pollutants can make a large contribution to the total SOA (Spracklen et al. 2011), which is another example of couplings in the climate system that should be investigated further.

17.4 Conclusions

We have illustrated and investigated two partly interacting climate feedback loops connected to terrestrial BVOC emissions, both driven by increased atmospheric CO_2 concentrations. The central point of first of these two loops is the plant gross primary production (GPP), which is hypothesized to be fed back positively via BVOC-induced organic aerosol formation and resulting changes in the ratio between diffuse and global solar radiation. The central point of the second loop is the ambient temperature which has been hypothesized to be fed back negatively via BVOCs-induced CCN production and resulting changes in the cloud properties.

The feedback loop associated with GPP was investigated here by analysing 15 years of atmospheric measurement data from a boreal forest site in Hyytiälä, Finland. A firm positive relation between all drivers forming the individual links in this feedback cycle was found, providing strong empirical support for the existence of such loop in a boreal forest environment. In future, we should aim to find out whether the same feedback loop is active in other terrestrial ecosystems as well and, using combination of measurement and modelling tools, to quantify the strength of this feedback.

The feedback loop associated with the ambient temperature increase was investigated here by using global model simulations. While subject to large uncertainties due to several simplifying assumptions, our simulations demonstrated that this second feedback loop may be regionally very important, and non-negligible even

in the global atmosphere. The overall strength of this feedback is likely to be increased in the future when anthropogenic emissions of primary aerosols and aerosol precursor gases are expected to decline.

From the atmospheric chemistry and aerosol system point of view, BVOCs are crucial both locally and globally because of their important role in both new particle formation and growth. By participating actively in chemical reactions, including the atmospheric oxidation capacity, BVOCs connect several feedback loops involving the biosphere and atmosphere. In order to quantitatively assess the impact of BVOCs on future biosphere-atmosphere-climate interactions and feedbacks, it is important to understand in more details the life cycle of BVOCs in the atmosphere.

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Chapter 18
State-of-the-Art of BVOC Research: What Do We Have and What Have We Missed? A Synthesis

Ülo Niinemets and Russell K. Monson

Abstract This book summarizes recent advancements in the resolution and quantification of the controls on tree BVOC emissions, including efforts toward synthetic projections using computer models. Major progress has been achieved in understanding the molecular mechanisms of volatile synthesis and emission, the role of emissions in plant stress tolerance and elicitation of emissions under biotic and abiotic stresses. Use of this rich source of insight not only allows for improvement of regional air quality estimations under current climate and atmospheric conditions, but it also allows for improvements to the models and observations needed to predict BVOC emissions under future climate and atmospheric conditions. As our understanding of physiological mechanisms, taxonomic distribution and multitrophic interactions in forest ecosystems increases further, we will be able to tackle some of the large-scale feedback loops between BVOC emissions, plant stress, and climate that have eluded us for so long.

18.1 Determinants of Diversity of BVOC Emitters and Emission Diversity

Trees are traditionally considered to be the key BVOC emitters and they make the greatest contributions to BVOC emissions worldwide. It has been a long-standing enigma as to why plants emit those BVOCs not involved in herbivore protection,

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in particular, isoprene, and light-dependent monoterpenes, and why not all, but only some species exhibit high rates of constitutive emissions? It is particularly enigmatic as to why this trait is so clearly associated with woody, principally tree, species. Studies with the plastidic isoprenoid pathway inhibitor, fosmidomycin, and with isoprene fumigation (Sharkey and Singsaas 1995; Singsaas et al. 1997; Loreto et al. 2001b), and work with transgenic plants with repressed (Behnke et al. 2007, 2010; Rosenkranz and Schnitzler 2013) or constitutively increased (Vickers et al. 2009; Velikova et al. 2011) isoprene emission suggests that isoprene emission increases tolerance to recurrent cycles of extremely high temperature, drought and oxidative stress. Similarly, experiments with suppression of constitutive monoterpene emission by fosmidomycin-feeding followed by monoterpene fumigations (Loreto et al. 1998, 2001a; Delfine et al. 2000; Copolovici et al. 2005; Llusià et al. 2005) have demonstrated that monoterpenes can fulfil analogous functions in leaves.

