Plant Volatiles in Defence

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ABSTRACT

Plant odours are the most ubiquitous volatiles in nature. This chapter deals with the biochemistry and molecular biology of plant volatiles that are emitted from vegetative tissues during pathogen- or herbivore-induced stress and the contribution of these volatiles to plant defences. While mechanical tissue wounding causes the non-specific release of volatiles, herbivore-specific elicitors from their saliva distinctly alter the volatile signature. These volatiles acquired diverse roles in ecological interactions. Firstly, they can be toxic to, or repel conspecific or other herbivores from already infested plants. This function is referred to as 'direct defence'. Secondly, volatiles function as prey-associated signals for foraging carnivorous arthropods. This is referred to as 'indirect defence'. Finally, volatiles elicit metabolic changes in unattacked neighbouring plants resulting in priming and induction of defences already before herbivores have arrived. This is referred to as 'plant–plant communication'. Although volatile production by native plants and crops is highly variable under different growth conditions and during development, its function in direct and indirect defences is remarkably robust. With the current state of knowledge, it is now possible to manipulate these direct and indirect defences through breeding or transgenic approaches. Taken together, plant volatiles play profound roles in plant–herbivore and plant–pathogen interactions and are promising targets for improved crop protection.

I. INTRODUCTION TO VOLATILE ORGANIC COMPOUNDS (VOCs) FROM PLANTS

Plants secrete organic and inorganic compounds during growth, development and reproduction from most of their tissues into the environment. For example, plant leaves secrete specific waxes and proteins onto their surface as a protective coating against pathogens (Shepherd and Wagner, 2007), leaf hairs secrete sticky substances that make it hard for small insects to move around on the plant surface (Wagner, 1991), and plant roots secrete a wide range of small molecules that act as signals for rhizobacteria and mycorrhizal fungi (Bertin *et al.*, 2003). Many plant secretions are volatile and diffuse rapidly through air or soil. Volatile products often have been considered as waste or metabolic overflow, since secretion implies that these products will, in most cases, be lost (Harrewijn *et al.*, 1995). Although many of them might once have occurred as waste products during evolution, they have clearly acquired novel functions in the plant's physiology and ecology and likely have evolved these functions from there on.

Plant-secreted volatile organic compounds (VOCs) create the plant's 'headspace', the blend of volatiles filling the arbitrary space surrounding a plant, after they have evaporated from the plant surface. It has been estimated that up to 36% of the assimilated carbon is released back into the atmosphere (Kesselmeier *et al.*, 2002) in the form of volatile products, although Halitschke *et al.* (2000) concluded that the resource requirements for the volatile release are minor. VOCs can be small and highly volatile molecules (e.g. ethylene (ET), methanol, isoprene), but also be compounds with moderate volatility ('oils'), such as mono- and sesquiterpenes or aromatics such as methyl salicylate (MeSA) or lipoxygenase (LOX)-derived C_6-volatiles such as hexenal, often collectively referred to as 'green-leaf volatiles' (GLVs), although some authors only consider the latter to be true GLVs. Many of these VOCs are released during the primary processes of growth and development. In addition, emission of VOCs is influenced by abiotic factors, such as nutrient availability, temperature or the spectral composition of light, especially photosynthetically active radiation (Peñuelas and Llusià, 2001, 2004). Although most plants release VOCs constitutively, the composition and quantity of the emitted blend changes markedly in response to biotic stresses, especially after herbivore and pathogen attack.

Plants display a wide variety of mechanisms to resist being consumed by pathogens or herbivores. Some of these mechanisms provide direct protection against these attackers, such as the production of toxins, feeding deterrents or repellents, many of which can be volatile. These defences are targeted directly at the herbivores or pathogens. In addition, plants have evolved mechanisms that recruit natural enemies of herbivores, and this attraction is often mediated by induced VOCs that function as prey-associated signals for experienced foraging carnivores. This phenomenon is referred to as indirect defence. Currently, it is widely acknowledged that such tritrophic interactions between plant, herbivore and carnivore occur throughout the plant kingdom (Dicke *et al.*, 2003). The observation that a plant's metabolic status may be encrypted in the VOCs it releases led, more than two decades ago, to the hypothesis that those volatiles might also facilitate plant–plant information exchange and alert neighbouring plants that herbivores or pathogens are in the area, making it worthwhile to initiate defences prior to actual infection or infestation (Baldwin *et al.*, 2006).

In this chapter, we will elaborate on the role of induced VOCs in plant defences, that is, on their role as toxins, repellents and attractors of 'bodyguards', and also on their properties to change the metabolism and defence status of neighbouring plants. We will first discuss the biochemical processes involved in the formation of various VOCs and the within-plant spatial organisation of their production and storage. Next, we will discuss the hormonal control of their production and release by individual plants and the herbivore- and pathogen-derived signals—elicitors—that initiate production and release during stress. We will then discuss the factors that cause VOC release to be variable and focus on the impact of this variation on responses of nearby herbivores and predators. Finally, we will elaborate on plant–plant eavesdropping, that is, the priming and induction of defence responses in plants that perceive VOCs from induced neighbouring plants.

II. HERBIVORE-PRODUCED ELICITORS AND SUPPRESSORS OF PLANT VOC EMISSION

Plants produce a wide variety of organic compounds that are in principle volatile if they are exposed to the open air. Many of these are intermediates in biosynthetic pathways of those secondary metabolites that actually are released, in small amounts, into the headspace, and many of these have no known primary function and are often stored, for example in trichomes, to serve a defensive function (Gershenzon and Dudareva, 2007). A relatively small, but biologically significant portion of these VOCs is aerially released, possibly because of metabolic overflow, although autotrophic organisms, unlike heterotrophic organisms, are not known to make use of these types of catabolic processes that deliver much waste. Emission of volatiles from vegetative tissue is in some cases associated with heat regulation or scavenging of radicals (Chanjirakul *et al.*, 2007; Vallat *et al.*, 2005), but it remains to be seen whether these functions are common. Although plants emit volatiles already under their normal growth conditions, emission is elevated notably during herbivory and pathogenesis, as well as in response to abiotic stress, such as drought, and altered in response to, for example, decreased nutrient status and increased UV light. Together, these factors can lead to a dramatic increase in the amount of volatiles (quantitative) and/or alteration of the types of volatiles (qualitative) in the plant's headspace.

Herbivores rupture cells and membranes during feeding. The degree and type of damage can differ highly among different feeding styles. Chewing herbivores, such as caterpillars, miners and borers, cause more loss of tissue and damage to walls and membranes than stylet feeders, such as phloem-feeding aphids. Mesophyll-feeding stylet feeders, such as mites and thrips, leave cell walls and plasma membranes largely intact, but can empty cell contents. Phloem-feeding stylet feeders, such as aphids and whiteflies, cause marginal damage to mesophyll cells and walls, but cause a shift in a plant's source–sink flow.

Mechanical wounding has been shown to induce accumulation of jasmonic acid (JA) and to elicit the release of volatiles, but these represent only part of the volatile mixture characteristic of different plant–herbivore interactions. Thus, herbivore-specific signals give rise to the release of additional volatiles. The fact that treatment of wounded plant tissue with herbivore oral secretions, that is, regurgitant, is sufficient to mimic herbivore-induced JA accumulation and defence gene expression, confirmed this assumption. In addition, some of the active components (elicitors) have been identified and in some cases turned out to be inducers of plant volatiles as well (Gaquerel *et al.*, 2009). The first identified herbivore-derived elicitor was the large cabbage white (*Pieris brassicae*)-derived enzyme β-glucosidase, which induced sufficient volatiles in cabbage to attract the parasitic wasp *Cotesia glomerata* (Mattiacci *et al.*, 1995). The second elicitor described was a fatty-acid conjugate, *N*-(17-hydroxylinolenoyl)-L-glutamine, named volicitin (Alborn *et al.*, 1997), which is formed in the gut of beet armyworm (*Spodoptera exigua*) larvae after ingestion of plant material via a 17-hydroxylation reaction of plant fatty acids and subsequent conjugation with insect-derived glutamine. Volicitin has also been found in secretions of tobacco budworm (*Heliothis virescens*) and corn earworm (*Helicoverpa zea*) (Mori *et al.*, 2001). Additional volatile-inducing fatty-acid conjugates were found to be produced by the tobacco hornworm (*Manduca sexta*) (Halitschke *et al.*, 2001) and sulphooxy fatty acids, such as caeliferin A16:0, by the American grasshopper (*Schistocerca americana*) (Alborn *et al.*, 2007). These fatty-acid conjugates come into contact with ruptured plant tissue during insect regurgitation. Apparently, plants possess membrane receptors that recognise such fatty-acid conjugates to trigger defence responses and volatile production, as suggested for maize (*Zea mays*) (Truitt and Paré, 2004). In wild tobacco (*Nicotiana attenuata*, also called coyote tobacco), the *Ma. sexta* regurgitant compound 2-hydroxyoctadecatrienoic acid (2-HOT), derived from plant linolenic acid by the action of plant α-dioxygenase proteins in the alkaline insect mid-gut, is responsible for induction of the major volatile sesquiterpene *trans*-α-bergamotene (Gaquerel *et al.*, 2009). Moreover, a peptide fragment derived from cowpea (*Vigna unguiculata*) chloroplastic ATP-synthase, called inceptin (Schmelz *et al.*, 2007), has been identified from fall armyworm (*Spodoptera frugiperda*) secretions. It induces several herbivore-induced plant volatiles, resulting in attraction of conspecific neonates that use these compounds as host plant location and recognition cues (Carroll *et al.*, 2008). When comparing the activity of these elicitors across plant species, Schmelz *et al.* (2009) found that volicitin exhibited the widest range of phytohormone- and volatile-inducing activity, spanning maize, soybean (*Glycine max*) and eggplant (*Solanum melongena*), whereas the action of inceptin was limited to cowpea and that of caeliferin to *Arabidopsis thaliana*. These findings suggest receptor-mediated elicitor specificity among plant species. Finally, oviposition by *Pi. brassicae* on Brussels sprouts (*Brassica oleracea*) induced metabolic changes that arrested the egg parasitoid *Trichogramma brassicae*. It appeared that the anti-aphrodisiac benzyl cyanide, released by mated female butterflies during egg deposition, accounted for this induction (Fatouros *et al.*, 2008).

The notion that herbivores produce specific elicitors of VOC emission indicated that plants can evolve to recognise a pest and to initiate indirect defences to augment direct defences. Indirect defences comprise the attraction to, and/or arrest of foraging carnivores to plants via, for example, emission of volatiles that signal the presence of prey, and also by providing shelter or alternative food sources such as extra-floral nectar. Hence, it is not surprising that there are indications that some herbivores, possibly due to selective pressure caused by predation, can suppress indirect defences (Bede *et al.*, 2006; Kant *et al.*, 2008). Takabayashi *et al.* (2000) found distinct intra-specific variability in the generalist two-spotted spider mite (*Tetranychus urticae*) in the sense that of two genetically distinct lines of the herbivore (a ‘red’ tomato line and a ‘green’ cucumber line) only one induced indirect defence by feeding on tomato. Subsequently, individuals of the Kanzawa spider mite (*Tetranychus kanzawai*) were found to differentially affect induced volatiles in Lima bean (*Phaseolus lunatus*) (Matsushima *et al.*, 2006), while different individuals of *Te. urticae* collected from the same natural population either induced or repressed emission of volatiles from tomato (*Solanum lycopersicum*) (Kant *et al.*, 2008). Moreover, in Canadian goldenrod (*Solidago altissima*) plants, the generalist caterpillar *Heliot. virescens* elicited strong indirect defensive responses, whereas the gall-inducing tephritid fly *Eurosta solidaginis* did not. The suppression by the tephritid fly appeared sufficient to repress volatiles induced by the generalist caterpillar as well (Tooker *et al.*, 2008). Since direct and indirect defences are metabolically tightly linked, it is well possible that adaptations that lead to suppression of indirect defences might be the same as those that lead to suppression of the direct ones.

