

Signaling and Communication in Plants

James D. Blande
Robert Glinwood *Editors*



Deciphering Chemical Language of Plant Communication

 Springer

Signaling and Communication in Plants

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Preface

The concept that plants interact with other organisms by emitting volatile chemicals is well established. Through the emission of volatile chemicals, plants advertise their physiological condition, which can provide valuable information to organisms that detect the odorous bouquet. Take, for example, volatiles emitted by herbivore-damaged plants; they can be received by neighbouring plants that increase their defences, foraging herbivores that can opt to forage either in the same area or elsewhere depending on perceived competition, predatory insects for which the chemicals indicate the presence of their prey, and numerous other members of the community. Volatile chemicals, as well as being emitted by plants, can be detected by plants and elicit various responses. Therefore, plants are not only communicators delivering a volatile presentation to an audience, but are members of an audience receiving chemically encoded information from other sources.

In the last decades, efforts to understand and decipher the chemical language of plants have increased substantially. In this book, we traverse three parts that deliver cutting-edge knowledge on several critical components of volatile-mediated plant communication. Part I covers the production and emission of volatile chemicals and the complexity of chemical messages that plants deliver. Attention is given to the temporal dynamics of plant volatile emissions, the role of abiotic factors in regulating emissions and the impact of multiple stresses as interacting inducers of emissions. A picture begins to build about the complexity of the volatile bouquets emitted by plants and how they can be viewed as an informative chemical language. Throughout the book, there is a focus on chemical ecology, which comes to the fore in Part II. In Part II, a clear picture is developed of the myriad interactions mediated by plant volatiles, spanning interactions between plants and herbivores, predatory and parasitic insects, hyperparasitoids, vertebrates, other plants, pollinating insects, microorganisms and mutualists. Interactions occurring both above- and below-ground are featured. In Part III, there are two chapters on recent developments to understand the detection and processing of volatile signals by plants. Plant electrophysiology and volatile uptake and conversion are the key concepts explored, which complement and add to the ecology of plant–plant interactions covered in

Part II. Some chapters in the book, particularly Chaps. 4, 8 and 12, provide detailed information on current methodologies and offer perspectives on future applications to advance the field of chemical ecology and further elucidate the chemical language of plants. We finish with a synthesis of the key findings within the book and some further ideas for future research directions.

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Part I
Plant Volatiles: Complexity of Chemical
Messages

Chapter 1

Temporal Dynamics of Plant Volatiles: Mechanistic Bases and Functional Consequences

Meredith C. Schuman, Henrique A. Valim, and Youngsung Joo

Abstract Plant volatiles comprise thousands of low-molecular weight, hydrophobic molecules that are classified as ‘secondary’ (specialized) metabolites, but are closely related to ‘primary’ (general) metabolites such as fatty acids, amino acids, sterols and carotenoids. In addition to having important physiological functions, these specialized small molecules have a large influence on plants’ ecological interactions. By emitting particular blends of volatiles, plants can provide detailed information about their current physiological and ecological states and even manipulate other organisms. In fact, the timing of volatile biosynthesis and emission may be as critical to function as the amount and composition of volatile blends. Here, we critically review the known and hypothesized effects of phenological changes in plant volatile emission, their regulation and importance for function.

1.1 Introduction

In life, time is of the essence. This is no mere cliché but rather an ecologically sound generalization: nearly all multicellular eukaryotes, as well as photosynthetic prokaryotes—the cyanobacteria—possess internal clocks that permit the coordination of their metabolism and activity with diurnal cycles of abiotic factors such as light,

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temperature and moisture (Bell-Pedersen et al. 2005; Yerushalmi and Green 2009). Perhaps as an emergent property of the coordination between individual organisms and abiotic cycles, circadian clocks also allow organisms to coordinate with each other's diurnal activity patterns [Wang et al. 2011; Goodspeed et al. 2012 (but see Jander 2012); Zhang et al. 2013]. Ontogenetic events also determine timing and prioritization of phenotypes due to, e.g. developmental necessity, adaptation to environmental changes or the transition from vegetative growth to reproduction. And while timing is important in general for ecology, it is crucial in the production and emission of plant volatiles.

1.1.1 A Brief Introduction to Plant Volatiles

Volatile compounds are small molecules (generally <300 Da) which are sufficiently lightweight and low polarity to have high vapour pressures under normal environmental conditions (reviewed in Dudareva et al. 2006; Baldwin 2010). These molecules may come from any of several biosynthetic pathways that are closely linked to pathways or products of general metabolism, i.e. fats and other lipids, amino acids and proteins (reviewed in Dudareva et al. 2006; Goff and Klee 2006; Baldwin 2010). The biosynthetic classes of plant volatiles and their known structures and functions are described in detail in Table 1.1, and example structures are shown in Fig. 1.1. In addition to the compounds shown, large amounts of methanol ($\mu\text{mol min}^{-1}$) can be produced from the demethylation of pectin in cell walls, and this process is induced by wounding and herbivory (von Dahl et al. 2006).

Plant volatiles have important roles within plant tissues in physiology, signalling and defence. When emitted through the cuticle, stomata or wounded tissue or from specialized structures (reviewed in Widhalm et al. 2015), they may be perceived by a host of other organisms as well as by remote parts of the plant (Heil and Silva Bueno 2007; reviewed in Baldwin 2010; Dicke and Baldwin 2010). The composition of volatile blends can convey detailed information about the physiological and ecological status of plants—such as the presence of open flowers, attack by herbivores, infection by microbes and production of ripe fruit—which may be used by microbes, animals and other plants, both detrimental and beneficial (reviewed in Dicke and Baldwin 2010). The timing of both production and emission of floral and vegetative volatiles is thus essential to their function in within-plant signalling, as well as in orchestrating interactions with other organisms, and may determine their potential for exploitation by enemies.