Thus, there appears to be an important role for constitutive emissions in abiotic stress tolerance. The question remains, however, as to why this trait is expressed in only some tree species. Multiple monoterpene synthase genes are present in all plant species, and plants that store monoterpenes in their leaves, and emit these compounds, occur among both woody and herbaceous species. In other species without storage structures, monoterpene emissions can be triggered (induced or upregulated) by multiple biotic and abiotic stresses (Loreto and Schnitzler 2010; Niinemets 2010; Trowbridge and Stoy 2013). Yet, the constitutive emissions of de novo monoterpene emissions in species without specialized storage are relatively rare. Obviously, modification in the regulatory elements in the promoter sequence of given monoterpene synthase genes can change the mode and location of monoterpene synthase expression, but information concerning the promoter sequences of monoterpenes is limited (Rajabi Memari et al. 2013).

Isoprene synthase genes analysed so far have been found to have high sequence homology with certain monoterpene synthase genes (Miller et al. 2001; Sharkey et al. 2005) and even a bifunctional acyclic monoterpene (myrcene) synthase/isoprene synthase gene has recently been described (Sharkey et al. 2013). Given the presence of multiple monoterpene synthases, and accordingly, the potential for multiple origins of isoprene synthase, it is even more surprising why a trait that improves tolerance to abiotic stress has not evolved in all plant species. Exposure to heat, drought and oxidative stress occurs broadly across taxonomic groups. Fineschi et al. (2013) in their chapter analyse recent phylogenetic evidence (Monson et al. 2013; Sharkey et al. 2013) and suggest that the probability of occurrence of isoprene emission is strongly driven by climatic conditions and atmospheric level of CO_2, as well as phylogenetic inertia. The benefits of isoprene emission are larger when photosynthesis is suppressed due to the combination of low atmospheric CO_2 concentration and stress (thus, increasing the need for non-photochemical energy quenching), whereas stress amelioration by a molecule that is volatile provides its greatest benefit when the stress is episodic, not chronic. Thus, the evolutionary diversification of isoprene emission is particularly favoured in atmospheres with low [CO_2] and in relatively humid environments which may nevertheless occasionally experience extremely high temperatures and water stress

(Fineschi et al. 2013). In fact, phylogenetic evidence suggests multiple events of loss and evolution of isoprene emission (Monson et al. 2013). On the other hand, emission has a cost in terms of photosynthetic carbon loss, especially under stress, and thus, isoprene emission seems to be confined to plant genera that occur frequently in habitats such as open, early-successional forests where photosynthesis is higher due to greater light availability, while emissions of less volatile monoterpenes are more common among late-successional species that often tend to grow under and form a dense canopy (Harrison et al. 2013).

There clearly seem to be cost-benefit effects operating on the appearance and disappearance of isoprene, and light-dependent monoterpene emissions. A second intriguing question is: what determines the blend of different monoterpenes? Monoterpene synthase specificity varies, with some synthases capable of producing a greater diversity of products, and others producing a single product (Degenhardt et al. 2009; Rajabi Memari et al. 2013). Although the structure of several key terpene synthases has been characterized (Hyatt et al. 2007; Köksal et al. 2011; McAndrew et al. 2011; Zhou et al. 2012), there is currently no objective way to predict terpene synthase properties on the basis of sequence homology and active site structure (Degenhardt et al. 2009). Even very high sequence and structural homology does not necessarily imply that the two paralogs have the same or even similar terpene product profiles. From an evolutionary perspective, this implies that terpene profiles can be altered with minimum changes in sequence (Kampranis et al. 2007), making the terpene emission profile very 'plastic' in terms of evolutionary diversification. Evidence that different monoterpenes can differently alter plant abiotic stress tolerance (Copolovici et al. 2005) further underscores the importance of understanding the role of emission blends.

From the plant-to-plant and plant-to-insect communication perspective, the role of differences in induced terpene emission blends has been known for a long time (Dicke and Bruin 2001; Dicke and Baldwin 2010; Trowbridge and Stoy 2013 for reviews), and thus, the capacity for changes in the emission profiles with minor changes in genome may be a common evolutionary outcome, and in fact, a necessity to cope with continual changes in herbivory pressure. Although we do not generally think about constitutive emissions in the infochemical context, the transgenic introduction of constitutive isoprene emissions into an otherwise non-emitting plant resulted in greater herbivory resistance (Loivamäki et al. 2008), suggesting a function for isoprene emissions beyond that of enhancing abiotic stress tolerance. Given that the emissions of many constitutive BVOCs are enhanced under mild stress (Niinemets 2010) and that priming by mild stress often results in reduced host quality, constitutive BVOCs might serve further functions we have not yet identified. There is no reason to believe that the evolutionary origins of traits, such as terpene emissions, are linked to isolated, individual effects on fitness. Synergy among adaptive benefits is possible, and should be explored in future studies of fitness in transgenic plants with variable levels and types of BVOC emissions.