While volatile-mediated attraction of natural enemies of herbivores clearly benefits plants, it is much harder to imagine that indirect defences also operate against pathogenic micro-organisms. Nevertheless, pathogen infection also often appears to be accompanied by changes in the plant’s headspace. Several bacterial pathogens, for example, *Pseudomonas syringae* and *Xanthomonas campestris*, differentially induce volatile emission upon infection of *Arabidopsis* and tobacco (*Nicotiana tabacum*), but there is no evidence that specific elicitors other than known avirulence factors are involved

(Cardoza *et al.*, 2002; Huang *et al.*, 2003, 2005). Yet, also β-glucans, chitin and nodulation (Nod)-factors were shown to induce sesquiterpene emission in *Medicago truncculata*, with characteristic volatile bouquets for each elicitor (Leitner *et al.*, 2008). Although the function of these volatiles remains to be elucidated, it is clear that a secondary infection by a pathogen following herbivory might have an influence on indirect defences against the primary insect attacker.

For experimental purposes, herbivory is often simulated. Roughly, two types of induction are used in experiments: (a) a single artificial stimulus, for example, a wound inflicted with scissors or a haemostat with or without addition of purified or chemically synthesised elicitors or insect saliva, and (b) a continuous stimulus, for example, repetitive artificial stimuli (Mithöfer *et al.*, 2005) as a mimic of actual herbivore feeding. A single stimulus usually results in a 'burst' of volatiles, reminiscent of the burst of JA (Paschold *et al.*, 2006), which fades with time. This method is typically used in experiments to study the impact of an external treatment or transgene on the qualitative composition of the induced volatile blend. Alternatively, repetitive or continuous stimuli are usually applied when the temporal dynamics of emission or behavioural responses of insects are studied. Experimentally mimicking the feeding rhythm and pattern of insects is hampered by the fact that different insects have very different feeding strategies and adjust these depending on cues that emanate from their food source: absence or presence of such cues, for example, due to a transgene, can alter such behaviour dramatically (Halitschke *et al.*, 2008; Kessler *et al.*, 2004).

III. BIOSYNTHESIS OF PLANT VOCs

The majority of the different volatile organic molecules that are released by plants are (1) linoleic acid/octadecanoid pathway-derived jasmonates and C_6-compounds; (2) phenylalanine (Phe)-derived aromatic products; (3) isoprene-derived products such as terpenoids (Fig. 1); (4) methanol; and (5) ET.

A. LINOLEIC ACID/OCTADECANOID PATHWAY-RELATED COMPOUNDS

The octadecanoid (C_{18}) pathway starts with linolenic acid and proceeds through dioxygenation in the plastids by C13-LOX via dehydration by allene oxide synthase (AOS) and cyclisation by allene oxide cyclase (AOC) to form oxophytodienoic acid (OPDA), the precursor of JA (Fig. 1). Multiple β-oxidation cycles in the peroxisomes result in the formation of JA. JA,

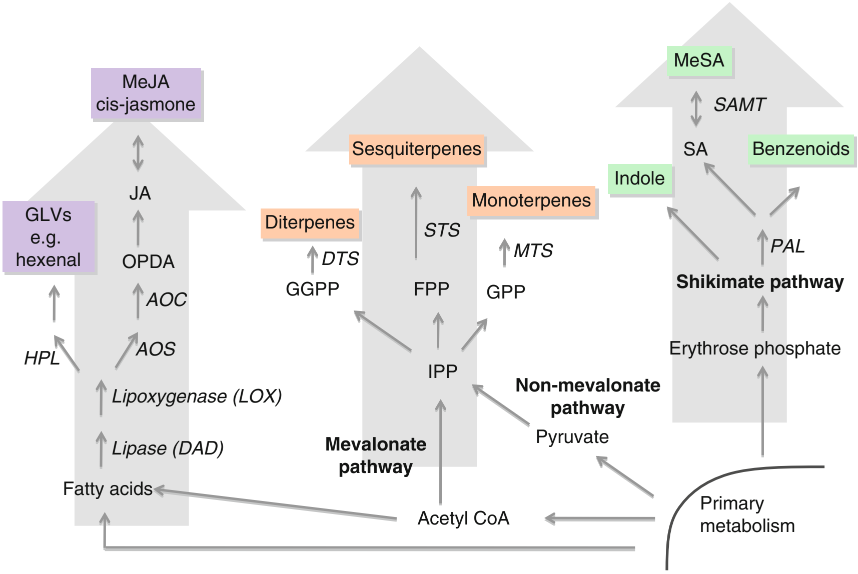

Fig. 1. Biosynthesis of plant volatiles. Metabolic pathways leading to the formation of fatty-acid derivatives, terpenoids via the mevalonic or non-mevalonic pathways, and Phe-derived volatiles via the shikimate pathway. Abbreviations: *AOC*: *ALLENE OXIDE CYCLASE*; *AOS*: *ALLENE OXIDE SYNTHASE*, *DAD*: *DEFENDER AGAINST APOPTOTIC DEATH* (a phospholipase); *DTS*: *DITERPENE SYNTHASE*; FPP: farnesyldiphosphate; GGPP: geranylgeranyldiphosphate; GLVs: green-leaf volatiles; GPP: geranyldiphosphate; *HPL*: *HYDROPEROXIDE LYASE*; IPP: isopentenyl pyrophosphate; JA: Jasmonic acid; *LOX*: *LIPOXYGENASE*; MeJA: methyl jasmonate; MeSA: methyl salicylate; *MTS*: *MONOTERPENE SYNTHASE*; OPDA: oxophytodienoic acid; *PAL*: L-*PHENYLALANINE AMMONIA-LYASE*; SA: salicylic acid; *SAMT*: *SALICYLIC ACID METHYLTRANSFERASE*; *STS*: *SESQUITERPENE SYNTHASE*.

and some of its amino acid derivatives, particularly JA-isoleucine, are key signalling compounds in plant defence (Howe *et al.*, 1996; McConn *et al.*, 1997). Alternatively, JA-methyltransferase (JMT) can catalyse the transfer of a methyl group from *S*-adenosyl-L-methionine (SAM) to the carboxyl group of JA. Methylated JA (MeJA) is volatile. MeJA, and the decarboxylated JA-derivative *cis*-jasmone, occur widely in diverse plant species and their endogenous levels increase dramatically during insect attack, in parallel with those of other JA derivatives (Katsir *et al.*, 2008). Pure MeJA has been shown to act as an elicitor of defences in undamaged plants, as it activates the expression of defensive genes, such as proteinase inhibitors (PIs) (Farmer and Ryan, 1990), and elicits VOC emissions (Kessler *et al.*, 2006) after adsorption and subsequent conversion to endogenous JA-amino acid conjugates (Tamogami *et al.*, 2008).

Biosynthesis of C_6-volatiles is closely associated with that of the C_{12} stress hormone traumatin (Zimmerman and Coudron, 1979) and probably makes use of the same linolenic acid pools as the octadecanoid pathway. C_6-aldehydes and C_6-alcohols and their esters are commonly emitted by green plant tissues (Paré and Tumlinson, 1999). C_6-volatiles are produced in relatively low amounts by undamaged plants, but their emission increases in response to wounding and herbivory (Hatanaka, 1993; Matsui, 2006; Turlings *et al.*, 1995). Upon tissue disruption, hydrolysis of galactolipids from chloroplast membranes provides free fatty acids necessary for the formation of C_6-volatiles (Matsui *et al.*, 2000). Hence, C_6-volatiles are derived from fatty acid metabolism and are synthesised usually from C_{18} α-linolenic and linoleic acids through dioxygenation by a C13-LOX and subsequent cleavage by a hydroperoxide lyase (HPL), a member of the cytochrome P450 family. C_6-aldehydes can be metabolised further by alcohol dehydrogenase (ADH) possibly in conjunction with an isomerisation factor (IF) (Hatanaka *et al.*, 1992) to form the corresponding C_6-alcohols. These can then be further modified by acyltransferases or alkenal reductases (D'Auria *et al.*, 2002; Mano *et al.*, 2002). There are indications that C_6-volatiles determine host plant acceptance or rejection by feeding or ovipositing herbivores (Halitschke *et al.*, 2008). The group of Junji Takabayashi dedicated a lot of time to study the inducing effects of C_6-volatiles on resistance in *Arabidopsis* against grey mould caused by the fungus *Botrytis cinerea*. They found that C_6-volatiles have bactericidal and fungicidal properties, as reviewed by Matsui (Matsui, 2006), and also against *B. cinerea* (Kishimoto *et al.*, 2008; Matsui *et al.*, 2006). Thus, C_6-volatiles also have anti-microbial properties against fungi and bacteria (Croft *et al.*, 1993; Nakamura and Hatanaka, 2002; Ongena *et al.*, 2004).

B. PHENYLALANINE-DERIVED VOLATILES

Biosynthesis of aromatic volatiles relies on precursors from primary processes involved in the production of aromatic amino acids, which are also the precursors of lignins and anthocyanins. Volatile phenylpropanoids and C_6-benzenoids are the most abundant constituents of floral scents and play an important signalling role in attracting pollinators (Knudsen and Tollsten, 1993; Negre *et al.*, 2003). Most research on phenylpropanoid-derived volatiles has been carried out with petunia (Schuurink *et al.*, 2006), roses (Guterman *et al.*, 2002) and snapdragon (Dudareva and Pichersky, 2000). The synthesis of phenylpropanoid-derived compounds proceeds from chorismate and isochorismate by the shikimate pathway (Catinot *et al.*, 2008). Chorismate is subsequently converted into the amino acid Phe, the universal building block for all phenylpropanoids and benzenoids, while isochorismate is converted into salicylate (SA) (Ament *et al.*, 2004; D'Auria *et al.*, 2003; Vlot *et al.*, 2008).

Many benzenoids originate from the *trans*-cinnamic acid (*t*-CA) route, where the enzyme L-phenylalanine ammonia-lyase (PAL) deaminates Phe to produce *t*-CA (Fig. 1). This part of the biosynthetic pathway is shared with the production of anthocyanins and lignins. Volatile benzenoids, such as eugenol, methyleugenol, chavicol and methylchavicol, are formed from *p*-coumaric acid. Phenylacetealdehyde is produced directly from Phe by phenyl-acetealdehyde synthase and can subsequently be converted to 2-phenylethanol. Benzoic acid (BA), a key component in benzenoid formation, is made from *t*-CA via multiple pathway branches: the CoA-dependent β-oxidative branch (analogous to the β-oxidation giving rise to fatty acid-derived JA), the CoA-independent non-β-oxidative pathway branch, or a combination of the two (Boatright *et al.*, 2004). Both BA and SA can be methylated by the BA/SA methyltransferases (called BAMT and SAMT, respectively) into volatile MeBA and MeSA. The enzymes responsible for the methylation of JA, SA and BA belong to the SABATH family, named after the first three genes identified: *SAMT*, *BAMT* and *THEOBROMINE SYNTHASE* (D'Auria *et al.*, 2003). However, when *BAMT* is silenced in petunia, production of other benzenoids does not increase, indicating a more complex network of regulation than currently known. The petunia benzenoid pathway is regulated by the transcription factor ODORANT1, which regulates expression of genes early in the shikimate pathway, that is, 5-enol-pyruvylshikimate-3-phosphate synthase (Verdonk *et al.*, 2005).

