Biochemistry of Plant Volatiles[1]

Natalia Dudareva*, Eran Pichersky, and Jonathan Gershenzon

Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907 (N.D.); Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109 (E.P.); and Max Planck Institute for Chemical Ecology, Beutenberg Campus, D–007745 Jena, Germany (J.G.)

Plants have a penchant for perfuming the atmosphere around them. Since antiquity it has been known that both floral and vegetative parts of many species emit substances with distinctive smells. The discovery of the gaseous hormone ethylene 70 years ago brought the realization that at least some of the compounds emitted may have physiological significance without any distinctive smell to humans. At present, more than 1,000 low M_r organic compounds have been reported to be emitted from plants, although a comprehensive list is available only for floral volatiles (Knudsen et al., 1993).

Our knowledge of the occurrence and distribution of plant volatiles has been significantly extended in the last 15 years thanks to the adoption of simple, sensitive methods for headspace sampling and the availability of relatively inexpensive bench-top instruments for gas chromatography-mass spectrometry. The substances reported are largely lipophilic products with molecular masses under 300. Most can be assigned to the following classes (in order of decreasing size): terpenoids, fatty acid derivatives including lipoxygenase pathway products, benzenoids and phenylpropanoids, C_5-branched compounds, and various nitrogen and sulfur containing compounds. Nearly all of these classes are emitted from vegetative parts as well as flowers (Knudsen et al., 1993), and some are even emitted from roots (Steeghs et al., 2004). A major discovery of the last decade is that plants commonly emit much greater amounts and varieties of volatiles after herbivore damage, and not just from the site of injury (Pare and Tumlinson, 1999).

Major progress in plant volatile research, as in other areas of plant biology, has come from the use of molecular and biochemical techniques. A large number of genes encoding enzymes of volatile biosynthesis have recently been reported. In vitro characterization of the heterologously expressed enzymes, especially determination of their substrate and product specificity, has helped clarify the pathways of volatile formation. In addition, investigation of the spatial and temporal patterns of gene expression has provided new information on the factors regulating the emission of plant volatile compounds. In this update, we survey the latest advances on the biosynthesis and regulation of plant volatiles, beginning with a brief review of the function of these substances.

FUNCTION OF PLANT VOLATILES

Perhaps the greatest mysteries surrounding volatiles concern their function in the life of the plant. While it is generally assumed that compounds emitted from flowers serve to attract and guide pollinators (Reinhard et al., 2004), only scattered attempts have been made to demonstrate the ability of individual substances to attract specific pollinators. Many floral volatiles have anti-microbial or anti-herbivore activity (DeMoraes et al., 2001; Friedman et al., 2002; Hammer et al., 2003), and so could also act to protect valuable reproductive parts of plants from enemies.

Among vegetative volatiles, the most intensively studied substance is isoprene, a simple five-carbon terpene emitted from the foliage of many woody species (Sharkey and Yeh, 2001b). The function of isoprene is still controversial, and this compound may act to increase the tolerance of photosynthesis to high temperatures by stabilizing the thylakoid membranes (Sharkey et al., 2001a) or by quenching reactive oxygen species (Loreto and Velikova, 2001). The release of volatiles from vegetative organs following herbivore damage seems to be a general property of plant species. Contributions to this special issue cover herbivore-induced volatiles from cabbage (*Brassica oleracea*; Vuorinen et al., 2004b), cucumber (*Cucumis sativus*; Mercke et al., 2004), *Lotus japonicus* (Arimura et al., 2004b), and maize (*Zea mays*; Degen et al., 2004). These substances have been demonstrated to serve as indirect plant defenses. That is, they attract arthropods that

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* Corresponding author; e-mail dudareva@hort.purdue.edu; fax 1–765–494–0391.

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prey upon or parasitize herbivores, thus minimizing further damage to plant tissue (Pare and Tumlinson, 1999; Dicke and Van Loon, 2000). In some cases, herbivore-induced volatiles may also act as direct defenses, repelling (DeMoraes et al., 2001; Kessler and Baldwin, 2001) or intoxicating (Vancanneyt et al., 2001) herbivores and pathogens (Andersen et al., 1994). The possibility that these substances also act in plant-plant communication has been discussed (Arimura et al., 2000; Dicke and Bruin, 2001; Engelberth et al., 2004).

Herbivore-induced volatiles could additionally have physiological roles within the plant, with their release being a consequence of their volatility and membrane solubility. Like isoprene, some herbivore-induced monoterpenes and sesquiterpenes have the potential to combine with various reactive oxygen species (Hoffmann et al., 1997; Bonn and Moortgat, 2003), and so could protect against internal oxidative damage (Delfine et al., 2000; Loreto et al., 2004b). In fact, ozone fumigation has recently been reported to promote the emission of herbivore-induced volatiles (Vuorinen et al., 2004a). Yet, it is still unclear why oxidative stress is likely to be significantly higher after herbivore damage. Further studies are needed to help elucidate the roles of these and other plant volatiles. The growing number of reports on genes involved in volatile formation, as described in the following sections, should enable investigators to manipulate volatile emission and test its function in plants.

BIOSYNTHESIS OF VOLATILE TERPENES

Terpenes, as the largest class of plant secondary metabolites, have many volatile representatives. The majority of hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), and even some diterpenes (C_{20}) have high enough vapor pressures at normal atmospheric conditions to allow significant release into the air. The basic pathway of volatile terpenoid biosynthesis is conveniently treated in three phases: (1) formation of the basic C_5 units, (2) condensation of two or three C_5 units to form C_{10}, C_{15}, or C_{20} prenyl diphosphates, and (3) conversion of the resulting prenyl diphosphates to end products.