So far, genetically engineered plants with introduced isoprene (Sasaki et al. 2007; Vickers et al. 2009; Velikova et al. 2011) or mono- and sesquiterpene (Wu et al. 2006) emissions were only available for herb model systems. In this book, for the

first time, transgenic birch (*Betula pendula*) lines are introduced (Rosenkranz and Schnitzler 2013), providing an exciting new tree model system to test the impact of constitutive introduction of isoprene emission on plant abiotic and biotic stress resistance.

18.2 Molecular, Pathway and Genetic Controls on Emissions

Phenomenological evidence of light and temperature controls on isoprene emission (Sanadze and Kalandadze 1966; Sanadze 1969; Tingey et al. 1981, 1987 Evans et al. 1982; Monson and Fall 1989; Monson et al. 1991) constituted the indirect support that isoprene must be formed enzymatically. This was proven with the discovery of isoprene synthase (Silver and Fall 1991), but at that time it was believed that isoprene emission occurred through the use of substrates generated in the mevalonate-dependent pathway in the cell cytosol, and thus, it was difficult to explain why isoprene emission was so tightly linked to photosynthesis. Especially to the point that the isotopic label from CO_2 was detected in emitted isoprene molecules within only a few minutes after the start of labelling (Sanadze et al. 1972; Delwiche and Sharkey 1993). This enigma was not resolved until it became widely known that 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DOXP) pathway located in the chloroplasts is responsible for the bulk of volatile isoprenoids emitted (Lichtenthaler 1999; Zeidler and Lichtenthaler 2001).

Discovery of the MEP/DOXP pathway has catalyzed rapid development in resolving the molecular and pathway controls on isoprene emission (Li and Sharkey 2013b; Monson 2013). The key issue in understanding these controls has been whether the emission is limited by isoprene synthase activity or by the concentration of its immediate substrate, dimethylallyl diphosphate (DMADP), in chloroplasts. As isoprene synthase requires Mg^{2+} ions as an enzymatic co-factor, as do all known type I terpene synthases with the active site located in the α-domain of the protein (Aaron and Christianson 2010; Köksal et al. 2010; Rajabi Memari et al. 2013), it has been suggested that light-dependent changes in Mg^{2+} are responsible for the light response of isoprene emission (Silver and Fall 1991). On the other hand, in early studies there was evidence of correlation of isoprene emission rate with cellular ATP and carbon intermediate levels (Loreto and Sharkey 1993). However, researchers faced a significant challenge in resolving the role of these alternative hypotheses in regulating the light-dependence of emission because of difficulties in estimating the size of the DMADP pool, and especially that of the chloroplastic pool. Methods for estimating the DMADP pool from cellular extracts were developed, but these did not have the potential to completely separate those fractions of the pool occurring in the cytosol versus the chloroplast (Fisher et al. 2001; Rosenstiel et al. 2002). Development of methods based on ^{13}C-labelling of the DMADP pool (Loreto et al. 2004) and the kinetics of post-illumination isoprene emission (Rasulov et al. 2009a) provided conclusive evidence that DMADP pool size is controlled by light availability (Loreto et al. 2004; Rasulov et al. 2009a), while isoprene synthase

activity does not change during light–dark transients, at least during the initial tens of minutes to a few hours after changing the light level (Rasulov et al. 2009b). Recent work has suggested that the key factor determining the chloroplastic DMADP pool size in the face of changes in the photosynthetic photon flux density is either ATP availability or the activities of several MEP pathway reductive enzymes that can accept electrons directly from the photosynthetic electron transport chain (Rasulov et al. 2011; Li and Sharkey 2013a). This does not mean that the availability of ATP controls the emissions *per se* as the overall requirement for ATP of isoprene emission is small compared with photosynthesis, but rather it suggests that a high effective K_m for ATP exists within the MEP pathway. This, combined with the previously-discovered exceptionally high K_m for DMADP exhibited by extracted isoprene synthase as well as in vivo (Schnitzler et al. 2005; Rasulov et al. 2009a; Li and Sharkey 2013b), implies that light-dependent changes in DMADP are immediately reflected in alterations in isoprene emission rates. Despite major progress in understanding these chloroplastic relations as controls over the light-dependency of isoprene emissions, there is also a great need for studies of isolated protoplasts and chloroplasts, as well as targeted transgenic manipulation, to validate some of the hypothesized metabolic connections.