C. TERPENOIDS

Terpenes are composed of isoprenes, and while many terpenoids are precursors of primary plant products such as plant hormones, photosynthetic pigments as well as electron carriers and structural components of membranes, several were found to function as defensive metabolites (Logan *et al.*, 2000). The majority of herbivore-induced terpenoids are monoterpenes (C_{10} compounds), sesquiterpenes (C_{15}), diterpenes (C_{20}) and homoterpenoids (C_{11} or C_{16}), many of which can be found in the plant headspace as well as within plant tissues (Ament *et al.*, 2004; Boatright *et al.*, 2004; Leitner *et al.*, 2005; Logan *et al.*, 2000; Verdonk *et al.*, 2005).

Terpene biosynthesis takes places via two compartmentalised pathways (Fig. 1). The cytosolic mevalonate (MVA) pathway produces sterols and predominantly sesquiterpenoids, whereas the plastidial 2-*C*-methyl-D-erythritol 4-phosphate (MEP or non-MVA) pathway is involved in the biosynthesis of chlorophyll, tocopherols, vitamins, hormones such as gibberellins (GAs) and abscisic acid (ABA), and of mono- and diterpenoids. All terpenes are generated through condensation of universal C_5-compounds: isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). In the cytosolic MVA pathway, isoprene units are generated from acetyl-CoA and sesquiterpenes are produced from C_{15}-farnesyldiphosphate (FPP) by the enzyme FPSynthase. In the MEP pathway, isoprenes are produced from pyruvate and glyceraldehyde-3-phosphate via several enzymatic steps in the plastid. Subsequently, C_{10}-geranyldiphosphate (GPP) and C_{20}-geranylgeranyldiphosphate (GGPP) are the precursors for monoterpenes and diterpenes, respectively, produced via the enzymes GPSynthase or GGPSynthase.

Although the MVA and MEP pathways are separated by the plastidial membranes, cross-talk between the two pathways via precursor exchange has been suggested (Hemmerlin *et al.*, 2003; Lichtenthaler, 1999). Moreover in basil and snapdragon, it appears that the production of terpenes depends almost exclusively on the MEP pathway (Dudareva *et al.*, 2004; Xie *et al.*, 2008).

The diversity of volatile terpenes found in nature originates from the activity of terpene synthases (TPSs) and modifying cytochrome P450 enzymes. TPSs have differentiated into multi-gene families and can catalyse the synthesis of single terpenoids, or of multiple terpene products in specific ratios (Bohlmann *et al.*, 1998; Cheng *et al.*, 2007; Segura *et al.*, 2003). As a consequence, TPSs are responsible for the formation of the most abundant and structurally diverse group of plant metabolites. To date, hundreds of TPSs have been functionally characterised, several of which are induced by biotic stress conditions (Herde *et al.*, 2008; Lin *et al.*, 2008; Van Schie *et al.*, 2007).

D. METHANOL

Plants produce and emit methanol (MeOH), which impacts $OH^{\cdot}$ radical concentrations and photochemical ozone. Emission of MeOH is associated with growth, ageing of plant tissues and leaf abscission (Huve *et al.*, 2007), as well as tissue damage. Wound-induced release of MeOH increased dramatically when larvae of *Ma. sexta* attacked *N. attenuata* plants. MeOH emission was sustained for 24 h after herbivore feeding and was substantially higher than the release of *E*-2-hexenal. It appeared that herbivore-induced MeOH results from a pH shift at the wound site due to larval oral secretions (pH 8.5–9.5). It coincided with increased transcript accumulation and activity of leaf pectin methylesterases. It has been suggested that herbivore-induced MeOH is actually beneficial to herbivores, since it has a negative effect on the induction of some well-known defence marker genes and increases the total weight gain of the attacking larvae (Von Dahl *et al.*, 2006).

E. ETHYLENE

Ethylene is a volatile plant hormone. The biology and genetics of ET responses were primarily dissected by studying the lack of effect of ET on dark-grown mutant seedlings (the 'triple response') (Guzman and Ecker, 1990). Active in minute amounts, ET is essential for plant development, fruit ripening and senescence. Most plants induce ET emission in response to herbivory or in response to pathogen infection. The biosynthesis of ET starts with the conversion of methionine to SAM (Ado-Met), which is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by the rate-limiting ET-biosynthetic enzyme ACC synthase (ACS). The enzyme ACC oxidase (ACO) finally produces ET. ET biosynthesis can be induced by exogenous ET, auxins or cytokinins. ET receptors are encoded by multiple genes and are located in the endoplasmic reticulum (Hall *et al.*, 2007). ET is known to modulate various defence responses against pathogens and insects (Van Loon *et al.*, 2006).

IV. VOLATILE METABOLISM IN PLANT TRICHOMES

A. TRICHOME FUNCTION AND OCCURRENCE

Production of aromatics and C_6-volatiles occurs mostly in plastids, while terpenoids are produced in both plastids and the cytosol. How these compounds are transported from there to their site of release is unknown. C_6-volatiles in the plant headspace are likely to result from *de novo* synthesis. In contrast, terpenoids and aromatics after synthesis are often stored in specialised organs. Storage organs can be oil ducts or glandular trichomes, which are distinct and relatively autonomous cellular structures largely separated from the rest of the plant, possibly because of the phytotoxic properties of high levels of secondary metabolites, only to be released in response to biotic stresses (Wagner, 1991). In general, trichomes are small, single-celled or large, multi-cellular structures with diverse morphology: non-glandular trichomes are simply leaf hairs without specialised storage function (Werker, 2000), whereas glandular trichomes consist of cells equipped to store volatile products and phenolics. It is suggested that these cells might often contain plastids, fix carbon themselves and function relatively independently from the rest of the plant (Wagner, 1991).

Glandular trichomes are present on leaf and stem surfaces of many plant species, but can also be found on petioles, seed coats, flower petals and pedicels. In many plant species, trichome development starts early during leaf differentiation and trichome density decreases with leaf expansion. The sub-cuticular trichome storage cavities at the tip are loaded with metabolites from secretory cells that lie below. How volatile components are released from trichomes is not known. Possibly, they evaporate when trichomes are ruptured, for example, by crawling insects. However, there are also likely active mechanisms for release, since induced emission of volatiles often occurs systemically, that is, also from healthy undamaged tissues. Per gram fresh weight or unit area, young leaves are far more active in the release of volatiles than older leaves, although this could very well be the result of differences in trichome density. Also, the qualitative composition of exudates produced by glandular trichomes may be different in old as compared to young leaves (Maffei *et al.*, 1989), as described in section VI. Trichome densities are not simply genetically determined and depending solely on developmental stage, since in wild tomato (*Lycopersicum hirsutum*) they can fluctuate over the seasons, consequently influencing the production of specific exudates (Antonious *et al.*, 2005).

Whereas trichomes are found on plant above-ground surfaces, root hairs can be considered a type of below-ground 'root epidermal trichomes' (Werker, 2000). The development of root hairs and trichomes has been shown to be under the same genetic control and regulation (Kellogg, 2001). Like trichomes, root hairs exude secondary metabolites, for instance carotenoid-derived strigolactones, which are involved in host recognition in plant–mycorrhizal and plant–parasite interactions (Lopez-Raez *et al.*, 2008), and fatty acid-derived lipid resorcinols and benzoquinones in sorghum, which are proposed to suppress growth of competing seedlings (Dayan *et al.*, 2007).

B. TRICHOME METABOLOMICS AND TRANSCRIPTOMICS

To elucidate the molecular genetic basis of trichome developmental processes, such as cell differentiation, the focus has been mainly on the unicellular and non-glandular trichomes of *Arabidopsis*. These likely have a primary function in focussing light onto the leaf surface and act as structural barriers against small herbivores (Kryvych *et al.*, 2008; Szymanski *et al.*, 2000). However, in general, glandular trichomes function as chemical barriers since they synthesise, store and secrete high concentrations of secondary metabolites and defensive proteins. As a target for metabolic engineering, the glandular trichomes of species like basil (*Ocimum basilicum*) and mint (*Mentha x piperita*) have been studied intensively (Aziz *et al.*, 2005;

Gang *et al.*, 2001; Wagner *et al.*, 2004). Glandular trichomes are characterised by a glandular head, but come in different types, shapes and sizes, some of which can co-occur on a single plant or on a single organ (Simmons and Gurr, 2005). The collection of metabolites stored in the trichome glandular head can vary greatly among plant species. For example, tomato trichomes contain mostly acyl sugars, fatty acid-derived methylketones and sesquiterpenes (Fridman *et al.*, 2005; Van der Hoeven *et al.*, 2000), whereas the glandular trichomes of mint contain predominantly monoterpenes such as *p*-methane (Alonso *et al.*, 1992; Lange *et al.*, 2000) and *N. tabacum* trichomes contain predominantly diterpenes (Amme *et al.*, 2005). In contrast, sweet basil trichomes produce phenylpropanoids abundantly (Gang *et al.*, 2001), whereas in the trichomes of hop cones, the female inflorescences, different terpenophenolic metabolites and prenylflavonoids are present (Stevens and Page, 2004).

The genes involved in the development of non-glandular trichomes and those playing a role in the biosynthetic pathways that load the extra-cellular cavity of glandular heads with secondary metabolites, have been identified primarily through mutant screening and analyses of expressed sequence tags (ESTs). For many plant species, cDNA libraries of glandular trichomes have been constructed, which were used to analyse the correlation between the presence or abundance of transcripts and the production of specific compounds in order to link genes to metabolites. For instance, Nagel *et al.* (2008) analysed hop trichomes and found 100 ESTs representing all seven enzymes of the plastidial MEP pathway, whereas only 10 ESTs matched six genes related to the cytosolic MVA pathway. Through analysis of the mint EST-database of Lange *et al.* (2000), it was found that 25% of the total ESTs were associated with essential oil metabolism, including all genes of the monoterpene biosynthetic pathway, and these data were in concurrence with earlier findings that the MVA biosynthetic route in mint is blocked (McCaskill and Croteau, 1995). Moreover, sweet basil produces aromatic phenylpropenes in addition to terpenes, and this correlated very well with the abundances of ESTs associated with specific enzymes involved in the production of precursors for the phenylpropanoid and terpenoid pathways isolated from sweet basil trichomes (Gang *et al.*, 2001; Xie *et al.*, 2008). Finally, Aziz *et al.* (2005) confirmed the presence of ESTs corresponding to enzymes for all steps in the biosynthesis of flavonoids in alfalfa trichomes. Taken together, in many cases glandular trichomes were found to be equipped well enough to support independent production of many secondary metabolites, although clear-cut evidence for such independence is still lacking (Wagner, 1991).