The formation of basic C_5 units, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) proceeds via two alternative pathways: the long known mevalonate pathway from acetyl-CoA and the methylerythritol phosphate pathway from pyruvate and glyceraldehyde-3-phosphate, discovered only in the last 10 years (for review, see Rodriguez-Concepcion and Boronat, 2002). The methylerythritol phosphate pathway, localized in the plastids, is thought to provide IPP and DMAPP for hemiterpene, monoterpene, and diterpene biosynthesis, while the cytosol-localized mevalonate pathway provides C_5 units for sesquiterpene biosynthesis. However, metabolic ''cross-talk'' between the two pathways is prevalent (Schuhr et al., 2003), particularly in the direction from plastids to cytosol (Laule et al., 2003). This issue contains two contributions concerning the regulation of the basic pathways in relation to isoprene formation. Although produced largely by the plastidial pathway, isoprene also seems to arise from extra-plastidial sources, but there is apparently no cross-talk between the two pathways in its formation (Loreto et al., 2004a). The plastidial pathway is controlled by tight feedback regulation on its first step, deoxyxylulose-5-phosphate synthase (Wolfertz et al., 2004).

In the second phase of terpene biosynthesis, IPP and DMAPP condense to form geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate, the precursors of monoterpenes, sesquiterpenes, and diterpenes, respectively. These reactions are catalyzed by short-chain prenyltransferases (Koyama and Ogura, 1999; Liang et al., 2002). FPP is synthesized by a large family of homodimeric prenyltransferases called FPP synthases. However, the situation regarding GPP formation is more complex. While the GPP synthases of Arabidopsis (Bouvier et al., 2000) and grand fir (*Abies grandis*; Burke and Croteau, 2002) are homodimers, like other short-chain prenyltransferases, those reported from peppermint (*Mentha* × *piperita*) leaves (Burke et al., 1999) and the flowers of snapdragon (*Antirrhinum majus*) and *Clarkia breweri* (Tholl et al., 2004) are unusual heterodimeric enzymes, with each subunit being a member of the prenyltransferase protein family.

The third phase of terpene volatile biosynthesis involves the conversion of the various prenyl diphosphates, DMAPP (C_5), GPP (C_{10}), FPP (C_{15}), and geranylgeranyl diphosphate (C_{20}), to hemiterpenes (isoprene and 2-methyl-3-buten-2-ol), monoterpenes, sesquiterpenes, and diterpenes, respectively. These reactions, carried out by a large family of enzymes known as terpene synthases (Cane, 1999; Wise and Croteau, 1999), produce the primary representatives of each skeletal type. The investigation of terpene synthases is a very active area of plant volatile research and this issue contains four contributions describing the isolation of genes of this type from Norway spruce (*Picea abies*; Martin et al., 2004), Arabidopsis (Chen et al., 2004), cucumber (Mercke et al., 2004), and *L. japonicus* (Arimura et al., 2004b). These gene sequences give new insights into the evolutionary origin and genetic regulation of terpene synthases. One of the most outstanding properties of these enzymes is their proclivity for making multiple products from a single substrate. Hence, there has been much curiosity about the carbocationic reaction mechanism. The elucidation of the first crystal structures of plant terpene synthases (Starks et al., 1997; Whittington et al., 2002) now puts this work on a much stronger experimental footing. Many terpene volatiles are direct products of terpene synthases, but others are formed through transformation of the initial products by oxidation, dehydrogenation, acylation, and other reaction types. These are discussed in the following section.

MODIFICATION REACTIONS THAT ENHANCE THE VOLATILITY OF COMPOUNDS

The terpene pathways are essentially biosynthetic, building up a carbon skeleton, and the immediate products formed by the large family of terpene synthases discussed above are mostly hydrocarbons, although sometimes they contain a hydroxyl group (e.g. linalool synthase produces linalool, a tertiary alcohol). Such compounds are already fairly volatile. In contrast, most other volatile compounds are produced through the shortening of a carbon skeleton, often followed by further modification, or simply by modification of the existing carbon skeleton. Compounds that are already somewhat volatile may also be modified, resulting in enhanced volatility or changed olfactory properties. The majority of these modifications involve the reduction or removal of carboxyl groups, the addition of hydroxyl groups, and the formation of esters and ethers. Each type of modification is catalyzed by a group (or several groups) of related enzymes constituting protein families. Some of these protein families had been previously recognized from biochemical research into nonvolatile compounds but some were only recently identified as part of the research into the biosynthesis of plant volatiles. Modifications for which enzymatic reactions and enzymes have been identified in plants are described below.

Oxidation by Cytochrome P450 Enzymes

The P450 cytochrome oxidases have been well characterized from a multitude of plant and animal species, and are involved in numerous metabolic pathways (Schuler, 1996). Not surprisingly, these enzymes have been found to be involved in many of the reactions of volatile biosynthesis. The basic skeleton of the monoterpenes and sesquiterpenes, discussed above, is often modified by hydroxylation. For example, 3-hydroxylation of limonene by a P450 enzyme is the first step in the biosynthesis of menthol (Fig. 1A; Lupien et al., 1999), a volatile flavor compound found in mint, whereas a 6-hydroxylation of limonene by another P450 enzyme is the first step in the biosynthesis of another volatile spice, carvone, in the caraway (*Carum carvi*) fruit (Bouwmeester et al., 1999). A P450 enzyme is also responsible for the conversion of the sesquiterpene 5-epi-aristolochene to capsidiol, a dihydroxylated volatile compound (Ralston et al., 2001). Some homoterepene compounds, for example the C11 compound 4,8-dimethyl-1,3,7-nonatriene which is often emitted from injured tissues, are believed to be derived from terpenes by cleavage catalyzed by P450 enzymes (Fig. 1A), but such enzymes have not yet been identified conclusively (Boland and Gabler, 1989; Degenhardt and Gershenzon, 2000).