In this chapter, we provide an overview of what is currently known about the importance of timing in plant volatile biosynthesis and emission; the roles of plant volatiles we briefly refer to in this overview are elaborated throughout this book. The word importance has no precise definition in biology. We use importance to refer to the biological reasons underlying timing: why and how the production and emission of plant volatiles is timed in particular ways. Biologically, why and how can be precisely defined as distinct levels of analysis at which biological

Table 1.1 Biosynthetic classes of plant volatiles and their biosynthesis, proposed functions and structural variety

Class	Compounds	Biosynthesis	Functions	Volatility (BP °C) ^a	Number of known structures
Fatty acid derivatives	Jasmonates	Jasmonates are oxylipins synthesized from 16:3 and 18:3 fatty acids dioxygenated at C13 by 13-lipoxygenase (13-LOX) (Wastmack 2007)	Methyl jasmonate is among the main components of the scent of the jasmine flower, where it was first discovered (Demole et al. 1962). It is a volatile plant hormone which may be involved in plant–plant interactions (Karban et al. 2000; Preston et al. 2001; Kessler et al. 2006), as may the volatile (<i>Z</i>)-jasmane (Birkett et al. 2000)	Methyl jasmonate 302.9 (<i>Z</i>)-Jasmane 292	Four stereoisomers
	Green leaf volatiles	GLVs are synthesized via the cleavage of 13-LOX products by hydroperoxide lyase (HPL) to yield hexan-1-al (from 18:2 fatty acids) or (<i>Z</i>)-3-hexen-1-al (18:3). (<i>Z</i>)-3-Hexen-1-al can be isomerized to (<i>E</i>)-2-hexen-1-al spontaneously or by an isomerase; the hexenals can be converted to alcohols by alcohol dehydrogenases, and the alcohols can be esterified (Matsui et al. 2006)	Green leaf volatiles make up the 'cut grass' smell typical of wounded plant tissue (Hatanaka et al. 1987), contribute to the odour of fruits and flowers (Dudareva et al. 2006) and are emitted from roots and are important recognition and flavour components for animal consumers (Halitschke et al. 2004). GLVs may contribute to plant defence as antimicrobials (Deng	(<i>Z</i>)-3-Hexen-1-al 127.3 (<i>Z</i>)-3-Hexen-1-ol 156.5 (<i>Z</i>)-3-Hexen-1-yl acetate 174.2	At least 32 known from plants: four aldehydes (hexan-1-al, (<i>Z</i>)-3-hexen-1-al, (<i>E</i>)-2-hexen-1-al, and (<i>E</i>)-3-hexen-1-al) which provide substrate for four alcohols and at least 24 esters (acetates, propanoates, butyrates, isobutyrate, valerate and isovalerate)

(continued)

Table 1.1 (continued)

Class	Compounds	Biosynthesis	Functions	Volatility (BP °C) ^a	Number of known structures
	Nine-carbon volatile aldehydes, alcohols and esters	Nine-carbon derivatives are synthesized from 9-lipoxygenase (9-LOX) products of 18:2 and 18:3 fatty acids cleaved at the ninth carbon by HPL and, like GLVs, include aldehydes, alcohols and esters. Some HPLs specifically cleave 9- or 13-hydroperoxides, whereas others cleave both 9- and 13-hydroperoxides. 9-HPL products from 18:2 fatty acids contain one double bond, and those from 18:3 fatty	et al. 1993) or antifungals (Shiojiri et al. 2006a), direct (Vancanneyt et al. 2001) and indirect (Shiojiri et al. 2006a) anti-herbivore defences, and between (Baldwin et al. 2006; Paschoold et al. 2006)-and within-plant (Frost et al. 2008) cues or signals 9-HPL products and their derivatives are odour and flavour components of flowers and fruits (Vancanneyt et al. 2001) and may be involved in seed development in almond (Mita et al. 2005). 9C aldehydes have antifungal properties (Matsui 2006)	(E,E)-3,6-Nonadienal 201.8 (E,E)-3,6-Nonadienol 214.7 (E,E)-3,6-Nonadienyl acetate 247.4	At least 15: five aldehydes which can be converted to five alcohols, which can be esterified; only the acetate esters are well represented in literature

Terpenoids	Terpene hydrocarbons: Most volatile terpenoids have a five- (hemiterpene), ten- (monoterpene), or 15- (sesquiterpene) carbon skeleton	acids contain two double bonds (De Domenico et al. 2007)	Terpene hydrocarbons are synthesized from the 5-carbon precursor isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) produced via one of two pathways in plants: the 2-C-methyl-derythritol 4-phosphate (MEP) pathway in plastids or the mevalonic acid (MVA) pathway in the cytosol. Generally, hemiterpenes and monoterpenes are synthesized in the plastid and sesquiterpenes in the cytosol; some sesquiterpenes may be synthesized in the mitochondrion from cytosolic substrate (Rodríguez-Concepción 2006; Kappers et al. 2005). Emission is usually light-dependent (Lerdau and Gray 2003)	Terpene hydrocarbons are components of flower, fruit, green tissue and root odours. Many are allelopathic (Junya Mizutani 1999) or may act in plant defence as antimicrobials or antifungals (Cowan 1999; Khosla and Keasing 2003), direct (cytochrome P450-inducing) (Brattsten 1983) and indirect (Degenhardt et al. 2003) antiherbivore defences or attract pollinators (Schiestl 2010). Most react with atmospheric ozone (Calogirou 1999) and could be involved in plant oxidative stress responses (Vickers et al. 2009)	Isoprene 34.1 Monoterpenes ca. 140–180 (Harborne 1973) Sesquiterpenes: >200 (Harborne 1973)	Isoprene is the only hemiterpene. At least 1000 different monoterpenes and approximately 5000 different sesquiterpenes are known (Seigler 2008). Most are monoterpenes or polycyclic
	Some terpenoids have an irregular number of carbons (8–18); these are called homoterpenes or	The homoterpene trans, trans-4,8,12-trimethyltrideca-1,3,7,11-tetraene [(<i>E,E</i>)-	<i>(E,E)</i> -TMTT and (<i>E</i>)-DMNT are herbivore-induced volatiles in many plants and can attract	<i>(E,E)</i> -TMTT 293.2 <i>(E)</i> -DMNT 195.6	<i>(E,E)</i> -TMTT and (<i>E</i>)-DMNT are the only homoterpenes known to be widespread in plants.	

(continued)

Table 1.1 (continued)

Class	Compounds	Biosynthesis	Functions	Volatility (BP °C) ^a	Number of known structures
	apocarotenoids depending on their origin and are derived from the cleavage of larger terpenoids	TMTT (C16) is derived from the diterpene geranylinalool (C20) in the plastid (Herde et al. 2008), and the homoterpene trans-4,8-dimethyl-1,3,7-nonatriene [(E)-DMNT] (C11) is derived from the sesquiterpene (3S)-trans-nerolidol (C15) in the cytosol (Boland and Gäbler 1989) by oxidative degradation, possibly catalyzed by cytochrome P450 enzymes (Dudareva et al. 2006). Apocarotenoids (C8-C18) are cleaved from carotenoids in the plastid by carotenoid cleavage oxygenases (CCOs) (Auldridge et al. 2006; Walter et al. 2010)	parasitoids and predators to plants with feeding herbivores (Dudareva et al. 2006). Apocarotenoids are flavour and odour components of flowers, fruit and green tissue (Camara and Bouvier 2004). In flowers, apocarotenoids increase apparency both to pollinators and predators and may be attractive or repellent; in fruit, they are associated with ripening (Bouvier et al. 2005). Some have antifungal properties (Maffei 2010)		Additionally, three different homoterpenes are emitted from elm leaves following oviposition by the elm leaf beetle <i>Xanthogaleruca luteola</i> (Wegener and Schulz 2002), and other structures are known in insects
	Oxidized terpenes and derivatives	Terpenoid hydrocarbons may be further modified by, e.g. cytochrome P450 enzymes, and the	Oxidized terpenes and derivatives are also components of flower, fruit, green tissue and root	Boiling points are higher than the corresponding terpene hydrocarbons	Thousands