The increasing knowledge of the genes involved in the production of volatiles in trichomes has opened up new possibilities for crop improvement as well as for applications in biotechnology through pathway engineering. Since it appears that trichomes are relatively independent from the rest of the plant, and can be used to produce and store secondary metabolites in otherwise toxic concentrations, they provide an ideal environment for such genetic metabolite engineering. This was advocated by Wang *et al.* (2001), who demonstrated that it is possible to enhance insect resistance in plants by down-regulation of a trichome-specific cytochrome P450 hydroxylase gene responsible for cembratriene-diol production, thereby decreasing the product and increasing the concentration of its specific diterpene precursor cembratriene-ol. Hence, targeted trichome-specific pathway engineering is essentially possible. Less specific, but also promising, is engineering trichomes via manipulations of a plant's responsiveness to phytohormones. The tomato protein Coronatin insensitive 1 (COI1), which is required for plant responsiveness to JA, was shown to be essential for the production of glandular trichomes on immature fruit and to modulate the density of type-VI trichomes, the most common glandular trichome morphotype, on leaves and sepals. Consequently, mutations in *COI1* altered the biosynthetic capacity of glandular trichomes and decreased the plant's resistance to herbivores (Li *et al.*, 2004). Taken together, engineering the trichome appears a realistic goal and may have many advantages over engineering green tissues, since the metabolic changes can essentially be localised to the trichome tissue only.

V. VOLATILE DEFENCE HORMONES MeJA, MeSA AND ETHYLENE

The two plant volatiles MeJA and MeSA are particularly well-documented because they are believed to act as volatile stress hormones. MeJA is a derivative of the stress hormone JA. Therefore, its formation is closely associated with JA-dependent stress responses. Its endogenous occurrence has been described in detail (Baldwin *et al.*, 1997; Seo *et al.*, 2001; Von Dahl and Baldwin, 2004), but MeJA is found only occasionally in the headspace of (induced) vegetative tissues (Farmer and Ryan, 1990; Hopke *et al.*, 1994; Meyer *et al.*, 2003). Notably sagebrush, *Artemisia tridentata*, is known for its constitutive aerial release of MeJA, which increases upon wounding. Farmer and Ryan (1990) discovered that like pure MeJA, naturally released MeJA from sagebrush was sufficient to induce accumulation of PIs in down-wind tomato leaves. This prompted the question whether volatile MeJA might play a role in plant–plant interactions, since unharmed plants may benefit by building up defences when their neighbours are attacked by herbivores before they are attacked by these herbivores themselves. Although plant–plant communication mediated by volatiles still receives a lot of attention, it is unlikely that MeJA plays a general role in this phenomenon simply because, despite its endogenous accumulation, it is rarely emitted by green plant tissues. Also for sagebrush–neighbour interactions under natural conditions, a role for volatile MeJA is doubtful (Karban 2007a,b; Preston *et al.*, 2001, 2002, 2004). However, for artificial induction of JA-dependent defences, that is, to simulate herbivory under experimental conditions, the compound MeJA has proven to be highly suitable (Wu *et al.*, 2008). Simulated herbivory and the use of mutant plants have shown beyond doubt that JA and/or its derivatives are essential signalling molecules for the production and release of herbivore-induced volatiles such as terpenoids. For example, the tomato JA biosynthesis mutant *defenceless 1 (def1)* has normal housekeeping levels of JA and normal trichome densities, but is deficient in the emission of JA-induced terpenoids and MeSA (Ament *et al.*, 2004; Halitschke and Baldwin, 2003; Li *et al.*, 2004) (Table I). For plant–plant interactions, another volatile JA-derivative, *cis*-jasmone, might be a more realistic mediator, since its occurrence as a herbivore-induced volatile is more general, while it has inducing properties in exposed plants similar to those of MeJA (Birkett *et al.*, 2000; Bruce *et al.*, 2008; Pickett *et al.*, 2007).

Whereas MeJA and *cis*-jasmone elicit JA-dependent responses, MeSA elicits SA-specific responses. SA is well-known for its role in inducing the accumulation of pathogenesis-related (PR) proteins and the induction of systemic acquired resistance (SAR) during pathogenesis (Glazebrook, 2005). Reminiscent of MeJA, whose activity is most likely due to its conversion to endogenous JA (Wu *et al.*, 2008), the action of MeSA is probably due to its conversion to SA after uptake by the plant (Chen *et al.*, 2003; Park *et al.*, 2007; Seskar *et al.*, 1998; Shulaev *et al.*, 1997). However, unlike MeJA, MeSA is commonly found in herbivore-induced plant headspaces. Whereas SA is essential as a precursor (Ross *et al.*, 1999), JA signalling is essential for the induction of the SA-methylating enzyme SAMT (Ament *et al.*, 2004). MeSA has been shown to be a critical not only *in planta* mobile signal for the establishment of SAR (Vlot *et al.*, 2008), but is also released into the air by stressed vegetative tissues. Synthetic MeSA was found to be a potent inducer of the SA-related defence marker gene *PR-1* (Shulaev *et al.*, 1997) and, as an odour, to act as an elicitor of foraging responses in predatory mites (De Boer and Dicke, 2004).

The previous implies that treating a plant with MeJA or MeSA is equivalent to treating it with JA or SA. It is well-known that SA/MeSA-signalling and JA/MeJA-signalling have antagonistic properties, since a response induced by one of the hormones is often associated with loss of responsiveness to the other. Intra-cellularly, this antagonistic cross-talk is mediated by the

TABLE I

Mutant and Genetically Modified Plants with Altered Volatile Production and Associated Biological Responses

Receiver Plant	Gene or mutant line	Type	Details	Volatile	Response	References
Arabidopsis	*all84*	Knockout mutant	Octadecanoid pathway mutant	Decreased *Pieris*-induced (*Z*)-3-hexenal	Decreased prey-finding success of parasitoid *Cotesia glomerata*	Shiojiri *et al.*, 2006a
Arabidopsis	S-12	Co-suppressed *35S::Lox2*	JA-biosynthesis mutant	Unknown	Decreased attractiveness of *Pieris rapae*-infested *Arabidopsis* to parasitoid *Cotesia rubecula*	Van Poecke and Dicke, 2002
Arabidopsis	*NahG*	*NahG* over-expresser	SA-hydroxylase converts SA to catechol	Unknown	Decreased attractiveness of *Pieris rapae*-infested *Arabidopsis* to parasitoid *Cotesia rubecula*	Van Poecke and Dicke, 2002
Nicotiana attenuata	*NaNPR1*	Knock-down (inverted repeat)	Mediator in SA/JA cross-talk	Decreased induced *cis*-α-bergamotene emission	Decreased abundance of, and predation by, predator *Geocoris pallens*	Rayapuram and Baldwin, 2007

(*continues*)

TABLE I (*continued*)

Receiver Plant	Gene or mutant line	Type	Details	Volatile	Response	References
Nicotiana attenuata	*NaWRKY3*, *NaWRKY 6* and *NaWRKY3/6*	Knock-down (inverted repeat)	Transcription factor in JA signalling	Decreased induced *cis*-α-bergamotene emission	Decreased *Manduca sexta* egg predation	Skibbe *et al.*, 2008
Nicotiana attenuata	*NaSIPK* or *NaWIPK*	Gene silencing	Early defence signalling	Decreased *cis*-3-hexenol, α-terpineol, trans-α-bergamotene and β-duprezianene	Decreased *Manduca sexta* egg predation	Meldau *et al.*, 2009
Nicotiana attenuata	*NaLOX3*	Knock-down (anti-sense)	JA biosynthesis mutant	Decreased *Manduca*-induced *cis*-α-bergamotene emission	Decreased abundance of, and predation by predator *Geocoris pallens*	Halitschke *et al.*, 2008
Nicotiana attenuata	*NaHPL*	Knock-down (anti-sense)	GLV biosynthesis mutant	Depleted in *Manduca*-induced C_6-volatiles	Decreased abundance of herbivore *Epitrix hirtipennis*	Halitschke *et al.*, 2008
Nicotiana attenuata	*NaHPL*	Knock-down (anti-sense)	GLV biosynthesis mutant	Depleted in *Manduca*-induced C_6-volatiles	*Manduca sexta* larval development slowed	Halitschke *et al.*, 2008

Nicotiana attenuata	*NaHPL x NaLOX3*	Heterozygous cross of *as-Lox3* and *as-Hpl*	Terpene and GLV biosynthesis mutant	Depleted in *Manduca*-induced C_6-volatiles and terpenoids	Reducing GLV emissions in *as-Lox3* plants by *as-Hpl* 'rescued' these plants from being heavily damaged by *Manduca sexta*	Meldau *et al.*, 2009
Potato	*Hpl*	Knock-down (anti-sense)	C_6-volatiles biosynthesis mutant	Decreased hexanal and 3-hexenal accumulation	Increased fecundity of aphid *Myzus persicae*	Vancanneyt *et al.*, 2001
Tomato	*def1*	Knock-out mutant	JA biosynthesis mutant	Depleted in several *Tetranychus urticae*-induced terpenes	Decreased attraction of predator *Phytoseiulis persimilis*	Ament *et al.*, 2004
Tomato	*def1*	Knock-out mutant	JA biosynthesis mutant	Depleted in several *Spodoptera*-induced terpenes	Decreased attraction of predator *Phytoseiulis persimilis*	Thaler *et al.*, 2002
Tomato	*spr2*	Knock-out mutant	JA biosynthesis mutant	Depleted in *Manduca*-induced terpenes	Increased oviposition of *Manduca sexta*	Sánchez-Hernández *et al.*, 2006

Abbreviations: *all84*, *aldehyde-less 84*; *def1*, *defenceless1*; *hpl*, *hydroperoxide lyase*; *LOX3*, *LIPOXYGENASE 3*; *NahG*, *Pseudomonas putida* PpG7 salicylate hydroxylase gene; *NPR1*, *NONEXPRESSOR OF* PR *GENES 1*; *SIPK*, *SALICYLIC ACID-INDUCED PROTEIN KINASE*; *spr2*, *suppressor of prosystemin-mediated responses 2*; *WIPK*, *WOUND-INDUCED PROTEIN KINASE*.

ankyrin-repeat protein Non-expressor of *PR* genes 1 (NPR1), but its mode of operation in not identical between plant species. Silencing *NPR1* in *Arabidopsis* resulted in loss of the antagonism (Beckers and Spoel, 2006), whereas in *N. attenuata NPR1* silencing antagonised JA accumulation and JA-related defences, making the plant more susceptible to *Sp. exigua* larvae (Rayapuram and Baldwin, 2007). Although the biological necessity for the metabolic trade-off between JA and SA is not clear, the general consensus is that it reflects the plant's need to time, prioritise and fine-tune poorly compatible processes that may consume considerable amounts of energy (Kahl *et al.*, 2000) and are embedded in tailored adaptive responses that sometimes involve the action of a herbivore's natural enemies.