Control of isoprene emission rate by intercellular CO_2, a response exhibiting an optimum at intercellular CO_2 concentrations between 120 and 200 μmol mol^{-1} and declining towards lower and higher CO_2 concentrations, has also been attributed to changes in chloroplastic DMADP concentration (Rasulov et al. 2009b; Monson 2013). There is, however, debate over the mechanism that links changes in CO_2 concentration to alterations in the chloroplastic DMADP pool size (Monson 2013). In one set of studies, alterations in the activity of the enzyme phosphoenol pyruvate (PEP) carboxylase in isoprene emitting leaves have been shown to result in reciprocal changes in the isoprene emission rate (Rosenstiel et al. 2003, 2004). Given that PEP carboxylase is a cytosolic enzyme that assimilates bicarbonate (in equilibrium with cytosolic CO_2 concentration), these results supported the hypothesis that the channeling of pyruvate substrate via phosphoenol pyruvate from the cytosol to the chloroplast controls the chloroplastic DMADP concentration, and therefore controls the response of isoprene emissions to changes in CO_2 concentration. In the chapter by Li and Sharkey (2013b), a new hypothesis has been proposed that links the import of PEP into the chloroplast to limitations in inorganic phosphate (P_i) availability, which is altered by the balance between the rates of photosynthesis as influenced by CO_2 concentration and sucrose biosynthesis in the cytosol. There is also evidence that changes in the intercellular CO_2 concentration can influence chloroplastic ATP concentration, once again due to changes in the rate of ATP turnover as photosynthetic rate and use of the immediate products of photosynthesis change with changing CO_2 concentration (Kiirats et al. 2009). This, in turn, can lead to changes in DMADP pool size and isoprene emission (Rasulov et al. 2009b, 2011). Thus, while we have an ample set of hypotheses 'on the table in front of us', there is much work to do to better resolve the precise mechanisms underlying the CO_2 sensitivity of isoprene emission in leaves.

Of course, it is not necessary to attribute all control to dynamics in the chloroplast DMADP concentration, but isoprene synthase activity can itself be an important control over the emission rate. There is a large variation in isoprene emission rates among leaves that have developed under different environmental conditions and in leaves of different age, reflecting differences in the amount of isoprene synthase (Harley et al. 1996; Sharkey et al. 1999; Hanson and Sharkey 2001; Wiberley et al. 2005, 2008; Niinemets et al. 2010a). This level of control has been defined as "genetic control" to reflect its underlying mechanisms (Monson 2013). Isoprene synthase promoter sequences have been described and multiple regulatory elements, including circadian control elements and temperature-dependent elements have been identified (Loivamäki et al. 2007; Cinege et al. 2009; Rosenkranz and Schnitzler 2013). This level of control is responsible for changes in isoprene synthase activity in response to past temperature, light and CO_2 conditions and we suggest that future work in resolving controls in isoprene emission should clearly distinguish between metabolic (changes in DMADP substrate) and genetic (changes in the expression of isoprene synthase genes) controls.

Finally, it is important to recognize that the controls over the emission of BVOCs can also occur beyond the processes of cellular metabolism and genetic expression. Physico-chemical properties of emitted volatiles themselves can alter the emission flux (Niinemets et al. 2004; Harley 2013). Stomata constitute the ultimate 'valve' for diffusion of BVOCs from leaves, and traditionally, based on the analogy to diffusion of CO_2 and water vapour in photosynthesis, stomata were considered to control BVOC emission flux (Harley 2013). However, no significant control by changes in stomatal conductance on isoprene and non-oxygenated monoterpene emissions has been observed (Sharkey 1991; Fall and Monson 1992; Loreto et al. 1996) while methanol emissions (Nemecek-Marshall et al. 1995; Harley et al. 2007) and oxygenated monoterpene emissions (Niinemets et al. 2002) were characterized by strong stomatal limitations. These discrepancies among the various compounds were not understood until dynamic models that considered the physico-chemical properties of volatiles were developed (Niinemets and Reichstein 2003; Harley et al. 2007). These models suggested that stomatal control of BVOC emissions was not possible in the steady-state conditions, because the rise in the partial pressure of the volatile compound in leaf intercellular air space, and accordingly the driving force for diffusion, always compensated for reductions in stomatal conductance (Niinemets and Reichstein 2003; Harley et al. 2007; Harley 2013). However, the question is how fast the diffusion gradient changes, i.e., how quickly steady-state conditions are established after changes in stomatal openness. Stomata have the potential to influence the rate of emission in transient, non-steady-state conditions, especially, for compounds with high solubility in the aqueous phase of the leaf, such as for methanol and oxygenated monoterpenes, while for compounds such as isoprene and non-oxygenated monoterpenes, with low solubility, stomata can have minimal influence on observed emission rates.