Cytochrome P450 enzymes are also very important in the biosynethsis of volatile phenylpropenes such as eugenol and the benzenoid vanillin. Both of these compounds, found in a wide variety of species both in flowers and in leaves, are derived from Phe and share with nonvolatile phenylpropanoids the earlier steps of 4-hydroxylation of cinnamate by 4CH, a P450 enzyme (Frank et al., 1996), and 3-hydroxylation by the newly discovered P450 enzyme that utilizes the shikimic or quinic ester of coumarate rather than coumaric acid or coumaroyl-CoA (Schoch et al., 2001; Gang et al., 2002a).

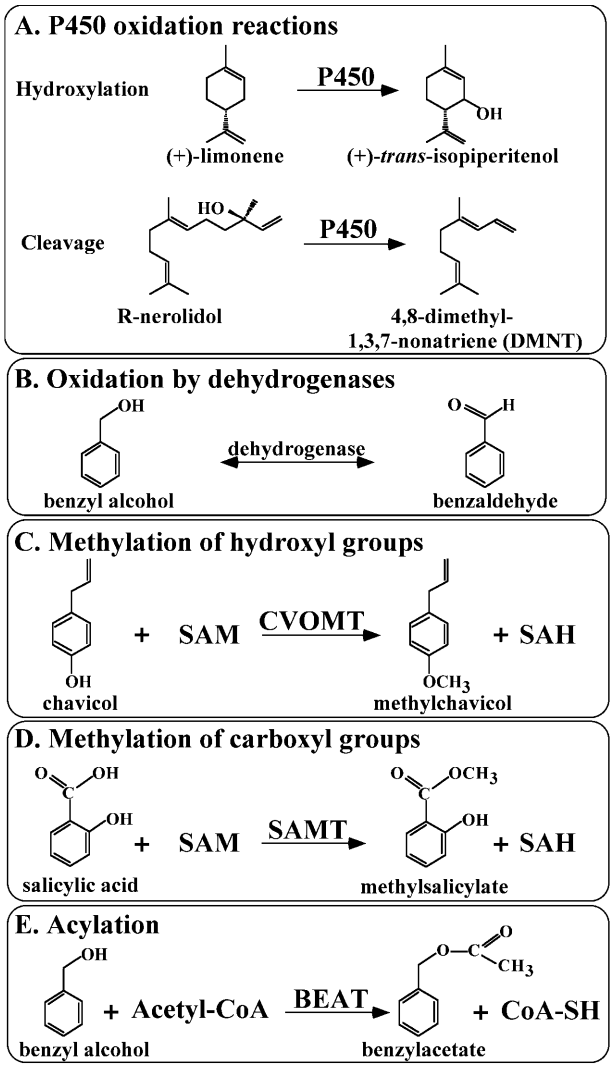

Figure 1. Representative modification reactions leading to the biosynthesis of compounds with enhanced or changed volatility and olfactory properties. SAM, *S*-adenosyl-L-Met; CVOMT, chavicol *O*-methytransferase; SAMT, *S*-adenosyl-L-Met:salicylic acid carboxyl methytransferase; and BEAT, acetyl-coenzyme A:benzyl alcohol acetyltransferase.

Cytochrome P450 enzymes are crucial in the biosynthesis of volatiles derived from fatty acids, and in particular, in the octodecanoic pathway. Two different P450 enzymes, 9-LOX and 13-LOX, can introduce a peroxide into linoleic acid (18:3) at the respective positions (Howe and Schilmiller, 2002). Subsequent cleavage of the hydrocarbon chain by hydroperoxide lyases produces nonadienal and 3-cis-hexenal, respectively. The later is an important component of green

leaf volatiles, the mixtures of compounds that are emitted when the leaf is damaged (Pichersky and Gershenzon, 2002). In addition to the C6 aldehyde, cleavage of linoleic acid at the 12 to 13 bond also produces a C12 compound that can subsequently be converted to jasmonic acid (Howe and Schilmiller, 2002). While jasmonic acid is not by itself volatile, its methylester is (see below).

Oxidation by Dehydrogenases

NADP/NAD-dependent oxidoreductases are another large and well-studied family of proteins with representatives found to be involved in the biosynthesis of volatiles. Such enzymes have been implicated in the interconversion of volatile alcohols and aldehydes (Fig. 1B). For example, apparently nonspecific alcohol dehydrogenases can convert short-chain aldehydes such as hexanal and 3-cis-hexenal to hexenol and 3-cis-hexenol, alcohols that are also found in damaged leaves (Bate et al., 1998). This lack of tight substrate specificity was used to alter the aroma profile of ripe tomato (*Lycopersicon esculentum*) fruit by genetic engineering (Prestage et al., 1999). Some terpene alcohols such as geraniol and carveol are converted to aldehydes by similarly nonspecific dehydrogenases (Hallahan et al., 1995; Bouwmeester et al., 1998). Geranial and neral (which are coproduced by the oxidation of geraniol; the mixture is termed citral), have lemony aroma and are found in many plants, and carvone gives caraway its distinct flavor. And benzyl alcohol, a major floral scent component in many Nicotianeae species (Raguso et al., 2003) and elsewhere, is also likely derived from benzaldehyde in a reversible reaction catalyzed by a member of the NADP/NAD-dependent oxidoreductases family (Fig. 1B; Boatright et al., 2004).