Phenylpropanoids and benzenoids	Acid, aldehyde and alcohol derivatives of L-phenylalanine	<p>products may be oxidized by dehydrogenases, esterified by acyltransferases, or reduced. Some terpene synthase enzymes incorporate a molecule of CO₂ to produce oxidized terpenoids as their initial product (Dudareva et al. 2006). Terpene alcohols may also be glycosylated, but the glycosides are not volatile</p> <p>L-phenylalanine is converted to trans-cinnamic acid via L-phenylalanine ammonia lyase (PAL). Further conversion of trans-cinnamic acid to other phenylpropanoids is shared with the lignin biosynthetic pathway through the steps of monolignol biosynthesis. Benzenoids originate from the same biosynthetic pathway, but the side chain of trans-cinnamic acid is enzymatically shortened</p>	<p>odours (Dudareva et al. 2004). They have similar ecological and physiological roles to those of terpene hydrocarbons but are more often directly toxic (Khosla and Keasling 2003)</p>	ca. 180–325 (Oyama-Okubo et al. 2005)	ca. 20% of all known plant volatiles (Qualley and Dudareva 2008)
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(continued)

Table 1.1 (continued)

Class	Compounds	Biosynthesis	Functions	Volatility (BP °C) ^a	Number of known structures
Amino acids and derivatives	Acids, aldehydes, alcohols, esters, nitrogen- and sulphur-containing VOCs derived from amino acids other than L-phenylalanine	<p>by two carbons. L-phenylalanine derivatives with a C2 side chain compete with phenylpropanoids and benzenoids for substrate and are synthesized via different pathways (Dudareva et al. 2006)</p> <p>Amino acids are deaminated or transaminated to form α-keto acids, which are carboxylated and may subsequently be reduced, oxidated or esterified. Amino acids may also be precursors for acyl coA molecules used in esterification reactions catalyzed by alcohol acyltransferases (Dudareva et al. 2006). Ethylene is derived from methionine and thus belongs in this category</p>	<p>Branched-chain amino acid (Leu, Ile, Val) derivatives are common in fruit. Amino-acid derived esters are found in flowers and fruits (Dudareva et al. 2006). Putrid sulphur-containing compounds, likely derived from methionine (Dudareva et al. 2006), may serve as direct defences (Berkov et al. 2000)</p>	<p>Ethylene -103.7 3-Methylbutan-2-ol 113.6 Butyl acetate 126.6</p>	

^aRoyal Society of Chemistry. acc. July 2010. ChemSpider: <http://www.chemspider.com/>

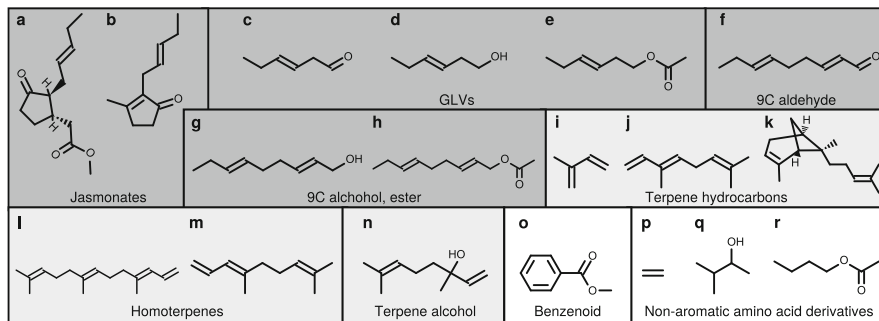


Fig. 1.1 Example structures from the biosynthetic classes of plant volatiles described in Table 1.1: the jasmonates methyl jasmonate (**a**) and (*Z*)-jasmonolide (**b**); GLVs (*Z*)-3-hexen-1-al (**c**), (*Z*)-3-hexen-1-ol (**d**) and (*Z*)-3-hexen-1-yl acetate (**e**); 9C compounds (*E,E*)-3,6-nonadienal (**f**), (*E,E*)-3,6-nonadienol (**g**) and (*E,E*)-3,6-nonadienyl acetate (**h**); terpene hydrocarbons isoprene (**i**), monoterpene β-ocimene (**j**) and sesquiterpene (*E*)-α-bergamotene (**k**); homoterpenes (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene [(*E,E*)-TMTT] (**l**) and (*E*)-4,8-dimethyl-1,3,7-nonatriene [(*E*)-DMNT] (**m**); monoterpene alcohol linalool (**n**); benzenoid methyl benzoate (**o**); and non-aromatic amino acid derivatives ethylene (**p**), 3-methylbutan-2-ol (**q**) and butyl acetate (**r**). Classes with the same type of biosynthetic precursors (fatty acids, IPP/DMAPP, amino acids) have identical background shading. Methanol, produced abundantly by demethylation of pectin in plant walls, is not shown

phenomena can be investigated: ‘why’ can be answered either in terms of functional outcomes or evolutionary history, and ‘how’ may be answered in terms of physiological or ontogenetic events (Tinbergen 1963; Sherman 1988).

Here, we focus on a functional level of analysis, including mechanistic and evolutionary dimensions when appropriate to provide a more complete biological picture. We begin from the assumption that observed traits are adaptive and reject this view only if evidence does not support it. There is abundant evidence against the alternative assumption that plant volatiles are waste products, released from plants more as a result of their physicochemical properties than of physiological mechanisms, which may opportunistically accrue functions (Niinemetts et al. 2004; Peñuelas and Llusà 2004; Rosenstiel et al. 2004). This is reminiscent of the more than 60-year-old theory that specialized plant metabolites generally are ‘flotsam and jetsam on the metabolic beach’, comprising waste products which may be opportunistically co-opted for functional roles, a theory which has not been supported for any plant specialized metabolite biosynthetically, physiologically, evolutionarily or functionally studied so far (Hartmann 2007). In fact, even the emission of plant volatiles through cuticles, formerly thought to occur passively, is likely to be actively regulated in order to avoid toxic concentrations of volatiles in membranes (Widhalm et al. 2015).