Once the importance of compound solubility, as a factor in determining the potential for stomatal limitations had been recognized, it was also recognized that existence of a certain capacity for non-specific storage of compounds in leaf

aqueous and lipid phases also implies that even in species without specialized storage structures, there are time-lags between compound synthesis and emission, complicating emission responses to environmental drivers (Noe et al. 2006; Harley 2013). On the other hand, constitutively non-emitting species can adsorb lipid-soluble volatiles released by neighboring plants (Noe et al. 2008; Himanen et al. 2010) and this can lead to greater fitness in terms of reduced herbivory pressure or improved thermal tolerance or priming for induced defences (Copolovici et al. 2005; Llusià et al. 2005; Frost et al. 2008; Himanen et al. 2010). Thus, physico-chemical characteristics of emitted compounds can have major effects on the species interactions in vegetation consisting of constitutive and non-constitutive emitters (Baldwin et al. 2006). This is an area that clearly needs high priority in future studies testing for the effects of plant-to-plant communications, or lack thereof in field experiments with transgenic models with modified emission profiles of the targeted compounds.

18.3 Stress and Emissions: From Constitutive to Induced Emissions

Being sessile in their life habits, plants have had to evolve multiple chemical mechanisms to signal other parts of the same organism, as well as other organisms, about the nature of an encountered stress and its magnitude; plants cannot simply move to a more favorable environment to avoid the stress. This constraint has led to a diversity of constitutive and induced capabilities in the production and emission of BVOCs and their use as ecological signalling cues (Possell and Loreto 2013; Trowbridge and Stoy 2013). Apart from the role of constitutive emissions in abiotic stress resistance and possible involvement in biotic interactions (Sect. 18.1), environmental and biotic stresses are known to induce emissions of a series of different volatile classes including emissions of oxygenated volatiles such as methanol and volatile products of lipoxygenase pathway such as various C6 aldehydes, also called green leaf volatiles, and induced emissions of mono-, sesqui- and homoterpenes (Beauchamp et al. 2005; Steindel et al. 2005; Loreto et al. 2006; von Dahl et al. 2006; Copolovici and Niinemets 2010; Holopainen and Gershenzon 2010; Loreto and Schnitzler 2010; Toome et al. 2010; Copolovici et al. 2012), reflecting the convergence of stress signalling pathways, likely at the level of stress-driven formation of reactive oxygen species (ROS) (Fujita et al. 2006). While the same BVOC classes are induced by many stresses, the volatile blends are often different for different stresses, and typically carry strong species-specific signatures (Dicke et al. 2009; Loreto and Schnitzler 2010). These variations in the blend of elicited volatiles have major impacts on plant-insect interactions (Pichersky and Gershenzon 2002; Bruce et al. 2005; Unsicker et al. 2009), and plant-plant interactions (Baldwin et al. 2006; Kessler et al. 2006). Thus, clearly there is a need to gaining insight into the "plant talk", but currently our learning extends to only a few herbaceous model systems, and even in these systems, our understanding is highly superficial.

In addition to ubiquitous stress-elicited volatiles, specific volatile emissions are characteristic to some stresses such as root-zone anoxia characterized by high emissions of ethanol and acetaldehyde (Kreuzwieser and Rennenberg 2013). These emissions characterize shifts in root zone heterotrophic metabolism from glycolysis with Krebs cycle to glycolytic fermentation and ethanol oxidation to acetaldehyde in leaves. There has been major progress in understanding the controls on ethanol and acetaldehyde emissions by the rate of transport of ethanol from roots to leaves and ethanol dehydrogenase activity (Kreuzwieser et al. 2000, 2001, 2004). However, it is still difficult to predict the magnitude of flood-driven emissions, especially under natural conditions (e.g., Bracho Nunez et al. 2009). The emissions are surprisingly poorly linked to species-specific flood tolerance, and specifics of flood treatment such as duration of flooding (Kreuzwieser and Rennenberg 2013). We may gain greater insight into differential responses of species to flood treatments through characterization of actual oxygen availability in the root zone and assessment of species-specific morpho-physiological traits that improve oxygen availability in root tissues, such as the capacity for aerenchyma and adventitious root formation. Broader taxonomic characterization of these traits and their efficacy toward ameliorating anoxic stress might allow us to predict anoxic stress induced BVOC emissions over large areas potentially impacted by flooding.