Methylation of Hydroxyl Groups

A large portion of plant volatiles contain a methylated hydroxyl group (i.e. a methoxyl group). The methyl group is usually added in a reaction catalyzed by a methyltransferase (MT) in which *S*-adenosyl-L-methionnine (SAM) serves as the methyl donor. All plant methyltransferases appear to share a similar SAM-binding domain; however, they fall into distinct families that share little primary sequence similarity elsewhere (Noel et al., 2003). A large family of methyltransferases with members involved in the synthesis of both volatile and nonvolatile "small molecules" (as opposed to proteins or nucleic acids) has been identified and designated as the Type I methyltransferase family (Noel et al., 2003). Members of this MT family have been shown to catalyze the 4-hydroxyl methylation of eugenol to form methyleugenol in flowers of *C. breweri* and in the glands of basil (*Ocimum basilicum*), and also the methylation of chavicol to methylchavicol in the basil glands (Fig. 1C; Wang et al., 1997; Lewinsohn et al., 2000; Gang et al., 2002b).

3,5-Dimethoxytoluene, a major scent compound in many hybrid roses, is produced from orcinol (3,5-dihydroxytoluene) in two successive methylation reactions catalyzed by two very similar MTs, orcinol OMTs (OOMT1 and OOMT2; Lavid et al., 2002; Scalliet et al., 2002). Both enzymes can carry out both reactions; however, OOMT1 is more catalytically efficient with orcinol while OOMT2 is more catalytically efficient with 3-methoxy,5-hydroxytoluene (Lavid et al. 2002). Chinese rose (*Rosa chinensis*) flowers make a similar compound with three methoxyl groups, 1,3,5-trimethoxybenzene, which is synthesized from 1,3,5-trihydroxybenzene. OOMT1 and OOMT2 can catalyze the methylation of the second and third intermediates (1-methoxy,3,5-dihydroxybenzene and 1,3-dimethoxy,5-hydroxybenzene) but not the methylation of 1,3,5-trihydroxybenzene, also known as phloroglucinol (Lavid et al., 2002; Scalliet et al., 2002). The enzyme that methylates this compound, phloroglucinol OMT (POMT), also belongs to the Type I MT family, but is only distantly related to OOMT1 and OOMT2 (Wu et al., 2004).

An important strawberry (*Fragaria* × *ananassa*) aroma compound, 2,5-dimethyl-4-methoxy-3(2H)-furanone, was shown to be produced by the action of another Type I MT. This MT appears to be able to methylate a wide range of substrates, including intermediates of the lignin biosynthetic pathway such as coniferal aldehyde and coniferal alcohol (Wein et al., 2002).

Eugenol, mentioned above as the substrate of a eugenol MT and an important volatile spice on its own, and vanillin, another important aroma compound, also contain a 3-methoxyl group on their benzene ring. The synthetic pathways of these compounds share the first few steps with the lignin pathway. Gang et al. (2002a) demonstrated that eugenol is derived from a lignin intermediate past the para and meta hydroxylation reactions and the 3-hydroxyl methylation, which is catalyzed by CCOMT (caffeoyl-CoA OMT). This is also likely to be the case for vanillin, although this has not yet been demonstrated conclusively. CCOMT is a member of the Type II MT family of plants (Noel et al., 2003).

Methylation of Carboxyl Groups

Some methyl esters are extremely wide spread in the plant kingdom. For example, methylsalicylate has been reported in numerous floral scents (Knudsen and Tollsten, 1993), and it is also commonly emitted from vegetative tissues under attack by insects or parasites (Van Poecke et al., 2001; Chen et al., 2003). An enzyme capable of methylating salicylic acid (SA), salicylic acid carboxyl methyltransferase (SAMT) was first reported from *C. breweri* flowers (Fig. 1D; Ross et al., 1999). It has since been identified from several other plant species (Fukami et al., 2002; Negre et al., 2002, 2003; Pott et al., 2002, 2004; Chen et al., 2003).

This enzyme, which uses SAM as the methyl donor, defines a new type of plant MT known as Type III or SABATH MT (after the first two letters of the names of the first three enzymes identified in this family). Some SAMT enzymes have been shown to be able to methylate also benzoic acid (BA), a compound identical to SA except for lacking the 2-hydroxyl group present in SA (for example, Pott et al., 2004). On the other hand, benzoic acid carboxyl methyltransferase (BAMT), the enzyme responsible for the snapdragon floral volatile methylbenzoate, cannot methylate SA (Dudareva et al., 2000; Murfitt et al., 2000).

There are 24 SABATH MTs encoded by the Arabidopsis genome, including one enzyme that methylates both SA and BA (Chen et al., 2003). It is not known if all of these MTs are involved in the biosynthesis of volatile compounds, but MTs belonging to the SABATH family have been shown to be responsible for the three consecutive metylation reactions in the biosynthesis of caffeine, a nonvolatile compound, in *Coffea arabica* (Uefuji et al., 2003). However, another Arabidopsis SABATH MT was shown to methylate jasmonic acid to form methyljasmonate (Seo et al., 2001). While this molecule may act as an internal signal molecule in Arabidopsis and other plant species, it is also emitted from injured plants (Howe and Schilmiller, 2002), and has also been reported in the floral scent of several plant species (Knudsen et al., 1993) where it is likely to be formed by similar enzymes.