In *N. attenuata*, ET mediates a switch from direct to indirect defence during feeding of direct-defence insensitive *Ma. sexta* caterpillars (Kahl *et al.*, 2000). Upon herbivory, *N. attenuata* rapidly accumulates the alkaloid nicotine and starts emitting terpenes somewhat later in time. It was found that ET repressed the accumulation of nicotine shortly after the emission of terpenes had started. It was suggested that the plant used this ET-mediated switch to facilitate nicotine-sensitive parasitoids of the herbivore by preventing the nicotine-tolerant prey to protect itself by sequestering nicotine.

VI. VOC SIGNALS ARE INFLUENCED BY ABIOTIC FACTORS AND PLANT DEVELOPMENTAL STAGE

The release of induced plant volatiles can be highly variable in time and space. This variation is roughly the resultant of the kinetics of the responses, diurnal rhythms, growth conditions, age and type of emitting tissue and the type of stress that elicits the emission.

The time of day is relevant for not only the quantitative, but also the qualitative emission pattern, since emission of volatiles exhibits diurnal photoperiodicity (Loughrin *et al.*, 1994; Turlings *et al.*, 1995). In tomato, emission of most terpenes is positively dependent on light (Maes and Debergh, 2003) and JA (Ament *et al.*, 2004), the sesquiterpene α-copaene being a notable exception. Loughrin *et al.* (1994) observed that *Sp. exigua* on cotton (*Gossypium hirsutum*) induced C_6-volatiles and cyclic and acyclic terpenes in a diurnal rhythm of higher emission during the day than during the night. After removal of the caterpillar, the emission of the cyclic terpenes ceased, but the C_6-volatiles and acyclic terpenes kept their rhythm of emission, albeit that they were released in smaller amounts. Similarly, the larvae of *Heliot. virescens*, *Ma. sexta* and *Helico. zea* induced release of several C_6-volatiles in *N. tabacum*, that is, (*Z*)-3-hexenyl butyrate, (*Z*)-3-hexenyl isobutyrate, (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenyl tiglate, exclusively during the dark phase, and (*E*)-2-hexenal in higher amounts than during the day. The nocturnal volatiles were used by adult *Heliot. virescens* to avoid plants already containing feeding larvae (De Moraes *et al.*, 2001). Schmelz *et al.* (2001) showed that the same treatments, that is, wounding with or without application of JA or volicitin, applied at different times of the day on excised maize leaves or intact plants, resulted in differential volatile emission during the subsequent light period. Excised leaves emitted three to eight times more sesquiterpenes than leaves on intact plants, and in different ratios. Induction had the strongest effect on excised leaves treated in the middle of the dark phase, whereas intact plants displayed little or no response to volicitin when they had been treated at the beginning of the light cycle.

Tissue type and age influence particularly quantitative emission profiles of induced volatiles. As a rule, on a weight basis, young tissue is more active than older tissue. Accordingly, the predatory mite *Phytoseiulus persimilis* preferred volatiles from young leaves infested with the spider mite *Te. urticae* over those emitted by old infested leaves, while their qualitative composition was similar (Takabayashi *et al.*, 1994). When older leaves of cotton were damaged by *Sp. exigua* caterpillars and started to produce volatiles, the undamaged younger leaves started to release some of these volatiles as well, showing systemic induction (Röse *et al.*, 1996). In sweet-scented tobacco (*Nicotiana suaveolens*), the quality and quantity of the floral volatile blend was altered by *Ma. sexta* larvae feeding on the leaves (Effmert *et al.*, 2008). Moreover, in a comprehensive analysis of the kinetics of *Sp. frugiperda*-induced volatiles in soybean, it was found that plants in the vegetative stage emitted 10-fold more volatiles per biomass than reproductive plants, that young leaves emitted three times more volatiles than old leaves and also that systemic induction in single leaves was stronger and faster (after one day) in the acropetal than in the basipetal direction (Rostás and Eggert, 2008). Hence, local responses affect the metabolism in distal tissues as well, but the magnitude of the response depends on tissue-specific parameters such as age and relative position.

Considering the nature of the plant's 'indirect' defences, it is clear that the receiving party, that is, a foraging carnivore or a host-searching parasitoid, must be able to discriminate volatile signals from background noise and to decipher the qualitative and quantitative information in the volatile blend to the best of their abilities. In principle, the concentration of volatiles decreases with increasing distance from the odour source, but evaporation gradients can be asymmetric due to air currents. When studying the impact of volatiles on insects, or on plants, working with realistic concentrations is not trivial. In insects behavioural assays, concentrations that are too high might elicit avoidance or escape or, alternatively, evoke behaviour where ‘something’ is preferred over ‘nothing’. Similarly, when studying induction of defences by volatiles in neighbouring plants, high concentrations might cause a general ‘stress’ reaction, which often will be difficult to separate from ‘defence’ since the two have much in common. Finally, when working with high amounts of volatile hormones, such as MeSA and MeJA, one might easily elicit responses that do not occur under natural conditions.

To study the impact of different amounts of MeJA on the defence chemistry of *N. attenuata*, Preston *et al.* (2004) determined that the concentration of the naturally constitutively released MeJA from neighbouring sagebrush plants remained constant within an area of 40 cm, but decreased rapidly over a distance of 1.5 m. They reasoned that the entire amount of MeJA released from one plant will not all be deposited onto a single neighbouring tobacco plant, and at realistic concentrations of synthetic MeJA (1–3 mg released per hour) they did not find evidence that MeJA elicits resistance or resistance-related metabolic changes. Still, volatile-mediated priming of resistance between sagebrush and *N. attentuata* does occur in nature, although its relevance to the plant’s survival strategy is still under debate. Co-occurrence of *N. attentuata* and sagebrush is less common in nature than one would expect if their relationship would have evolved to be truly beneficial for the tobacco plants (Karban 2007a; Kessler *et al.*, 2006).

Changes in the plant’s induced volatile metabolism depend not only on tissue type, age and time of day, but also on growth conditions. How abiotic factors influence the profile of herbivore-induced volatiles has been only marginally investigated. Gouinguené and Turlings (2002) tested the effect of soil humidity, air humidity, temperature, light and fertilisation (complete nutrient solution vs. demineralised water) on the induced terpene, indole and C_6-volatiles emission of maize plants. They concluded that plants standing in dry soil released more volatiles overall than in wet soil, and emission was optimal at 60% relative air humidity and a temperature between 22 °C and 27 °C. Emission appeared fully photophase dependent and fertilisation had a strong overall positive effect on the quantities released. Takabayashi *et al.* (1994) tested the impact of light conditions, time of year and water stress on the relative composition of the spider mite-induced volatile blend of Lima beans. While the blend from low-light plants consisted of 5% (*E*)-*β*-ocimene, in high light this increased to 21%, and predatory mites were attracted more towards high-light plants in a choice test. The attractiveness of high-light plants compared to low-light plants was clearest from April until September, and absent in winter. Plants grown under water stress conditions (3–3.5 pF_{soil}) compared to plants grown under normal moisture levels (1.5–1.8 pF_{soil}), but both at 60–70% relative air humidity, produced higher amounts of linalool, (*E*)-β-ocimene, 4,8-dimethyl-1,3,7-nonatriene (DMNT) and MeSA, and were more attractive to predatory mites. Induction of these volatiles in Lima bean (Gols *et al.*, 2003) and tomato (Ament *et al.*, 2004) depends on JA, which is produced by the 13-LOX pathway (Halitschke and Baldwin, 2003). Although poorly documented and largely inferred from transcript analyses, most results indicate that the activity of the 13-LOX pathway and JA accumulation are indeed induced by abiotic stress (Fujita *et al.*, 2006; Nemchenko *et al.*, 2006; Walia *et al.*, 2007). Ozone exposure triggered DMNT, 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) and (*Z*)-3-hexenyl acetate emission in Lima beans to a sufficient extent to attract predatory mites when unexposed clean plants were the alternative. Also, diamondback moth (*Plutella xylostella*)-infested ozone-exposed *Br. oleracea* plants attracted more predatory mites than un-infested ozone-exposed plants (Vuorinen *et al.*, 2004). The preference of the parasitoid *Cotesia plutellae* for diamondback moth-infested *Br. oleracea* in the presence of ozone remained unaffected, although some terpenes and C_6-volatiles were oxidised by ozone (Pinto *et al.*, 2007). This suggested that tritrophic interactions are not significantly affected by ozone and that herbivore-induced terpenes might function to quench ozone and reactive oxygen species (ROS) (Holopainen, 2004). Taken together, growth conditions and abiotic stress clearly alter induced volatile emission, but the volatile-mediated indirect defences appear quite robust.

VII. NATURAL VARIATION IN VOC PRODUCTION

The spectrum of herbivore-induced volatiles can vary across species, within species across cultivars and even between individuals within cultivars. Here, we will limit ourselves to studies where plants were compared directly within one study, since differences between methods/sampling techniques hamper making a sensible meta-analysis of the available literature.

Variation in the production of secondary metabolites across plant species is well-known and well-studied (Bennett and Wallsgrove, 1994) and could reflect diversification of plant defences, possibly as a result of ongoing arms races (Aubourg *et al.*, 2002; Benderoth *et al.*, 2006).

Plants of different families produce different volatiles when infested with the same herbivore species. Van den Boom *et al.* (2004) undertook a comprehensive across-species analysis, collecting the induced volatiles of 11 plant species during infestation with the generalist spider mite *Te. urticae* and comparing those to volatiles from mechanically damaged control plants. They concluded that almost all species produce novel compounds upon infestation, including MeSA, terpenes, oximes and nitriles, and that only two species, tobacco and eggplant, alter their emission only quantitatively after induction. Nevertheless, when seven of these species, including eggplant, were tested for attractiveness to *Te. urticae*'s natural enemy *Ph. persimilis*, all elicited a positive response to this predatory mite. In addition, when comparing the relative attractiveness of four *Te. urticae*-infested gerbera varieties to *Ph. persimilis*, this response appeared to be positively correlated with the amount of terpenes each produced and with the level of infestation (Krips *et al.*, 2001). Hence, although volatile production may be variable, the outcome of the tritrophic interaction apparently is not.

If variation in induced volatile production during crop breeding is generated by coincidence—since there is no targeted selection for it—one would expect that the degree of variability between cultivars of a crop is different from that between ecotypes of wild plants that are under natural selection. For example, when Takabayashi *et al.* (1991) measured the emission of volatiles from two commercial apple varieties, 'Summer Red' and 'Cox Orange Pippin', after infestation by two different species of spider mites, that is, *Te. urticae* and *Panonychus ulmi*, they found that differences between the two apple varieties infested by the same mite species were bigger than of a single apple variety infested by the two different mite species. This indicated that genetic differences between races of the same plant species might translate into markedly different induced volatiles. Turlings *et al.* (1998) observed considerable differences in the timing of volatile emission from two maize cultivars after induction with *Spodoptera littoralis* oral secretions, since one of the cultivars produced several monoterpenes and sesquiterpenes, which the other did not at all. Subsequently, Gouinguené *et al.* (2001) compared the induced volatile emissions among seven maize cultivars and five of their wild ancestors, including a comparative analysis of eight individuals from a single natural population of wild teosinte. They observed considerable differences, for example, absence or presence of the sesquiterpene β-caryophyllene, and quantitative differences in ratios for all groups except among the eight individuals of the same wild ancestor. In a follow-up study, Degen *et al.* (2004) compared the induced volatiles of 31 maize inbred lines, representing a large portion of the genetic diversity used by breeders, by a principal component analysis. The genotypes showed highly variable odour profiles and, again, β-caryophyllene stood out as being typical for European, but not American, varieties. There appeared to be no relation between the genetic distances of the lines and their odour-profile distances. Different natural populations of *N. attenuata* growing in the field appeared variable in their production of volatiles as well (Halitschke *et al.*, 2000) and, similarly, within natural populations of sacred thornapple (*Datura wrightii*) considerable heritable variation in the production of β-caryophyllene as well as trichome type was shown, the latter under control of a single gene (Hare, 2007). Taken together, variation in induced volatiles is common both between ecotypes in nature and between varieties of crops, suggesting that insects using such information for finding prey must cope with this variability irrespective of the circumstances.