In general, there is a reverse correlation between the magnitude of constitutive and induced emissions under stress (Loreto and Schnitzler 2010; Niinemets 2010; Possell and Loreto 2013). However, this response is typically observed after severe stress is reached, while the stress can initially enhance the constitutive isoprene and monoterpene emissions. Such initial enhancements have been reported for drought, heat and ozone stress (Sharkey and Loreto 1993; Loreto et al. 1998; Staudt and Bertin 1998; Pegoraro et al. 2004; Velikova et al. 2005; Calfapietra et al. 2013; Possell and Loreto 2013). Such enhancement lasts until the stress becomes severe enough to curb overall photosynthetic and respiratory metabolism. Our estimates of isoprene biosynthesis rate during abiotic stress, and the true enhancement, however, may be even higher than we originally estimated. Recent observations have revealed that a significant fraction of the synthesized isoprene may react with reactive oxygen species (ROS) inside leaves as part of the metabolic tolerance mechanism (Jardine et al. 2011). Such internal reactions of constitutive isoprene emission clearly require more attention in future studies. In fact, ‘overshoots’ of isoprene emission rates (higher than pre-stress rates) following the relief of stress have been occasionally observed (Possell and Loreto 2013 for a review), indirectly supporting the consumption of part of the synthesized isoprene during the stress. In this regard, the initial upregulation of both constitutive isoprene and monoterpene emissions by moderately high concentrations of the strong oxidant, ozone, is particularly interesting (Calfapietra et al. 2013) as this evidence suggests that the rate of constitutive emissions may be modulated at the level of ROS. Ultimately, severe drought, heat or ozone stresses lead to reductions of constitutive emissions and elicitation of induced emissions (Calfapietra et al. 2013; Possell and Loreto 2013), but here the puzzling issue is how the balance between constitutive and induced emissions is modulated by ROS. Is there a certain threshold beyond which the capacity for

constitutive BVOCs for ROS detoxification is not enough, triggering elicitation of stress signalling pathways or is the stress-dependent reduction in photosynthetic metabolism the factor that inevitably results in reduction of constitutive emissions and thereby enhanced ROS? These are the questions to which we, armed with modern molecular, cell, and integrative biology tools, will hopefully soon have a conclusive answer.

The issue with ozone pollution in current and future atmospheres opens a number of additional highly relevant issues. Many of the stress-triggered volatile signals are sensitive to changes in the oxidative nature of the atmosphere through which they are transmitted (Holopainen et al. 2013). This means that more reactive compounds can be important for signalling only over shorter distances, while less reactive compounds can be propagated as ecological signals over longer distances. This may be the requisite relation given that the atmosphere is oxidative in its fundamental nature, and compounds with higher reactivity may not be capable of carrying out their signalling role far from their emission source – especially if the adaptive role is to elicit top-down controls over herbivory (Trowbridge and Stoy 2013). We are just now beginning to understand the nature of multitrophic signalling systems (Baldwin et al. 2006; Dicke et al. 2009; Dicke and Baldwin 2010), and as we gain knowledge about these systems, which have evolved in atmospheres of fairly constant oxidative potential, we realize that the atmosphere is changing rapidly due to anthropogenic influences. Now, the challenge is to determine how these signalling pathways will change as the oxidative potential of the atmosphere continues to change (Holopainen et al. 2013).

18.4 What Is Lacking in the Emission Models?

In the atmospheric sciences, simple empirical algorithms dating back to the early 1990s (see the Preface) are often used to predict the source magnitude of tree-produced BVOCs and their emissions to the atmosphere. However, there have been major advancements in the understanding of the factors controlling volatile emissions at timescales from minutes-to-days-to-seasons and at spatial scales ranging from cellular-to-global. In particular, gene expression studies have provided important information on the regulation of key controlling enzyme activities (Sects. 18.1 and 18.2), while novel physiological (CO_2 effects on emissions), physico-chemical mechanisms (controls by volatility) have been discovered and quantitative algorithms for all of these effects have been developed for inclusion in large-scale computer models (Sects. 18.2 and 18.3, Grote et al. 2013).

Furthermore, while previous research and models have focused only on constitutive emissions, there has been increased understanding that BVOC emissions can be triggered by various biotic and abiotic stresses in nearly all previously observed species (Sect. 18.3, Niinemets et al. 2010b). Quantitative relationships between the severity of the stress and the rate of emission have been reported for several stresses (Beauchamp et al. 2005; Loreto et al. 2006; Copolovici et al. 2011, 2012),

suggesting that these interactions can be developed in models; although it might still be difficult to predict precise BVOC elicitation stress thresholds (Niinemets 2010). Furthermore, there is a scarcity of studies of stress versus induction relations in trees. For induced emissions, new quantitative models are just beginning to emerge (Grote et al. 2013 for a construction of a quantitative model for insect herbivory elicited emissions) opening the frontiers for future components of ecological complexity to be included in BVOC emissions modelling.