Acylation

Acylation, most often with an acetyl moiety but also with larger acyls such butanoyl or benzoyl acyls, to make volatile compounds is also common. In all known examples, such plant volatile esters are synthesized by a recently discovered family of plant acyltransferases called BAHD, after the first letter of the first four enzymes identified (St-Pierre and De Luca, 2000). The basic reaction catalyzed by these enzymes is the transfer of an acyl group from an acyl-CoA intermediate to the hydroxyl group of an alcohol (Fig. 1E). Many BAHD enzymes are involved in the synthesis of nonvolatile compounds such as acylated alkaloids or taxol derivatives (St-Pierre et al., 1998; Walker and Croteau, 2000), or in early steps of pathways that may lead to the synthesis of volatiles such as eugenol (Gang et al., 2002a). BAHD acyltransferases directly involved in volatile synthesis include benzyl alcohol acetyl-CoA transferase from *C. breweri* flowers, which produced benzyl acetate (Fig. 1E; Dudareva et al., 1998); benzyl alcohol benzoyl-CoA transferase, which produces benzylbenzoate in flowers of Clarkia (D'Auria et al., 2002) and petunia (*Petunia hybrida*; Boatright et al., 2004), and in tobacco mosaic virus-infected leaves of tobacco (*Nicotiana tabacum*; D'Auria et al., 2002); and 3-cis-hexen-1-ol acetyl CoA transferase, which produces 3-cis-hexenyl acetate (a green leaf volatile) and is induced in damaged leaves of Arabidopsis (D'Auria et al., 2002).

The BAHD enzymes often show wide substrate specificity for both the acyl moiety and the alcohol moiety. For example, the petunia benzyl alcohol benzoyl-CoA transferase enzyme can also transfer an acetyl moiety to the alcohol phenylethanol, producing phenylethylacetate (Boatright et al., 2004). Similarly, a BAHD enzyme from ripening strawberry (*Fragaria* spp) fruit, can use a series of acyl moieties such as acetyl, butanyl, and hexanyl, and transfer them to various alcohols such as heptanol, octanol, and geraniol (Aharoni et al., 2000; Beekwilder et al., 2004). An acyltransferase from banana (*Musa sapientum*) has similarly wide substrate specificity (Beekwilder et al., 2004), and a rose (*Rose hybrida*) flower BAHD enzyme can acetylate both geraniol and citronellol (Shalit et al., 2003). In such cases, the type of volatile formed in a given tissue depends more on the internal concentrations of the substrates than on the K_m and K_{cat} values of the enzyme for these substrates (Beekwilder et al., 2004; Boatright et al., 2004).

The Production of the C6-C1 Benzenoids from C6-C3 Phenylpropanoids

The shortening by two carbons of the three-carbon chain attached to the phenyl ring of phenylpropanoids leads to the formation of benzenoid compounds. The mechanism by which this is achieved is not fully understood. In vivo stable isotope labeling and computer-assisted metabolic flux analysis, described in this issue, revealed that both the CoA-dependent-β-oxidative and CoA-independent-non-β-oxidative pathways are involved in the formation of benzenoid compounds in petunia (Boatright et al., 2004). However, a recent discovery also indicates that in the case of 2-hydroxybenzoic acid (i.e. salicylic acid), a third pathway, via the isochorismate pathway, may also operate in plants, as it does in bacteria (Wildermuth et al., 2001).

REGULATION OF EMISSION OF VOLATILE COMPOUNDS

Emission of a particular volatile compound into the atmosphere depends on both the rate of its biosynthesis and the rate of its release. Substantial progress in the last decade in the isolation and characterization of genes responsible for the formation of volatile compounds has facilitated the investigation of the regulation of the biosynthesis of plant volatiles. It has been found that volatiles are synthesized de novo in the tissues from which they are emitted. Biosynthesis normally occurs in the epidermal cells of plant tissues from which they can escape into the atmosphere or rhizosphere after being synthesized (Dudareva et al., 1996; Dudareva and Pichersky, 2000; Kolosova et al., 2001b; Chen et al., 2004) or in the secretory structures or glandular trichomes as, for instance, was found in peppermint, *Artemisia annua*, and sweet basil (*Ocimum*

basilicum; McCaskill et al., 1992; Gang et al., 2001; Lu et al., 2002).

Formation of volatile compounds is spatially regulated. Of the plant organs in scented species, flowers produce the most diverse and the highest amount of volatile compounds, which peak when the flowers are ready for pollination. Vegetative tissue also releases small quantities of volatile organic compounds, which could be induced by mechanical damage or by herbivore- or pathogen infection (Loughrin et al., 1994; Pare and Tumlinson, 1997; Arimura et al., 2004a). In herbs, such as peppermint, significant amounts of volatile compounds accumulate in the leaf glandular trichomes and the emitted volatiles represent only a small fraction of the total pool produced (Gershenzon et al., 2000).