1.1.2 Why Are Plant Volatiles Synthesized and Emitted in Certain Tissues at Certain Times?

1.1.2.1 Function

In order to synchronize with abiotic and biotic factors, organisms need to have a sense of timing. Rhythmic behaviour can efficiently increase fitness by optimizing plant metabolism to abiotic circumstances dictated by the timing of sunrise and sunset (reviewed in Yerushalmi and Green 2009). Since different times of day bring different abiotic conditions and thus biotic communities, each plant has its own ‘specific timing’ to maximize its fitness (Raguso et al. 2003). This is realized not only in visually apparent behaviours like flower opening and leaf movement but also in the emission of plant volatiles, which often show distinct diurnal or nocturnal patterns in different tissues. Plants produce different amounts and combinations of volatiles over time, and these volatile blends have different physiological and ecological functions including:

- Within-plant signalling, which also leads to ‘eavesdropping’ on other plants (Baldwin and Schultz 1983; Rhoades 1983; Heil and Silva Bueno 2007; reviewed in Heil and Karban 2010)
- Pollinator attraction (Kessler et al. 2008)
- Seed dispersal (Bolen and Green 1997; Luft et al. 2003; Goff and Klee 2006; Klee and Giovannoni 2011)
- Deterrence of herbivores, although they can also be co-opted by herbivores as host location cues (Kessler and Baldwin 2001; De Moraes et al. 2001; Halitschke et al. 2008; reviewed in Bruce et al. 2005) and feeding stimulants (Halitschke et al. 2004; Meldau et al. 2009)
- Attraction of predators and parasitoids to defend against herbivores (Dicke 1986; De Moraes et al. 1998; Kessler and Baldwin 2001; Rasmann et al. 2005; Schuman et al. 2012; reviewed in Dicke and Baldwin 2010; McCormick et al. 2012)
- Mediating interactions with microbes (reviewed in Junker and Tholl 2013)
- Allelopathic inhibition of neighbours (Inderjit et al. 2009; reviewed in Glinwood et al. 2011)
- Tolerance of abiotic stress (reviewed in Holopainen and Gershenson 2010)

These roles can be viewed on a spectrum from internal to external, in terms of whether plant volatiles act within or between plant cells, or after emission into the environment, and are usually localized to particular tissues (Fig. 1.2).

1.1.2.2 Evolution

From the evolutionary perspective, the answer to ‘why’ plants synthesize and emit particular volatiles, at particular times and from particular tissues or structures, is the evolutionary trajectory resulting in the currently observed phenotype.

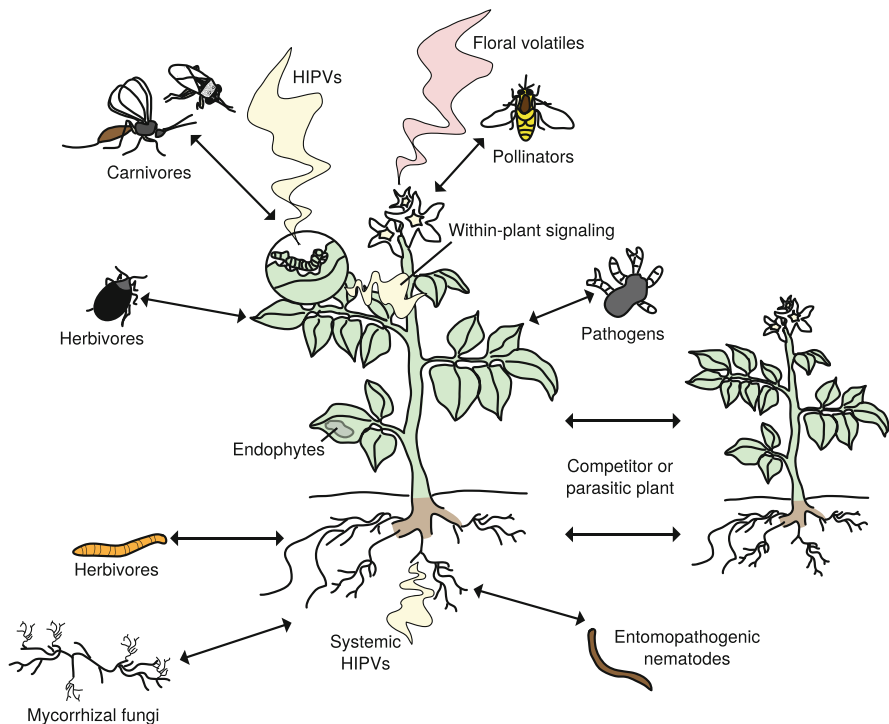


Fig. 1.2 Functional roles of plant volatiles. Modified with permission from Dicke and Baldwin (2010)

Evolutionary analyses usually study the appearance and mutation of biosynthetic enzymes (reviewed in Pichersky et al. 2006), though they may also focus on structures for biosynthesis, storage or emission (reviewed in Lange 2015). The enzymes of GLV and jasmonate biosynthesis are ubiquitous in plants, originating in the green algae (reviewed in Andreou et al. 2009; Lange 2015), and the mevalonate and methylerythritol phosphate pathways providing substrate for terpenoid volatiles can be found in bacteria (reviewed in Rodríguez-Concepción et al. 2013; Lange 2015). In contrast, many volatile end products are limited to particular plant lineages, indicating rapid diversification of downstream biosynthetic enzymes (reviewed in Pichersky et al. 2006). However volatiles from most biosynthetic classes are emitted from vegetative, floral and root tissues in higher plants (reviewed in Dudareva et al. 2004) (although roots are more difficult to analyse and thus less well studied).

The enzymes of plant volatile biosynthesis are derived from general metabolism. For example, the diverse family of terpene synthases is derived from an ancestral ent-Kaurene synthase (Trapp and Croteau 2001, reviewed in Chen et al. 2011). The enzymes of jasmonate and GLV biosynthesis carry out the controlled degradation of reactive free fatty acids; substrate for phenylpropanoid and benzenoid volatiles

comes from intermediate products of lignin biosynthesis, and other volatiles derive from salvage and degradation pathways of amino acids via α -keto acids (reviewed in Dudareva et al. 2006). Thus volatile metabolites are more or less closely linked to the dynamics, regulation and rhythms of general metabolism.

1.1.3 How Is the Induced, Diurnal and Ontogenetic Timing of Volatile Biosynthesis and Emission Regulated?

The roles of physiology and ontogeny in plant volatile synthesis and emission are depicted in Fig. 1.3.