The chapter by Grote et al. (2013) has provided a clear path through the historical development of the algorithms underlying most existing models of BVOC emission. Many of these models are well justified in the relation of their components to fundamental metabolic processes and their interactions with the environment. However, these models are often isolated as single-factor algorithms. There is room for further exploration of a mathematical framework within which processes can interact and within which higher-order feedbacks can be represented. There is also room for a 'systems' approach to modelling and for the exploration of new analytical expressions that may bring together, in mathematical form, the true synergies that exist in metabolic form (e.g., Harrison et al. 2013).

Apart from leaf-level improved algorithms, scaling up from leaf to canopy and landscape has received relatively little attention in BVOC community, reflecting the difficulties of measuring BVOC emission fluxes from ecosystems due to lack of suitable fast BVOC sensors (Hewitt 1999). Despite these difficulties, basic canopy radiative transfer features were described in several early algorithms (Guenther et al. 1994, 1995; Baldocchi and Meyers 1998). With development of methods aimed at canopy-scale flux measurements of reactive compounds such as relaxed eddy accumulation (Bowling et al. 1998; Ciccioli et al. 2003), and the invention of 'fast-response' BVOC sensors for use in measuring single compound eddy covariance (Guenther and Hills 1998) or for multiple compounds sampled interchangeably ("disjunctly") (Karl et al. 2002; Grabmer et al. 2004), and with development of recent technology for true eddy covariance measurement of multiple trace gases (Müller et al. 2010), ecosystem level trace gas fluxes are well within the realm of accurate measurements, allowing for the testing of scaled-up leaf models at the ecosystem and landscape scales (Guenther 2013; Niinemets et al. 2013). While the canopy parameterization schemes could be successfully validated in many cases (Guenther 2013 for a review), there are still inherent uncertainties associated with the temporal resolution of canopy flux data, standardized emission factors for dominant species, descriptions of canopy structure and key emitting species coverage such that different leaf-level algorithms, once consistently parameterized, can not always be conclusively compared (Niinemets et al. 2013). As it becomes hard to say what is the best model, the selection of a model is often driven by practical reasoning rather than by objective criteria of model performance. However, if we are to design models with the capability for projection of emissions into future climate regimes or in regimes of elevated atmospheric CO_2 concentration, we must design our models to explicitly accommodate the longer-term acclimation controls over emissions to climate as well as to atmospheric [CO_2].

Beyond the canopy and landscape scales, many uncertainties remain with models at the global scale (Arneth et al. 2008; Ashworth et al. 2013). Major components of the uncertainty include difficulties with scaled-up emission potential estimates, landcover types and climatic driver databases that affect emissions in non-linear fashion, but are often linearized during averaging to the global scale (Ashworth et al. 2013; Guenther 2013). At the global scale, novel methods to test model predictions by top-down inverse modelling, using for example formaldehyde columns as a proxy of isoprene formation, have been developed (Palmer et al. 2003, 2006; Ashworth et al. 2013). However, at this level of resolution, it becomes especially difficult to sort out the emission algorithm effects on predictions. Instead of showing the differences among the emission algorithms per se, the model intercomparisons conducted so far have highlighted major inconsistencies in model parameterization (Arneth et al. 2011; Ashworth et al. 2013). This certainly underscores the need for greater care in model parameterization and selection of climatic driver datasets.