Production and emission of volatile compounds is also a developmentally regulated process. Volatile emission in flowers and accumulation in leaves and fruits follow similar developmental patterns, increasing during the early stages of organ development (when leaves are young and not fully expended, fruit is not yet mature, or when flowers are ready for pollination) and then either remaining relatively constant or decreasing over the organs' lifespan (Bouwmeester et al., 1998; Dudareva and Pichersky, 2000; Gershenzon et al., 2000). The concurrent temporal changes in activities of enzymes responsible for the final steps of volatile formation, enzyme protein levels, and the expression of corresponding structural genes suggest that the developmental biosynthesis of volatiles is regulated largely at the level of gene expression (Dudareva et al., 1996; McConkey et al., 2000). It is still unclear to what extent transcriptional, posttranscriptional, translational, posttranslational, and other events contribute to this process.

In general, more than one biochemical pathway is responsible for a blend of volatile compounds released from different plant tissues. A comparative analysis of the regulation of benzenoid and monoterpene emission in snapdragon flowers revealed that the orchestrated emission of phenylpropanoid and isoprenoid compounds is regulated upstream of individual metabolic pathways and includes the coordinated expression of genes that encode enzymes involved in the final steps of scent biosynthesis (Dudareva et al., 2000, 2003). However, transcription factors that regulate multiple biosynthetic pathways leading to the formation of odor bouquet have not yet been discovered.

The level of the enzyme responsible for the final step of the biosynthesis of a particular volatile is not the only limiting factor. The target for the regulation of developmental production of volatile compounds also includes the level of supplied substrate in the cell (Dudareva et al., 2000). In the case of enzymes that are able to use several similar substrates, such as SAMT and acyltransferases, the level of supplied precursor controls the type of produced product (Negre et al., 2003; Boatright et al., 2004; Pott et al., 2004). The role of substrate in the regulation of the biosynthesis of volatile compounds was also recently confirmed by metabolic engineering. When the linalool synthase gene was introduced under the control of the cauliflower mosaic virus 35S constitutive promoter into petunia W115, the differences between organs in the amount of the synthesized linalool or its glycoside depended more on the availability of the substrate GPP in the tissue than on the expression of the linalool synthase gene (Lücker et al., 2001). In peppermint the up-regulation of 1-deoxy-D-xylulose-5-phosphate reductase, which catalyzes the conversion of 1-deoxy-D-xylulose-5-phosphate to methylerythritol phosphate, increased the flux to GPP and led to about a 50% increase in the essential oil production (Mahmoud and Croteau, 2001). Additional regulation of GPP formation can occur at the level of GPP synthase, as was shown in snapdragon where the small subunit can play a key role in GPP biosynthesis (Tholl et al., 2004). Feedback regulation of GPP synthase by product and substrate inhibition could also contribute to the regulatory control of the flux to GPP and subsequently to monoterpene production (Tholl et al., 2004). When enzymes competed for the same substrate as in the case of three monoterpene synthases (γ-terpinene cyclase, [+]-limonene cyclase, and [−]-β-pinene cyclase) introduced into tobacco plants, the magnitude of monoterpene emission in leaves was close to that predicted based on the K_m values of the enzymes for GPP, while the emission levels in flowers were comparable suggesting that the GPP pool did not limit monoterpene production (Lücker et al., 2004). These results show that while the investigation of the regulation of the final steps of volatile biosynthesis is an important starting point, a detailed understanding of the regulation of the flux through the entire biochemical pathway is essential for the complete understanding of production and emission of secondary volatile compounds.

Emission of volatile compounds from flowers and leaves of some plant species, as well as herbivore-induced volatiles, varies remarkably throughout the photoperiod. The release of floral volatiles in these species displays a rhythmic pattern with maximum emission during the day or night, which generally coincides with the foraging activities of potential pollinators, and is controlled by a circadian clock or regulated by light (Jakobsen and Olsen, 1994; Helsper et al., 1998; Kolosova et al., 2001a). Isoprene, as well as volatiles emitted from undamaged and herbivore-attacked leaves, exhibit a distinct diurnal emission pattern (Loughrin et al., 1994; Loreto et al., 1996; De Moraes et al., 2001; Lerdau and Gray, 2003; Martin et al., 2003; Arimura et al., 2004a) with some leaf volatiles' emission also controlled by a circadian clock, for example (−)-β-pinene in *Artemisia annua* (Lu et al., 2002). In flowers the rhythmic production and emission of some volatile compounds, such as the volatile ester methylbenzoate, is regulated primarily by the level of substrate availability (BA), which in turn could be regulated at the level of expression of genes encoding the key enzymes of its biosynthesis (Kolosova et al., 2001a). While regulation of isoprene emission

is well understood (Wolfertz et al., 2003), little is known to date about the molecular mechanisms responsible for diurnal emission of inducible vegetative volatiles. Often, there is a delay of several hours between the beginning of herbivore damage and the release of induced volatile compounds, which could be explained, in part, by the up-regulation of the expression of genes responsible for their biosynthesis (Arimura et al., 2004a). When volatile compounds are released immediately after herbivore damage, they arise from stored pools (Pare and Tumlinson, 1997).

Environmental factors such as light, temperature, and moisture status can greatly influence the emission of volatiles and the yield and composition of essential oils (Staudt and Bertin, 1998; Gershenzon et al., 2000). In addition, pollination induces the decrease in emission of floral volatiles which begins after pollen tubes reach the ovary (Negre et al., 2003).