1.1.3.1 Physiology

Physiology provides the immediate mechanisms. The close relationship of plant volatiles to general metabolic pathways dictates precursor availability according to diurnal or circadian control (Pokhilko et al. 2015), hormonal signalling and flux channelling (reviewed in Dudareva et al. 2006; Nagegowda 2010; Vranová et al. 2012). Direct volatile biosynthetic enzymes may be regulated by any or all of these cues, and many of the best-studied examples are of herbivory-induced volatile emission (reviewed in Howe and Jander 2008). Emission, like biosynthesis, is also tightly regulated—even the diffusion of plant volatiles across membranes and cuticles is likely to be an actively regulated process (Widhalm et al. 2015). Other than diffusion, emission may occur through stomata (Seidl-Adams et al. 2014), wounds (De Domenico et al. 2007), or specialized tissues such as flowers (reviewed in Muhlemann et al. 2014) or glandular trichomes (e.g. Schuurink 2007; but see Hare 2007). Emission rates are influenced by internal signalling (reviewed in Howe and Jander 2008) and other wounding-related processes (e.g. De Domenico et al. 2007) as well as temperature, humidity and light levels, both due to direct physical effects on volatility and—likely more importantly—to the effects of these factors on the physiological mechanisms of emission (e.g. Grote et al. 2014).

1.1.3.2 Ontogeny

Ontogeny describes the second mechanistic or the ‘how’ level at which we may investigate the regulation of plant volatile biosynthesis and emission, comprising in part the regulatory physiological system of each life stage and tissue of the plant. To a large extent, however, ontogenetic changes may accompany the development of new tissues and transitions to different life stages, e.g. from vegetative to reproductive (Diezel et al. 2011). Perhaps due in part to overlap in the emission profiles

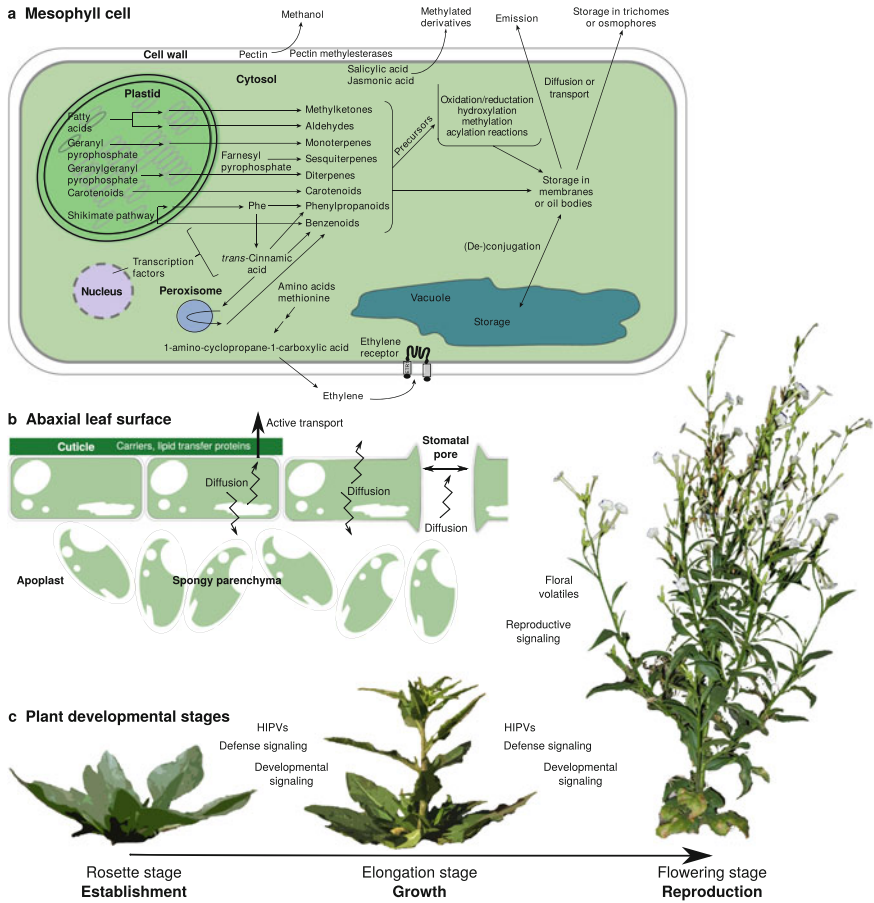


Fig. 1.3 Mechanisms of plant volatile biosynthesis and emission. **(a)** Physiological mechanisms, modified with permission from Baldwin (2010). **(b)** Depiction of possible paths for volatiles—once synthesized, released from conjugates or intracellular storage—to diffuse (over cell walls, through stomata) or be actively transported (through cuticles) to the headspace, using the abaxial leaf surface as an example. **(c)** Vegetative and reproductive stages of the ecological model plant *Nicotiana attenuata* visualized as changes to shoots and changing roles of plant volatiles. Roots and seeds are not shown as less is known about the structural changes or dynamic volatile emissions of these tissues. Plants modified from Schuman et al. (2014)

of different tissues or to the hormonal signalling functions of some volatiles, vegetative volatiles may interfere with the function of floral volatiles and vice versa (von Dahl et al. 2007; Diezel et al. 2011; Kessler et al. 2011; Desurmont et al. 2015). Ontogenetic changes in volatile emission may serve to avoid such interference. Furthermore, both within tissues and across life stages, the development of specialized cells and tissues may be required for the biosynthesis and emission of some volatiles, placing physiological limitations on ontogenetic timing

(e.g. Bate et al. 1998; reviewed in Dudareva et al. 2004; Rodríguez-Concepción et al. 2013).

1.2 The Importance of Timing in Plant Volatile Emission

In the following sections, we describe how several well-studied phenomena mediated by plant volatiles can be understood on the timescales of induction, diurnal and circadian rhythms and changes over ontogeny. We focus on functions but also address their regulation.

1.2.1 *Plant Volatiles as Induced Defences and Defence Signals*

It is well known that biosynthesis and emission of specific plant volatiles are induced by stress in every higher plant studied so far (reviewed in Holopainen and Gershenzon 2010), resulting in predictable volatile blends which can serve as reliable cues or signals: cues benefit the receiver with an unspecified effect on the sender, while signals benefit the sender and the receiver, with ‘benefit’ defined biologically as increase in Darwinian fitness (Greenfield 2002; Allison and Hare 2009). Stress-induced plant volatile blends may be highly specific to particular plant genotypes (e.g. Wu et al. 2008; Delphia et al. 2009; Schuman et al. 2009; reviewed in Wu and Baldwin 2010) and stress events (reviewed in Holopainen and Gershenzon 2010), including specificity to particular herbivores (reviewed in McCormick et al. 2012) and numbers of herbivores (e.g. Shiojiri et al. 2010). The ubiquity and specificity of stress-induced plant volatile emission indicates the importance of these compounds in structuring ecological communities, from effects on pollinators, herbivores, predators and microbes, to impact on global climate (reviewed in Kessler and Halitschke 2007; Armeth and Niinemets 2010; Dicke and Baldwin 2010; Holopainen and Gershenzon 2010). Herbivore-induced plant volatiles (HIPVs) are among the best-studied induced phenomena in the field of plant–herbivore interactions and have served as highly specific phenotypic read-outs in studies of herbivore elicitors (reviewed in Howe and Jander 2008).