To appreciate the role of herbivore-induced volatiles in tritrophic interactions, it is essential to understand how insects perceive and process volatile information. Electrophysiological studies indicate that VOCs are perceived by a wide variety of insect taxa. High sensitivities to C_6-volatiles have, for example, been reported in beetles (Blight *et al.*, 1995), moths (Anderson *et al.*, 1995), flies (De Bruyne *et al.*, 2001) and parasitoids (Smid *et al.*, 2002). Terpenoids such as linalool, β-caryophyllene, β-ocimene and geraniol are also well-perceived by moths (Anderson *et al.*, 1995; Rostelien *et al.*, 2005; Shields and Hildebrand, 2000), flies (De Bruyne *et al.*, 2001) and parasitoids (Smid *et al.*, 2002). Phenylpropanoids such as MeSA have been reported to be well perceived by flies (De Bruyne *et al.*, 2001), moths (Shields and Hildebrand, 2000), parasitoids (Smid *et al.*, 2002) and predatory mites (De Bruyne *et al.*, 1991).

The parasitic wasp *Cotesia marginiventris* was used to test whether quantitative or rather qualitative variability in beet armyworm-induced volatile production of maize plants impacts its success in finding caterpillars. Cowpea and 13 different Mexican maize varieties, producing quantitatively and qualitatively significantly different volatile blends, were tested in all combinations using dual choice tests. While naïve wasps preferred induced cowpea odours to maize odours, they did not discriminate between most of the maize varieties, even though these produced markedly different blends (Hoballah *et al.*, 2002). The absence of clear-cut fixed preferences raises an important question: how do foraging predators solve the problem of qualitative and quantitative variation in herbivore-induced VOCs?

Although some insects possess special sensitivities for only a sub-set of specific host plant volatiles, such as isothiocyanates for cruciferous plants in the cabbage seed weevil (*Ceuthorhyncus assimilis*) (Blight *et al.*, 1995), perception of a wide variety of VOCs appears to be common. Comparative electrophysiological studies in closely related species of *Drosophila* (Stensmyr *et al.*, 2003), *Heliotis* (Rostelien *et al.*, 2005) and *Rhagoletis* (Olsson *et al.*, 2006, 2009) all suggest that although behaviour towards odours among these sister species is different, their olfactory sensitivities hardly vary. Possibly, the stringent expression pattern of insect olfactory receptors allows for less genomic drift of olfactory receptor genes than the more random selection of vertebrate olfactory receptors (Nei *et al.*, 2008). Compared to vertebrates, the low rate of evolutionary change, combined with the variation in VOC emissions by host plants, may explain why insects perceive a wide variety of VOCs.

In theory, predatory arthropods might solve the problem of qualitative and quantitative variation in herbivore-induced VOC mixtures in two ways. Different individuals of the same species could vary in the expression of different olfactory receptors. Within the population, this variation might be maintained as a balanced polymorphism in the presence of spatial or temporal heterogeneity of different profitable VOC mixtures in the environment. Alternatively, all individuals may possess the same 'nose' but 'learn' about the relevance of specific VOC mixtures. Whereas in humans polymorphisms in odour perception are common (Nei *et al.*, 2008), we are not aware of such examples for insects. This could be a consequence of the relatively low rate of evolutionary change of insect olfactory receptors. Predatory arthropods have been reported to readily learn to distinguish herbivore-induced VOCs. For example, the parasitoid *Co. glomerata* learns so fast that it already develops a long-term memory for VOC mixtures after a single rewarding oviposition experience (Smid *et al.*, 2007). Herbivore-induced VOCs as such are not required to elicit attraction of predators as is evident from the observation that the predatory mite *Ph. persimilis* develops a preference for the odour of herbivore-infested plants over uninfested plants, not only when fed in the presence of the infested-plant odours, but also vice versa, when fed in the presence of uninfested plants (Drukker *et al.*, 2000; Van Wijk *et al.*, 2008).

These results indicate that learning by association plays an important role in shaping the behavioural response elicited by VOC mixtures in predatory arthropods. However, it follows that predators, in order to learn, can be misled by volatile signals that resemble those associated with another learning experience. Indeed, Shimoda and Dicke (2000) observed that the blind predatory mite *Ph. persimilis* can have difficulties in discriminating plants with prey, *Te. urticae*, or non-prey, *Sp. exigua*, on beans or cucumber. It was found that for predators such as predatory mites, their genetic background (Maeda *et al.*, 2001) and rearing history, that is, prior experiences (Maeda *et al.*, 2000), strongly determine their ability to make proper decisions under experimental circumstances. Hence, for optimal foraging of predators that use prey-induced plant volatiles for finding plants carrying the prey, the clarity of such signals above the background noise might be more important than long-term signal stability. This implies that temporal and spatial variation in induced plant volatiles might not be a big obstacle for smart carnivores.

VIII. VOC-MEDIATED SPECIFICITY OF INDIRECT DEFENCES

If blends of volatiles elicit specific foraging responses, do single volatiles have the same effect? If so, manipulating plants to produce a novel compound might increase their attractiveness for carnivores and enhance protection. For this purpose, transgenic plants over-expressing specific biosynthetic enzymes have been created. Table II lists transgenic lines that have an altered attractiveness for herbivores or carnivorous bodyguards as a result of manipulated VOC emission. Constitutive expression of several TPSs, that is, *FaNES1*, *MxpSS2* and *ZmTPS10*, altered the attractiveness of *Arabidopsis* plants in the desired fashion, except for the isoprene gene *PcISPS*, which led to decreased attractiveness to the parasitoid wasp *Anagrus nilaparvatae*. Larger amounts of released VOCs through over-expression of HPL, which increases release of C_6-volatiles, or by constitutive activation of JA signalling, increased the attractiveness of *Arabidopsis* and tomato to parasitoids as well (Table II).

The complementary approach, using mutant and knock-out lines, has also been taken. Table II lists mutants and transgenic lines in which expression of key genes of the JA biosynthesis and response pathways or the C_6-volatile pathway has been knocked out by point mutations or knock-down RNA interference. In most cases, these genetic alterations resulted in a reduction in VOCs release, which in some cases dramatically altered the interaction with herbivorous and carnivorous bodyguards. These results show that it is well possible to alter the behaviour of herbivores and carnivores by manipulating the plant's headspace. However, this leaves the question whether such manipulations can suffice to establish enhanced biological control. Given the evidence that many insects rapidly learn to avoid unrewarding stimuli, enhancing attraction will only work if alternative rewards are offered in the absence of prey. Hence, constitutive expression might not be the ultimate solution, and optimising the 'natural' process by engineering herbivore-inducible enhancers of indirect defences may be far more sustainable. However, besides the intriguing possibilities for improving crop protection, plants with altered inducible VOCs are also extremely useful for testing the effect of induced defences under more natural conditions.

Turlings *et al.* (1998) found differences in the volatile emissions induced in maize plants by a folivorous caterpillar (*Sp. littoralis*), a stemborer (*Ostrinia nubilalis*) and the maize aphid (*Rhopalosiphum maidis*), which prompted the question if specialist parasitoids and predators can discriminate between plants with and without prey, irrespective of the plant species. Indeed, the parasitic wasp *Cardiochiles nigriceps* discriminates between prey-induced volatiles in cotton, tobacco or maize, and non-prey-induced volatiles

TABLE II

Transgenic Plants with Modified Volatile Production and Associated Biological Responses

Receiver Plant	Donor plant	Transgene	Promoter	Details	Volatile	Response	Reference
Arabidopsis	Strawberry	linalool/nerolidol synthase *FaNES1*	35S	Targeted to plastids	Linalool and derivatives	Enhanced repellence of aphid *Myzus persicae*	Aharoni *et al.*, 2003
Arabidopsis	Strawberry	linalool/nerolidol synthase *FaNES1*	35S	Targeted to mitochondria	(*E*)-DMNT; (3*S*)-(*E*)-nerolidol	Enhanced attraction of predator *Phytoseiulus persimilis*	Kappers *et al.*, 2005
Arabidopsis	Gray poplar	Isoprene synthase *PcISPS*	35S		Isoprene	Loss of attraction of parasitic wasp *Diadegma semiclausum*	Loivamäki *et al.*, 2008
Arabidopsis	*Mentha x piperita*	Modified *MxpSS2*	35S		(*E*)-β-Farnesene	Enhanced repellence of aphid *Myzus persicae*; enhanced arrestment of parasitoid *Diaeretiella rapae*	Beale *et al.*, 2006; Prosser *et al.*, 2006
Arabidopsis	Maize	*ZmTPS10*	35S		Sesquiterpenes, predominantly (*E*)-β-farnesene and (*E*)-α-bergamotene	Enhanced preference of parasitoid *Cotesia marginiventris*	Schnee *et al.*, 2006

Arabidopsis	Rice	*OsBSMT1*	35S	Most of the 'volatiles' accumulated endogenously	MeSA and MeBA	Enhanced susceptibility to pathogens *Pseudomonas syringae* and *Golovinomyces orontii*	Koo *et al.*, 2007
Arabidopsis	*Arabidopsis*	*AtHPL*	35S	Also compared to knock-out	C_6-aldehydes	Enhanced resistance to pathogenic fungus *Botrytis cinerea*	Kishimoto *et al.*, 2008
Arabidopsis	*Arabidopsis*	*AtHPL*	35S		C_6-aldehydes	Increased attractiveness to parasitoid *Cotesia glomerata*; increased parasitation and mortality of *Pieris rapae*	Shiojiri *et al.*, 2006b
Arabidopsis	*Arabidopsis*	*AtJMT*	35S	Constitutive high MeJA production	Constitutive high endogenous MeJA accumulation	Enhanced resistance to fungus *Botrytis cinerea*	Seo *et al.*, 2001
Rice	Rice	*OsTPS3*	35S		Increase in (*E*)-β-caryophyllene; decrease in α-humulene	Increased attraction of parasitoid wasp *Anagrus nilaparvatae*	Cheng *et al.*, 2007
Tomato	Tomato	*PROSYSTEMIN*	35S	Constitutive high JA biosynthesis and responsiveness	Higher release of α-Pinene, β-myrcene, 3-carene, limonene and β-ocimene	Increased attractiveness to parasitoid wasp *Aphidius ervi*	Corrado *et al.*, 2007

Abbreviations: *BSMT*, S-*ADENOSYL-L-METHIONINE:BENZOIC ACID CARBOXYL METHYLTRANSFERASE*; *HPL*, *HYDROPEROXIDE LYASE*; *ISPS*, *ISOPRENE SYNTHASE 1*; *JMT*, S-*ADENOSYL-L-METHIONINE:JA-CARBOXYL METHYLTRANSFERASE*; *MxpSS2*, *SESQUITERPENE SYNTHASE 2*; *NES1*, *NEROLIDOL SYNTHASE 1*; *TPS*, *TERPENE SYNTHASE*.