To date very little is known about the release of synthesized volatile compounds from plant tissues. In general, the rate of release is a function of the physical properties of the compound itself (its volatility) and the properties of cellular and intracellular membranes (in case of monoterpenes which are synthesized in the chloroplast) through which the compound has to diffuse. Comparative analysis of volatile compounds emitted and present within the plant tissue revealed that the emission of volatiles is not merely a function of their differential volatility but could also involve a cytologically organized excretory process (Altenburger and Matile, 1990; Gershenzon et al., 2000). The membranes of the storage compartment (where it exists) or epidermal cell wall might be selectively more permeable to some volatile compounds or the emitted substances may be associated with an entirely different secretory compartment than the stored volatiles. Virtually nothing is presently known about metabolite trafficking between various subcellular compartments, the mechanism of the release process, and how these processes contribute to the regulation of volatile emission.

CONCLUSIONS

Plants produce a plethora of volatile compounds for both general and specialized functions. Recent advances in instrumentation, coupled with our present ability to isolate and characterize genes and the enzymes they encode from many diverse plant species, have greatly enhanced our understanding of how plants synthezise such compounds and regulate their production. The next level of understanding should surely come from studying the significance of these volatiles for plant physiology and their impact on the ecological interactions of plants with their environment.

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LITERATURE CITED

Aharoni A, Keizer LCP, Bouwmeester HJ, Sun ZK, Alvarez-Huerta M, Verhoeven HA, Blaas J, van Houwelingen A, De Vos RCH, van der Voet H, et al (2000) Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. Plant Cell **12:** 647–661

Altenburger R, Matile P (1990) Further observations on rhythmic emission of fragrance in flowers. Planta **180:** 194–197

Andersen RA, Hamilton-Kemp TR, Hildebrand DF, McCracken CT Jr, Collins RW, Fleming PD (1994) Structure-antifungal activity relationships among volatile C_6 and C_9 aliphatic aldehydes, ketones and alcohols. J Agric Food Chem **42:** 1563–1568

Arimura G, Huber DPW, Bohlmann J (2004a) Forest tent caterpillars (*Malacosoma disstria*) induce local and systemic diurnal emissions of terpenoid volatiles in hybrid poplar (*Populus trichocarpa* x *deltoides*): cDNA cloning, functional characterization, and patterns of gene expression of (−)-germacrene D synthase, PtdTPS1. Plant J **37:** 603–616

Arimura G, Ozawa R, Kugimiya S, Takabayashi J, Bohlmann J (2004b) Herbivore-induced defense response in a model legume: Two-spotted spider mites, *Tetranychus urticae*, induce emission of (*E*)-β-ocimene and transcript accumulation of (*E*)-β-ocimene synthase in *Lotus japonicus*. Plant Physiol **135:** 1976–1983

Arimura G, Ozawa R, Shomoda T, Nishioka T, Boland W, Takabayashi J (2000) Herbivory-induced volatiles elicit defense genes in lima bean leaves. Nature **406:** 512–515

Bate NJ, Riley JCM, Thompson JE, Rothstein SJ (1998) Quantitative and qualitative differences in C-6-volatile production from the lipoxygenase pathway in an alcohol dehydrogenase mutant of *Arabidopsis thaliana*. Physiol Plant **104:** 97–104

Beekwilder J, Alvarez-Huerta M, Neef E, Verstappen FWA, Bouwmeester HJ, Aharoni A (2004) Substrate usage by recombinant alcohol acyltransferases from various fruit species. Plant Physiol **135:** 1865–1878

Boatright J, Negre F, Chen X, Kish CM, Wood B, Peel G, Orlova I, Gang D, Rhodes D, Dudareva N (2004) Understanding *in vivo* benzenoid metabolism in petunia petal tissue. Plant Physiol **135:** 1993–2011

Boland W, Gabler A (1989) Biosynthesis of homoterpenes in higher plants. Helv Chim Acta **72:** 247–253

Bonn B, Moortgat GK (2003) Sesquiterpene ozonolysis: origin of atmospheric new particle formation from biogenic hydrocarbons. Geophys Res Lett **30:** 1585

Bouvier F, Suire C, d'Harlingue A, Backhaus RA, Camara B (2000) Molecular cloning of geranyl diphosphate synthase and compartmentation of monoterpene synthesis in plant cells. Plant J **24:** 241–252

Bouwmeester HJ, Gershenzon J, Konings MCJM, Croteau R (1998) Biosynthesis of the monoterpenes limonene and carvone in the fruit of caraway. I. Demonstration of enzyme activities and their changes with development. Plant Physiol **117:** 901–912

Bouwmeester HJ, Konings MCJM, Gershenzon J, Karp F, Croteau R (1999) Cytochrome P-450 dependent (+)-limonene-6-hydroxylation in fruits of caraway (*Carum carvi*). Phytochemistry **50:** 243–248

Burke CC, Croteau R (2002) Geranyl diphosphate synthase from *Abies grandis*: cDNA isolation, functional expression, and characterization. Arch Biochem Biophys **405:** 130–136

Burke CC, Wildung MR, Croteau R (1999) Geranyl diphosphate synthase: cloning, expression, and characterization of this prenyltransferase as a heterodimer. Proc Natl Acad Sci USA **96:** 13062–13067

Cane DE (1999) Sesquiterpene biosynthesis: cyclization mechanisms. *In* DE Cane, ed, Comprehensive Natural Products Chemistry. Isoprenoids Including Carotenoids and Steroids, Vol 2. Pergamon Press, Oxford, pp 155–200