1.2.1.1 Functions of Herbivore-Induced Plant Volatiles (HIPVs)

As semiochemicals, HIPVs can attract parasitoids of herbivores (De Moraes et al. 1998) and insectivorous carnivores (Dicke 1986; Kessler and Baldwin 2001) and deter herbivore oviposition (Kessler and Baldwin 2001; De Moraes et al. 2001), all of which may enhance plant fitness by increasing the apparency

of herbivores to predators (Feeny 1976). However, HIPVs and other plant volatiles also act as host location cues for herbivores (e.g. Halitschke et al. 2008; reviewed in Bruce et al. 2005; Dicke and Baldwin 2010). In *N. attenuata*, the release of HIPVs reduces herbivore loads by 50% or more on the emitting plants (Kessler and Baldwin 2001; Allmann and Baldwin 2010; Schuman et al. 2012) allowing GLV-emitting plants to produce twice as many flowers, buds and seed capsules as non-emitters in the presence of predators (Schuman et al. 2012). HIPVs can also contribute to defence signalling within plants, for example, eliciting the production of hormones and gene transcripts involved in defence and eliciting or priming defence traits (Heil and Silva Bueno 2007; Frost et al. 2008; reviewed in Heil and Karban 2010). Likely as a side effect of their ability to respond to their own HIPVs, plants can also elicit or prime defence responses after ‘eavesdropping’ on HIPVs from neighbours (reviewed in Baldwin et al. 2006; Heil and Karban 2010; Scala et al. 2013; and see Chap. 7); responses may be specific to plant genotype and interaction (e.g. Li et al. 2012). Neighbour volatiles have also been shown to alter biomass allocation and growth in barley (Ninkovic 2003; Kegge et al. 2015).

1.2.1.2 Regulation of HIPVs

HIPV emission is regulated by the same signalling systems that elicit other induced defence responses in plants (Fig. 1.3). For example, it has been shown that different elicitors in the regurgitant of *Manduca sexta* (tobacco hornworm) larvae elicit specific HIPVs in *Nicotiana attenuata* (wild coyote tobacco) (Gaquerel et al. 2009). Furthermore, in *N. attenuata*, mitogen-activated protein kinase (MAPK) signalling (Meldau et al. 2009), jasmonate signalling (Halitschke and Baldwin 2003; Schuman et al. 2009; Oh et al. 2012; Woldemariam et al. 2012, 2013), abscisic acid signalling (Dinh et al. 2013) and WRKY transcription factors (Skibbe et al. 2008) all have been shown to regulate HIPV emission. Some isoprenoids have been shown to react with and quench reactive oxygen species (ROS) (reviewed in Vickers et al. 2009; Holopainen and Gershenzon 2010). ROS are a component of early signalling events that induce stress hormone signalling, including abscisic acid and jasmonate signalling (reviewed in Maffei et al. 2007; Wu and Baldwin 2010), and are likely to be involved in the induction of HIPVs and other stress-responsive volatiles.

Interestingly, in *Arabidopsis* (*Arabidopsis thaliana*), it has been shown that HIPV emission is dependent on jasmonates but not on the known active form jasmonoyl isoleucine (JA-Ile) (Fonseca et al. 2009), indicating that a different jasmonate may activate genes controlling HIPV emission, while JA-Ile regulates other defence responses (Van Poecke and Dicke 2003; Wang et al. 2008). In *Phaseolus lunatus* (lima bean), synthetic JA-Ile analogues similar in structure with coronatine, the highly active jasmonate mimic produced by the biotrophic plant pathogen *Pseudomonas syringae*, strongly elicit HIPV biosynthesis as long as the carbonyl group on the 5-membered ring is intact and regardless of modifications to the amino acid moiety (Krumm et al. 1995). Application of methyl jasmonate has

also been shown to elicit volatile emission in other wild and domesticated plants including *Solanum peruvianum* (Peruvian wild tomato) (Kessler et al. 2011), *Datura wrightii* (sacred Datura) (Hare 2007), *N. attenuata* (Halitschke et al. 2000), *P. lunatus* (Ozawa et al. 2000) and *Gossypium hirsutum* L. (cotton) (Rodriguez-Saona et al. 2001). The volatile emission elicited by jasmonate treatment often partially, but not fully, overlaps with the profiles elicited by feeding of different herbivores (e.g. Dicke et al. 1999; Kessler and Baldwin 2001).

1.2.2 Diurnal and Circadian Rhythms of Stress-Induced Volatiles

Volatiles are generally emitted beginning from seconds to hours following induction, and the emission induced by a single event may last as little as minutes or as long as days (see, e.g. Loughrin et al. 1994; von Dahl et al. 2006; Allmann and Baldwin 2010; Schaub et al. 2010; Danner et al. 2012; Jardine et al. 2012; Kallenbach et al. 2014). Longer emission cycles may display diurnal rhythms (e.g. Loughrin et al. 1994; Arimura et al. 2008) (Fig. 1.4), which may allow plants to synchronize volatile emission temporally with biotic factors, e.g. herbivores, predators and parasitoids. This could increase plant fitness, because many insects also have their own predictable rhythmic behaviour. For example, circadian-regulated jasmonate accumulation can enhance plant resistance to herbivores with synchronized feeding activity (Goodspeed et al. 2012; but see Jander 2012). Also, nocturnal volatiles produced by host plants repelled oviposition of nocturnal moths (De Moraes et al. 2001; Allmann et al. 2013), and determined the behaviour of *Mythimna separata* (Northern armyworm) larvae (Shiojiri et al. 2006b).