(De Moraes *et al.*, 1998). Moreover, herbivore foraging behaviour is also influenced by such volatile information. Herbivores with different feeding habits, that is, the piercing–sucking western flower thrips (*Frankliniella occidentalis*), the chewing herbivore *Heliot. virescens*, or both simultaneously, elicited emission of different blends of VOCs in *N. tabacum*. In choice tests, herbivorous thrips consistently preferred uninduced plants over all other treatments and, hence, possibly use the same herbivore-induced volatiles that natural enemies use to find prey, to avoid competition with other herbivores (Delphia *et al.*, 2007).

Evidence that, despite all variation, herbivore-induced volatiles indeed mediate indirect defences under natural circumstances has been accumulating slowly. Drukker *et al.* (1995) observed that predatory anthocorid bugs aggregated near cages containing pear trees infested with pear psyllids (*Cacopsylla* spp.), and in laboratory olfactory choice assays Scutareanu *et al.* (1997) showed that the same bugs were attracted to the induced volatiles of *Cacopsylla*-infested pear leaves. Field application of synthetic herbivore-induced volatiles as a mimic of plant 'alarm' calls is currently being explored for its effectiveness to recruit and retain beneficial insects in the field (James, 2003; James and Grasswitz, 2005; James and Price, 2004). However, without testing this hypothesis under field conditions, it should not be assumed that predators that are attracted by herbivore-induced volatiles necessarily benefit the plant (Karban, 2007b). In a landmark study, Kessler and Baldwin (2001) showed that mimicking the natural emissions induced by leaf-feeding herbivores from *N. attenuata* in the field, that is, by application of *cis*-3-hexen-1-ol, linalool and *cis*-α-bergamotene, egg predation rate by the generalist predator *Geocoris pallens* was increased, while application of linalool also decreased lepidopteran oviposition rates. The authors estimated that herbivore-induced volatiles reduced the number of herbivores by more than 90%. Field-planted *N. attenuata* silenced for *LOX3* or *HPL* genes and deficient in α-bergamotene and GLV emission, respectively, not only were more vulnerable to *N. attenuata*'s adapted herbivores, but also attracted novel herbivore species, which fed and reproduced successfully (Kessler *et al.*, 2004). In a follow-up study, Halitschke *et al.* (2008) showed that predatory bugs use terpenoids and C_6-volatiles to locate their prey on herbivore-attacked plants, and thereby reduce herbivory, and also that plants producing C_6-volatiles are damaged more by flea beetles. Yet, although not obtained from field experiments, there are some indications that indirect defences can really increase a plant's fitness. Van Loon *et al.* (2000) showed that both *Arabidopsis* accessions L*er* and Col-0 infested with unparasitised larvae of *Pi. rapae* produced less seeds than when infested with parasitised larvae. Similar results were obtained using maize plants under attack by *Sp. littoralis*: these plants showed increased attractiveness to endoparasitoids, that is, *Co. marginiventris* and *Campoletis sonorensis*. Not only did parasitism significantly reduce feeding and weight gain of the host larvae, but also plants attacked by a single parasitised larva produced about 30% more seed than plants that were attacked by an unparasitised larva (Hoballah and Turlings, 2001). Finally, release of (*E*)-β-caryophyllene induced by *Diabrotica virgifera virgifera* beetles from the roots of *Z. mays* into the soil appeared to attract the beetle's entomopathogenic nematode *Heterorhabditis megidis* and decreased emergence of adult beetles to less than half (Rasmann *et al.*, 2005). Taken together, herbivore-induced volatiles are highly suitable cues for predatory insects to locate prey. Under many circumstances, this may increase host-plant fitness through reduced herbivory.

IX. VOCs AS ALARM SIGNALS FOR NEIGHBOURING PLANTS

Plant volatiles diffuse freely through the surrounding air and hence reach not only foraging insects, but also neighbouring plants. Rhoades (1983) found that field-grown willows next to herbivore-attacked conspecifics were less palatable to larvae than were unattacked trees growing next to unattacked willows. This prompted the question whether exudates of the attacked willows and possibly volatiles from leaves or roots, had 'induced' their unattacked neighbours. Baldwin and Schultz (1983) discovered that exposing undamaged, individually potted sugar maples and poplars to the VOCs of mechanically damaged relatives increased the trees' levels of tannins and phenolics. Since in this study the plants did not have root contacts, the observed effects could only have been mediated by above-ground airborne VOCs. These two studies initiated a whole new field of research on plant–plant interactions via induced volatiles and there is now a long list of researchers who have tried to discern which VOCs elicit such effects. In numerous follow-up studies in the laboratory and in the field, additional evidence for the occurrence of VOC-mediated defence activation among plants has been found (Baldwin *et al.*, 2006; Dicke *et al.*, 2003).

A. TRANSCRIPTIONAL RESPONSES TO VOC EXPOSURE

In the first two decades after the publications of Baldwin and Schultz (1983) and of Rhoades (1983) the field of VOCs-mediated plant–plant interactions was led primarily by the working hypothesis that herbivore-induced volatiles induce defences in neighbouring plants, and focussed more on the consequences, for example, for herbivore performance on receiver plants than on the mechanism of the response. Unbiased methods to study such mechanisms in receiver plants became available in the genomics era, especially after the invention of gene chip micro-arrays. The ease of collecting gene expression data has boosted our knowledge of the transcriptional changes that determine the downstream changes in protein and enzyme activity, herbivore performance and VOC-induced volatile production. For example, from field experiments it was already known that airborne molecules released from clipped sagebrush induced polyphenol oxidase activity in wild tobacco (Karban and Baxter, 2001; Karban *et al.*, 2000, 2003), but Kessler *et al.* (2006) showed that, both in the glasshouse and in nature, clipping the sagebrush led to considerable transcriptional changes in the wild tobacco. When micro-arrays are available, one can obtain a fairly good overview of the transcriptome upon plant exposure to a volatile without having to deal with responses at the phenotypic level (Kant and Baldwin, 2007). The goals of these analyses are sometimes straightforward, that is, to identify a marker for the response to a particular volatile, but often complex, such as prediction of the phenotype based on the response at the transcriptional level.

The number of studies analysing transcriptional responses of plants to volatiles has increased rapidly since the discovery that MeJA can induce PI expression in tomato and tobacco (Farmer and Ryan, 1990; Farmer *et al.*, 1992). MeJA is likely converted to JA *in planta* (Tamogami *et al.*, 2008). Interest in volatile signalling was boosted when it was discovered that tobacco plants reacting hypersensitively to tobacco mosaic virus (TMV) could induce *PR-1* expression in neighbouring uninfected tobacco plants through volatile MeSA, as described in section IV (Shulaev *et al.*, 1997). However, *in planta* MeSA is almost completely converted to SA, which acts as the endogenous signalling compound in inducing *PR-1* transcripts (Chen *et al.*, 2003; Park *et al.*, 2007; Seskar *et al.*, 1998; Shulaev *et al.*, 1997). Attention has therefore deviated from MeSA to other volatiles. The only other benzenoid tested for its ability to bring about a transcriptional change in plants is methylbenzoate (MeBA), of which 10 μM was applied to germinating *Arabidopsis* seeds for 8 h (Horiuchi *et al.*, 2007). Although considered phytotoxic at this concentration because of its inhibition of seed germination, MeBA changed the expression of more than 1% of the genes significantly. Several of these genes are related to the phytohormones auxin, cytokinin, ABA and ET. Interestingly, the germination of the auxin-resistant mutant *axr1* and cytokinin response mutant *cre1* was less affected by MeBA than that of the wild type, suggesting that some of the responses to MeBA are mediated by these hormones (Horiuchi *et al.*, 2007). Since MeBA is predominantly a floral volatile, it can be speculated that floral volatiles can have allelopathic activity.

In addition, a lot of attention has been paid to the most abundant class of plant volatiles, the terpenoids. In an influential paper Arimura *et al.* (2000) showed that β-ocimene, DMNT and TMTT, which are emitted in higher amounts by Lima bean leaves upon herbivory by spider mites, are capable of inducing the transcription of *PR*, *LOX*, *PAL* and *FPS* genes. Linalool did not induce transcription of these genes, indicative of specificity of the response. Moreover, the authors also showed that these genes were induced either transiently or at different time points. In an additional experiment with custom-made cDNA micro-arrays, the transcriptional response of uninfested, excised Lima bean leaves on the complete (predominantly terpenoid) bouquet of volatiles released by spider mite-infested excised leaves was investigated (Arimura *et al.*, 2000). The finding that ET biosynthetic genes were up-regulated in the detached receiver leaves led to the discovery that these genes were also up-regulated in intact receiver plants (Arimura *et al.*, 2002). Moreover, in leaves of intact receiver plants ET production, as well as JA production, increased upon exposure to volatiles from intact Lima bean plants infested with *Te. urticae*. Clearly, micro-array experiments paid off here in the discovery of the regulation of ET biosynthesis by a mixture of volatiles. Moreover, each individual volatile (terpenoid or C_6) of this mix could also induce expression of the ET biosynthetic genes. The logical follow-up studies with ET mutants were done, for some unknown reason, with *allo*-ocimene (Kishimoto *et al.*, 2005). Induction of a selected set of genes by *allo*-ocimene was similar in mutant ET-insensitive *etr1-1* and in wild-type *Arabidopsis*. This volatile also enhanced resistance against the fungus *Bo. cinerea* equally in the *etr1-1* mutant and in wild-type plants (Kishimoto *et al.*, 2006b). Thus, a role for ET in these processes has yet to be determined.

Most attention has been paid to the transcriptional response upon perception of C_6-volatiles. Bate and Rothstein (1998) investigated the response of *Arabidopsis* plants, using different C_6 volatiles and selected marker genes associated with defence. They demonstrated that various C_6-volatiles had differential effects on the expression of *LOX2*, albeit that a high concentration (10 μM) was used. These distinct effects of different C_6-volatiles on gene expression were confirmed by Arimura *et al.* (2002). With only four marker genes—*PR-2*, *SAMS*, *SAMDC* and *ACO*—they clearly demonstrated that *Z*-3-hexenol, *E*-2-hexenal and *Z*-3-hexenyl acetate had differential effects on their expression after 3 and 24 h. Kishimoto *et al.* (2006a) concluded that transcriptional responses of JA- and SA-related marker genes such as *AOS*, *LOX2*, *HPL*, *VEGETATIVE STORAGE PROTEIN 1* (*VSP1*), *PLANT DEFENSIN 1.2* (*PDF1.2*) and various *PRs* to the C_6 aldehydes *E*-2-hexenal or *Z*-3-hexenal were significantly reduced in the JA-response mutant *jar1–1*, the ET-response mutant *etr1–1* and the phytoalexin-defective *pad2–1*, but not in the SA-insensitive *npr1–1* mutant (Table III). Their study highlights an essential problem with this and other studies, that is, the lack of specific gene markers for C_6-volatiles. Conversely, these studies also raise the question how specific the traditional JA, ET and SA markers are. Studies in other plant species have confirmed the capability of C_6-volatiles to induce gene expression. *Z*-3-hexenol induces *MAIZE PROTEINASE INHIBITOR* (*MPI*), *PAL* and *LOX* in maize (Farag *et al.*, 2005) but *Z*-3-hexenol is rapidly converted to *Z*-3-hexenyl acetate that by itself does not induce *PAL* expression. Another study with maize showed that *Z*-3-hexenyl acetate induced the expression of several OPDA reductases (Frost *et al.*, 2008b). A recent study using *N. attenuata* plants genetically engineered to be 'mute' in different aspects of their volatile vocabulary revealed that the transcriptional responses of neighbouring 'eavesdropping' plants were not elicited by the presence of specific C_6-volatiles in the volatile bouquet but, rather, by their absence (Paschold *et al.*, 2006). This study underscores the need to keep an open mind about the nature of the information encrypted in the VOC bouquet.