Accurate consideration of these fundamental, long-term mechanisms and responses is essential to quantitatively explore the key feedbacks between global emissions and global change postulated recently. The global feedbacks involving BVOCs include BVOC effects on tropospheric ozone level altering the productivity of non-emitting species, BVOC effects on the formation of secondary organic aerosols (SOA) and cloud condensation nuclei (CNN) altering the amount of diffuse radiation and plant productivity and further modifying solar radiation penetration and the rate of atmospheric [CO_2] and temperature increase (Kulmala et al. 2004, 2013; Lerdau 2007; Sitch et al. 2007; Mercado et al. 2009; Arneth et al. 2010; Lathière et al. 2010; Ashworth et al. 2013). These global feedbacks surely constitute currently an area of high uncertainty, and have not been quantitatively tested to our knowledge. In this book, Kulmala et al. (2013) provide a first quantitative test of large-scale feedbacks involving BVOC emissions, plant productivity, SOA, clouds, atmospheric [CO_2] and temperature in boreal conifer forests that are known to be responsible for high aerosol loading in the atmosphere (Tunved et al. 2006). The analysis demonstrates that BVOC emissions can play a major role in large-scale biosphere-atmosphere interactions and that the postulated feedback loops between BVOC emissions and atmospheric processes are likely operative (Kulmala et al. 2013). Their chapter not only demonstrates that BVOC models are ultimately becoming robust enough to be used in Earth system models, but also indicates that the knowledge in physical science has been reaching forward to be able to use BVOC emissions to predict SOA and cloud formation. Clearly, there is still a long and possibly a winding path to true Earth system models capable of simulating large-scale climate-vegetation processes with high degree of certainty, but we believe that with this exercise an important milestone has been reached.

One issue yet to be reconciled in global emission models is the heterogeneity created in vegetation distributions due to urban and suburban development (Hewitt et al. 2009; Owen et al. 2013). Ecology in the future cannot pretend as though the globe is covered with natural forests, and ignore the fact that human settlement and agronomic activities will continue to re-distribute forests through the development of feedstocks for second-generation biofuels and through horticultural decoration.

Areas in which non-emitting species used to occupy vast tracts of land are now being converted into short-rotation forests to be used in harvesting cellulose for ethanol production. Most of the species used in these forests emit BVOCs of various types at relatively high rates (Owen et al. 2013). This trend will continue well into the current century, and is certain to modify our current projections of global BVOC emission patterns. It would seem imperative that new databases and inventories be developed that can inform the BVOC modelling community as to the location and rates of spread of agroforestry activities.

18.5 Outlook

The sources of volatile organics can be both anthropogenic and biological, but biological emissions, and in particular, emissions by trees, are more than an order of magnitude higher than anthropogenic emissions. Furthermore, biogenic, high molecular mass, compounds and their oxidation products have the potential to enter the aerosol phase of the atmosphere through condensation on the surfaces of secondary volatile organic aerosols. This process of aerosol production has profound implications for the global radiation budget and climate. In the chapters of this book, control over BVOC emissions has been discussed at numerous scales and within the context of ecological, evolutionary, atmospheric and climatic perspectives. A complete understanding of the primary influences and coupled feedbacks between tree BVOC emissions and future states of the Earth system will require even further synthesis among BVOC control processes.

The chapters in this book make clear that we have entered a new and broader realm of conversation and collaboration within the BVOC research community. Ecologists with interests in multitrophic interactions are interacting with atmospheric chemists interested in the degradation of ecological signalling pathways. Molecular biologists interested in gene sequences, induction of promoter activity and modulation of enzyme activity are interacting with physiologists and biochemists interested in the complexities of biochemical pathway controls and the tradeoffs between enzyme activity and substrate production as factors determining the shapes of emission responses to environmental variation. All of these communities are interacting with modelers interested in providing greater biological reality to their emission projections, at the local, regional and global scales. The emergence of new tools such as those used for transgenic manipulation, and the detection of novel chemical species, have opened up new opportunities to validate models for control over BVOC emissions at the scales of chloroplasts, leaves, canopies and even the entire globe. In all of these interactions, we see evidence of new intellectual energy as the community of scientists considering BVOC emissions continues to grow, in both the entrainment of established researchers and the recruitment of a new generation of researchers. In ending this volume, the editors cannot help but think that this surely must be one of the most intellectually stimulating and broad academic disciplines with which to be affiliated!

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Editors Biography

Professor Ülo Niinemets did his Ph.D. in plant ecophysiology at the University of Tartu, Estonia and conducted postdoctoral work at the University of Bayreuth, Germany; University of Antwerp, Belgium and Centro di Ecologia Alpina, Trento, Italy. His past international faculty appointments include Erskine fellow (2002, Canterbury University, New Zealand), annual G. P. Wilder Chair (2006–2007, University of Hawaii, USA) and F. C. Donders Chair (Utrecht University, The Netherlands). He is currently the head of the Department of Plant Physiology at the Estonian University of Life Sciences where he is in charge of a research team dealing with quantification and predictive modelling of plant carbon gain and trace gas exchange from leaf to ecosystem, landscape and biome scales under globally changing climates. He has collaborated with more than 450 scientists from 40 countries and has co-authored more than 200 international articles and book chapters. He serves the community as an editor or editorial board member of multiple international scientific journals and as a steering committee member of several international programs.

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