1.2.2.1 Rhythmic Volatile Emission in Response to Biotic and Abiotic Stress

Most diurnal plant behaviours are synchronized to daily rhythms of abiotic factors like light and temperature, denoted 'zeitgeber' (time givers). In particular, many isoprenoids and terpenoids show strong diurnal rhythms because 75 % of carbon for isoprenoid synthesis originates from photosynthesis, and the methylerythritol phosphate (MEP) pathway has strong diurnal and circadian rhythms (Schnitzler et al. 2004; Dudareva et al. 2005; Pokhilko et al. 2015). In many tree species, monoterpene production positively correlates with light intensity and temperature (Tingey et al. 1980; Harley et al. 2014; Jardine et al. 2015). It should be noted that the lower volatility of sesquiterpenes (Table 1.1) can lead to experimental artefacts, e.g. resulting from their temperature-dependent adsorption to and re-release from collection cuvettes, which can obfuscate emission patterns (Schaub et al. 2010). However herbivore-induced emission of the monoterpene β -ocimene in *P. lunatus*

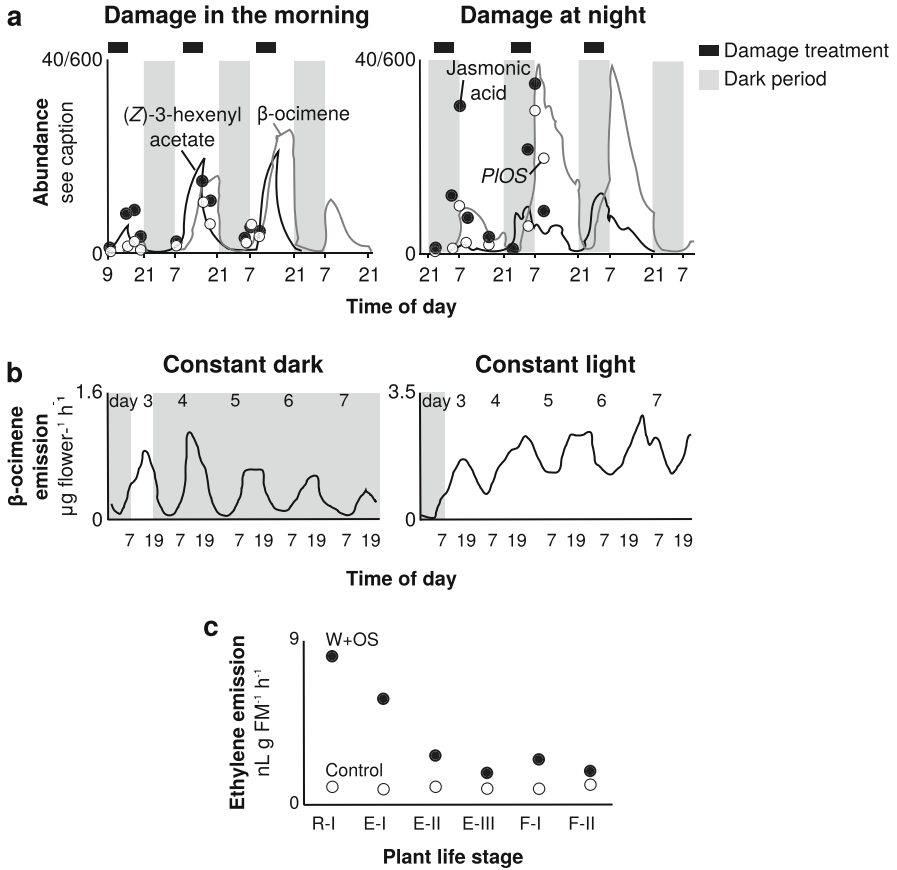


Fig. 1.4 Examples of phenological changes in plant volatile emission. **(a)** The monoterpene β -ocimene is emitted from *Phaseolus lunatus* leaves during light periods following simulated herbivore damage (MecWorm treatment), while the GLV (Z)-3-hexenyl acetate is emitted immediately upon damage regardless of light or dark period (volatiles shown on the scale of 0–40 $\text{ng g FM}^{-1} \text{min}^{-1}$). Jasmonic acid and *PIOS* (β -ocimene synthase) transcript accumulation reflect dynamics of β -ocimene emission (jasmonic acid scale: 0–600 ng g FM^{-1} , *PIOS* transcripts relative to *PIACT1* transcripts). Scales in the left and right panel are the same, indicating that jasmonate accumulation and β -ocimene emission are greater after night-time damage. Drawn from data in Fig. 3 of (Arimura et al. 2008). **(b)** Circadian oscillation in the emission of β -ocimene from flowers of *Antirrhinum majus* (snapdragon) cv Maryland true pink. Overall, more β -ocimene is emitted under constant light conditions (note difference in scale between left and right panels). Drawn from data in Fig. 2 of (Dudareva et al. 2003). **(c)** Ethylene emission induced after treatment of mature, non-senescent *Nicotiana attenuata* leaves with mock herbivory (wounding and *Manduca sexta* oral secretions, W+OS) is attenuated as plants age; control, untreated leaf at same position. Stages: R-I rosette; E-I beginning elongation, first inflorescence visible but not fully developed; E-II and E-III elongated, consecutive days, buds develop rapidly but do not yet open; F-I first corolla elongation; F-II first fully opened flowers. Drawn from Fig. 1 in (Diezel et al. 2011)

leaves showed a diurnal rhythm, while emission levels strongly decreased under artificial dark treatment (Arimura et al. 2008) (Fig. 1.4). Although many volatile emission rhythms strongly diminish or disappear without environmental cues, some volatiles retain oscillation under free-running conditions, demonstrating circadian regulation (Hsu and Harmer 2013). Isoprene emission, for example, has a strong circadian rhythm, presumably to protect plants from heat stress and maintain photosynthesis and also to mitigate the effects of oxidative stress in response to abiotic stress-mediated ROS production (reviewed in Sharkey et al. 2008; Loreto and Schnitzler 2010). Stress-induced plant volatile emission and its responsiveness to diurnal and seasonal zeitgeber contribute significantly to atmospheric pollution and global climate (Arneeth and Niinemets 2010).

1.2.2.2 Are There Examples of Circadian Regulation of HIPVs?

So far no leaf HIPVs have been reported to have circadian-regulated emissions. Perhaps synchronization via the circadian clock is more common either when dictated by abiotic factors as discussed above or in mutualistic interactions. The evolutionary strategy between plants and herbivores is a diffuse arms race: each side responds to selection pressure for counter-adaptation from the other side (Fox 1981). For example, plants are thought to diversify their production of defensive metabolites in response to herbivore adaptation to older defences (Speed et al. 2015). So if plants have developed rhythmic traits to synchronize with herbivore behaviour, the herbivore may experience selection pressure to change the behaviour and escape the synchronization. Thus we would predict it is uncommon to find cases of circadian-mediated synchronization between HIPVs and herbivore activity, e.g. as direct defence responses. However, if two species have mutualistic interactions, both sides could benefit from synchronization, and this may be one way in which mutualistic interactions increase the stability of communities (Georgelin and Loeuille 2014). If there are cases of the circadian regulation of HIPVs, these might originate in physiological roles of these volatiles, if the resulting rhythms are also compatible with their defensive roles. For example, a rhythm dictated by the oxidative stress produced by photosynthesis may also be compatible with the activity patterns of certain herbivores or their natural enemies, or insects may learn to recognize typical, physiologically dictated and thus tightly conserved rhythms of plant volatile emission.