Although the notion that pure VOCs can elicit specific responses in plants is now generally accepted, it remains unclear how such VOCs are perceived by plants. The volatiles, ET, MeSA and MeJA, may simply adhere to the leaf and diffuse from the surface into the epidermal cells, where upon additional modification or not, they may play direct signalling roles. However, for most induced plant volatiles conversion into known phytoactive substances seems unlikely. VOC receptors have not yet been identified, with the notable exception of the ET receptors. In an attempt to identify components of C_6-volatile perception and signalling pathways, a mutagenesis screen was performed on *Arabidopsis* using a root elongation assay, as *E*-2-hexenal inhibits root elongation in wild-type plants. Analysis of the first mutant found, *her-1*, revealed a link between *E*-2-hexenal-induced responses and γ-aminobutyric acid (GABA) metabolism (Mirabella *et al.*, 2008).

B. PRIMING OF PLANT DEFENCES BY VOLATILES

The phenomenon of VOC-mediated defence activation among plants raises intriguing questions about how plants discriminate between signals and noise. The activation of defence responses requires energy and activation of defences is therefore, by definition, undesirable in the absence of herbivory. Solutions to this problem have been proposed; for example, it would make sense if plants did not activate their complete defence arsenal after the first signs of trouble but were to 'prime' their defence metabolisms so as to be in a

TABLE III
Transcriptional Responses Elicited by Volatiles

Species	Mutants/ transgenics	Volatile	Receiver	Read-out	Reference
Arabidopsis	-	MeBA	Germinating seeds	26k Micro-array	Horiuchi *et al.*, 2007
Arabidopsis	*jar1-1*	C_6-volatiles	Plants	*CHS*, *PAL*, *PRs*, *LOX*	Bate and Rothstein, 1998
Arabidopsis	*etr1-1*	Terpenoids, C_6-volatiles	Plants	*AOS*, *HPL*, *PRs*, *VSP1*,*CHS*, *COMT*, *DGK1*, *GST1*, *LOX2*, *PR-2*	Kishimoto *et al.*, 2005, 2006a,b
Arabidopsis	*etr1-1*, *jar1-1*, *npr1-1*, *pad2-1*	C_6-volatiles	Plants	*AOS*, *LOX2*, *HPL*, *VSP1*, *PDF1.2*, *PRs*	Kishimoto *et al.*, 2006a,b
Lima bean	–	Terpenoids	Excised leaves	*PRs*, *LOX*, *PAL*, *FPS*	Arimura *et al.*, 2000
Lima bean		Plant volatiles	Excised leaves	2k cDNA-micro-array	Arimura *et al.*, 2000
Lima bean	–	Terpenoids, plant volatiles	Plants	Ethylene biosynthetic genes	Arimura *et al.*, 2002
Lima bean	–	C_6-volatiles	Plants	*SAMS*, *ACO*, *SAMDC*, *PR-2*	Arimura *et al.*, 2002
Maize	–	C_6-volatiles	Plants	*HPL*, *FPS*, *PAL*, *LOX*, *IGL*, *MPI*	Farag *et al.*, 2005
Maize	–	Plant volatiles	Plants	Differential cDNAs	Ton *et al.*, 2007

(*continues*)

TABLE III (*continued*)

Species	Mutants/ transgenics	Volatile	Receiver	Read-out	Reference
Nicotiana attenuata	*hpl*, *lox3*	Plant volatiles	Plants	1k oligo-micro-array	Paschold *et al.*, 2006
Nicotiana attenuata		Sagebrush volatiles	Plants	PI accumulation upon *Manduca sexta* feeding; feeding damage and mortality rate	Kessler *et al.*, 2006
Poplar	–	C_6-volatiles	Plants	5.4 k cDNA-micro-array	Frost *et al.*, 2008a,b
Tobacco	–	MeSA	Plants	*PR-1*	Shulaev *et al.*, 1997
Tobacco, Tomato	PI-reporter line	MeJA	Plants	*PI*	Farmer *et al.*, 1992

Abbreviations: *ACO*: *1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE*; *AOS*: *ALLENE OXIDE SYNTHASE*; *CHS*: *CHALCONE SYNTHASE*; *COMT*: *CAFFEIC ACID O-METHYLTRANSFERASE*; *DGK1*: *DIACYLGLYCEROL KINASE 1*; *etr1*: *ethylene resistant 1*; *FPS*: *FARNESYL PYROPHOSPHATE SYNTHETASE*; *GST1*: *GLUTATHIONE* S-*TRANSFERASE 1*; *HPL*: *HYDROPEROXIDE LYASE*; *IGL*: *INDOLE-3-GLYCEROL PHOSPHATE LYASE*; *jar1*: *jasmonate resistant 1*; *LOX*: *LIPOXYGENASE*; *MPI*: *MAIZE PROTEINASE INHIBITOR*; *npr1*: *non-expressor of* PR *genes 1*; *pad2*: *phytoalexin deficient 2*; *PAL*: *PHENYLALANINE AMMONIA-LYASE*; *PDF1.2*: *PLANT DEFENSIN 1.2*; *PI*: *PROTEINASE INHIBITOR*; *PR*: *PATHOGENESIS-RELATED*; *SAMDC*: S-*ADENOSYL METHIONINE DECARBOXYLASE*; *SAMS*: S-*ADENOSYL METHIONINE SYNTHASE*; *VSP1*: *VEGETATIVE STORAGE PROTEIN 1*.

temporal state of 'enhanced alertness' (Conrath *et al.*, 2006). Then, the induction of defences, observed under many experimental conditions, might in some cases have been an artefact caused by using unrealistically high concentrations of pure volatiles (Preston *et al.*, 2001, 2004). Priming is thought to entail low-cost metabolic changes (Van Hulten *et al.*, 2006). These may be small and therefore difficult to detect experimentally, but could enable a plant to launch defence responses more rapidly and more strongly when attacked. By allowing themselves to be primed after eavesdropping on a neighbour, plants might very well prevent resources from being wasted on unnecessary induction of responses, and thereby realise an overall fitness benefit (Kessler *et al.*, 2006; Ton *et al.*, 2007).

A specific function for C_6-volatiles in priming defences was demonstrated by Engelberth *et al.* (2004), who showed that upon herbivore-regurgitant treatment both JA production and volatile release were higher in maize plants that had been previously exposed to C_6-volatiles. Within 30 min, C_6-volatile perception led to a transient increase in JA levels, which were fully reset after 3 h. Since this effect occurred with all three C_6-volatiles tested, that is, *Z*-3-hexenal, *Z*-3-hexen-1-ol and *Z*-3-hexenyl acetate, the specificity of this priming response needs further testing. A follow-up study (Ton *et al.*, 2007) confirmed that volatiles released from *Sp. littoralis*-infested maize plants could indeed prime receiver maize plants. This study showed that this occurred not only at the level of gene activation, but also at the level of herbivore performance: caterpillars remained smaller on primed maize plants. These primed maize plants also emitted more volatiles, leading to greater attraction of the parasitic wasp *Co. marginiventris*. Although this result is not directly related to priming, this is clearly an example of increased indirect defences induced by volatiles. Since the complete headspace of caterpillar-infested maize plants were used, specific roles of individual volatiles still need to be assessed in order to investigate if there are specific components that establish priming. However, this will not always be easy to address since some volatiles can be converted by the plant to other volatiles, as is the case for *Z*-3-hexenol into *Z*-3-hexenyl acetate (D'Auria *et al.*, 2007; Farag *et al.*, 2005; Mirabella *et al.*, 2008; Paré *et al.*, 2005). Still, Frost *et al.* (2008a) clearly showed that *Z*-3-hexenyl acetate, in turn, not only primed expression of genes mediating oxylipin signalling and direct defences induced upon feeding by the gypsy moth (*Lymantria dispar*), but also JA levels and volatile emission in hybrid poplar.

In *Arabidopsis*, the terpenoid ocimene could prime for enhanced lignification and production of the phytoalexin camalexin upon infection with *Bo. cinerea* (Kishimoto *et al.*, 2006b). Additional studies in priming by volatiles were done in Lima bean plants. Here the priming effect on induced indirect defences, that is, the production of extra-floral nectar as an alternative food source for predatory arthropods, was determined under laboratory (Choh and Takabayashi, 2006a,b) as well as field conditions (Heil and Kost, 2006). Although plants were exposed to an artificial blend of volatiles they clearly showed that extra-floral nectar production increased much more rapidly in wounded than in non-wounded plants. Another field study that focused on the role of volatiles in within-plant signalling in Lima bean plants led to similar results (Heil and Silva Bueno, 2007). Apparently, within-plant priming by volatiles can overcome vascular constraints, as also indicated by Frost *et al.* (2007) for hybrid poplar. Although in the latter study tubing was used to transport volatiles from one leaf to another, it clearly demonstrated that leaves can respond to naturally occurring, very low concentrations of volatiles by becoming primed for their inducible volatile production. Interbranch signalling could be a feature of perennial shrubs with newly developed lateral branches that have little or no vascular connections, such as in blueberries (Rodriguez-Saona *et al.*, 2003) and sagebrush (Karban *et al.*, 2006). Sagebrush can be primed by volatiles released from neighbouring *Art. tridentata* both in a laboratory setting and in field-based experiments (Kessler *et al.*, 2006), resulting in an accelerated production of trypsin-inhibiting PIs and lower total herbivore damage. It is likely that the low doses of volatiles required for priming will entail at most a small fitness cost, but this still needs to be investigated.

In conclusion, herbivore-induced plant volatiles play a significant role in complex ecological interactions, and function as signals that alert prey-searching carnivores to the presence of food. Such foraging carnivores might, in turn, contribute positively to the plant's defensive state and thereby to the plant's fitness. However, these volatiles also serve other functions that benefit the plant, that is, as scavengers of free radicals, to dispose of metabolic wastes, as toxins to pathogens or repellents of herbivores. For the purpose of enhancing crop protection through improvement of volatile-mediated biological control, genetic engineering of plants to manipulate volatile production is a promising approach. However, for designing efficient pest control strategies it will be essential to understand what motivates a predator to use a particular odour during foraging, that is, whether it is an innate preference or an association with its prey after a positive experience. Finally, plant defences in undamaged plants can be activated by exposure to herbivore-induced volatiles or synthetic mimics, but how plants regulate such responses and to what extent they depend on the ability to recognise volatile signals from neighbours when coping simultaneously with competitors, herbivores, pathogens and abiotic stresses, are questions still open for the future.

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