1.2.3 *Circadian Timing of Floral Volatile Emission*

It is perhaps not surprising that floral volatiles provide the best-known examples of robustly regulated, rhythmic plant volatile emission. Many flowering plants require assistance from pollinators to transfer pollen between flowers, and species with self-compatible flowers often nevertheless benefit from out-crossing mediated by

pollinators (reviewed in Rosas-Guerrero et al. 2014). Flowers thus produce attractant volatiles for pollinators to enhance chances of out-crossing (Kessler et al. 2008). Many plants that require insect pollinators have evolved very specific floral traits for this purpose (reviewed in Raguso 2004). They also produce particular floral volatiles at specific times to synchronize with the activity of their pollinators (reviewed in Muhlemann et al. 2014). *N. attenuata* produces benzyl acetone during the night to attract nocturnal moths, *M. sexta* and *Hyles lineata* (whiteline sphinx) (Kessler et al. 2008). Whereas *Petunia* spp. (petunia) flowers mainly produce benzenoids to attract night pollinators (Hoballah et al. 2005); *Antirrhinum majus* (snapdragon) cv. Maryland true pink flowers emit larger amounts of methyl benzoate during the day to attract bees (Kolosova et al. 2001).

Circadian regulation has been shown for many floral volatiles. Bee-pollinated *A. majus* flowers produce the monoterpene myrcene, (*E*)- β -ocimene and linalool specifically during the day, a rhythm maintained under constant light conditions (Dudareva et al. 2003) (Fig. 1.4) and also emit more methyl benzoate during the day (Kolosova et al. 2001), while moth-pollinated *Nicotiana suaveolens* (Australian tobacco) and *Petunia* cv *Mitchell* flowers produce benzenoid volatile compounds at night, a rhythm maintained under constant dark conditions (Kolosova et al. 2001). Recently, evidence that the circadian clock directly regulates floral volatiles was reported in *Petunia hybrida* and *N. attenuata* (Yon et al. 2016; Fenske et al. 2015). Both studies showed that *late elongated hypocotyl* (LHY), a morning element of the circadian clock, is a main regulator in the peak timing of floral volatile emission. These findings suggest that the role of the circadian clock in flowers may be conserved in the Solanaceae. LHY may also transcriptionally regulate isoprene synthase to produce strong circadian rhythms of isoprene emission in grey poplar (Loivamäki et al. 2007). Circadian rhythms of plant volatiles are the consequence of circadian regulation of substrate flux and biosynthetic genes at transcriptional and enzymatic levels (Kolosova et al. 2001; Dudareva et al. 2005; Fenske et al. 2015; Pokhilko et al. 2015). In addition, circadian emission patterns of plant volatiles may also be influenced by the circadian regulation of emission mechanisms such as stomatal opening or transport through membranes (Lehmann and Or 2015; Widhalm et al. 2015) (Fig. 1.3).

1.2.4 Ontogenetic Changes in Plant Volatiles: Theory and Observation

1.2.4.1 Application of Plant Defence Theory to Ontogenetic Patterns

The composition of specialized metabolites in plants, including volatiles, varies not only in response to stress and diurnal events but also throughout lifecycles, across plant life histories and by type of herbivore pressure exerted on plants (Barton and Koricheva 2010). Plant defence theories have long sought to explain this variation with different degrees of success, and because many studies of variation in plant

volatile emission are based on plant defence theory, it is important to mention them here. The most commonly employed have been optimal defence (OD) theory (McKey 1974; Rhoades and Cates 1976; McKey 1979; Rhoades 1979), apparency theory (Feeny 1976) and growth-differentiation balance (GDB) theory (Loomis 1932; Loomis 1953; Herms and Mattson 1992). Among these, GDB has been least effective in predicting ontogenetic changes described in literature. The view of ontogenetic changes in GDB is largely mechanistic but oversimplified, treating growth and differentiation as general processes at a level of understanding achieved in the first half of the twentieth century (Loomis 1932, 1953) and considering light and nutrient availability while ignoring other abiotic and biotic stimuli (Stamp 2003). As one study of defensive monoterpenes described, GDB is ‘...a source-driven model that does not, in its simplest form, consider changes in the need (demand) for growth or differentiation products’ (Lerdau et al. 1994).

Apparency and OD theory have been more useful, despite not focusing on ontogeny, because many hypotheses related to the ontogenetic distribution of plant volatiles can also be posed as functional hypotheses. Further, apparency and OD theory provide some testable functional hypotheses, while the hypotheses posed by GDB theory mix mechanistic and functional levels of analysis and are thus not testable (Sherman 1988; Baldwin 1994; Stamp 2003). Functional studies largely treat ontogenetic stages as black boxes, with no exploration of the mechanistic background for differences which make ontogenetic stages distinctive, though not discrete. In contrast, the mechanistic literature is mostly restricted to developmental biology, though there are studies of the development of tissues, specialized storage and secretory structures which cross the boundary between developmental biology and the study of plant defence (reviewed in Dudareva et al. 2004), and studies of developmental regulation of biosynthetic enzymes in floral volatile emission (e.g. Pichersky et al. 1994; Bate et al. 1998; Dudareva et al. 2000). The literature on functions of plant volatiles over ontogeny is our focus here because we feel a critical discussion of the functional literature is lacking.

In a meta-analysis of 116 studies reporting ontogenetic patterns in plant defence traits across 153 plant species interacting with 30 herbivore species, Barton and Koricheva (2010) found that patterns over ontogeny in plant response variables (concentrations of secondary metabolites, measures of physical defence and tolerance) depended on plant life form, type of herbivore and type of response, and thus a generalization about patterns in defence over ontogeny could not be supported. The plant-age hypothesis (Bryant et al. 1992) explicitly applies predictions of OD theory to ontogenetic changes in plants, ‘predicting that that extrinsic factors, namely, selection by herbivores, lead to high levels of defence in juveniles, followed by decreases as plants mature and become less susceptible to the fitness reductions of these attacks’ (Barton and Koricheva 2010). Yet within a life stage, OD predicts that younger tissues should always induce higher direct and indirect defences, if they make a larger contribution to the plant fitness (reviewed in Meldau and Baldwin 2013). Of all tissues, then, reproductive tissues and seed capsules should be the best defended, representing the culmination of the plant’s labours, as the rest of the plant senesces and concentrates metabolites in the reproductive