Chen F, D'Auria JC, Tholl D, Ross JR, Gershenzon J, Noel JP, Pichersky E (2003) An *Arabidopsis thaliana* gene for methylsalicylate biosynthesis, identified by a biochemical genomics approach, has a role in defense. Plant J **36:** 577–588

Chen F, Ro D-K, Petri J, Gershenzon J, Bohlmann J, Pichersky E, Tholl D (2004) Characterization of root-specific Arabidopsis terpene synthase responsible for the formation of the volatile monoterpene 1,8-cineole. Plant Physiol **135:** 1956–1966

D'Auria JC, Chen F, Pichersky E (2002) Characterization of an acyltransferase capable of synthesizing benzylbenzoate and other volatile esters in flowers and damaged leaves of *Clarkia breweri*. Plant Physiol **130:** 466–476

Degen T, Dillmann C, Marion-Poll F, Turlings TCJ (2004) High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. Plant Physiol **135:** 1928–1938

Degenhardt J, Gershenzon J (2000) Demonstration and characterization of (*E*)-nerolidol synthase from maize: a herbivore-inducible terpene synthase participating in (3*E*)-4,8-dimethyl-1,3,7-nonatriene biosynthesis. Planta **210:** 815–822

Delfine S, Csiky O, Seufert G, Loreto F (2000) Fumigation with exogenous monoterpenes of a non-isoprenoid-emitting oak (*Quercus suber*): monoterpene acquisition, translocation, and effect on the photosynthetic properties at high temperatures. New Phytol **146:** 27–36

De Moraes CM, Mescheer MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel nonspecific females. Nature **410:** 577–580

Dicke M, Bruin J (2001) Chemical information transfer between plans: back to the future. Biochem Syst Ecol **29:** 981–994

Dicke M, Van Loon JJA (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. Entomol Exp Appl **97:** 237–249

Dudareva N, Cseke L, Blanc VM, Pichersky E (1996) Evolution of floral scent in *Clarkia*: novel patterns of *S*-linalool synthase gene expression in the *C. breweri* flower. Plant Cell **8:** 1137–1148

Dudareva N, D'Auria JC, Nam KH, Raguso RA, Pichersky E (1998) Acetyl-CoA:benzyl alcohol acetyltransferase: an enzyme involved in floral scent production in *Clarkia breweri.* Plant J **14:** 297–304

Dudareva N, Martin D, Kish CM, Kolosova N, Gorenstein N, Faldt J, Miller B, Bohlmann J (2003) (*E*)-β-Ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: function and expression of three terpene synthase genes of a new TPS-subfamily. Plant Cell **15:** 1227–1241

Dudareva N, Murfitt LM, Mann CJ, Gorenstein N, Kolosova N, Kish CM, Bonham C, Wood K (2000) Developmental regulation of methylbenzoate biosynthesis and emission in snapdragon flowers. Plant Cell **12:** 949–961

Dudareva N, Pichersky E (2000) Biochemical and molecular genetic aspects of floral scents. Plant Physiol **122:** 627–633

Engelberth J, Alborn HT, Schmelz EA, Tumlinson JH (2004) Airborne signals prime plants against insect herbivore attack. Proc Natl Acad Sci USA **101:** 1781–1785

Frank MR, Deyneka JM, Schuler MA (1996) Cloning of wound-induced cytochrome P450 monooxygenases expressed in pea. Plant Physiol **110:** 1035–1046

Friedman M, Henika PR, Mandrell RE (2002) Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica.* J Food Prot **65:** 1545–1560

Fukami H, Asakura T, Hirano H, Abe K, Shimomura K, Yamakawa T (2002) Salicylic acid carboxyl methyltransferase induced in hairy root cultures of *Atropa belladonna* after treatment with exogeneously added salicylic acid. Plant Cell Physiol **43:** 1054–1058

Gang DR, Beuerle T, Ullmann P, Werck-Reichhart D, Pichersky E (2002a) Differential production of meta hydroxylated phenylpropanoids in sweet basil (*Ocimum basilicum* L.) peltate glandular trichomes and leaves is controlled by the activities of specific acyltransferases and hydroxylases. Plant Physiol **130:** 1536–1544

Gang DR, Lavid N, Zubieta C, Chen F, Beuerle T, Lewinsohn E, Noel JP, Pichersky E (2002b) Characterization of phenylpropene *O*-methyltransferases from sweet basil: facile change of substrate specificity and convergent evolution within a plant OMT family. Plant Cell **14:** 505–519

Gang DR, Wang JH, Dudareva N, Nam KH, Simon JE, Lewinsohn E, Pichersky E (2001) An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. Plant Physiol **125:** 539–555

Gershenzon J, McConkey ME, Croteau RB (2000) Regulation of monoterpene accumulation in leaves of peppermint. Plant Physiol **122:** 205–213

Hallahan DL, West JM, Wallsgrove RM, Smiley DWM, Dawson GW, Pickett JA, Hamilton JGC (1995) Purification and characterization of an acyclic monoterpene primary alcohol:$NADP^+$ oxidoreductase from catmint (*Nepeta racemosa*). Arch Biochem Biophys **318:** 105–112

Hammer KA, Carson CF, Riley TV (2003) Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J Appl Microbiol **95:** 853–860

Helsper JPFG, Davies JA, Bouwmeester HJ, Krol AF, van Kampen MH (1998) Circadian rhythmicity in emission of volatile compounds by flowers of *Rosa hybrida* L. cv. Honesty. Planta **207:** 88–95

Hoffmann T, Odum JR, Bowman F, Collins D, Klockow D, Flagan RC, Seinfeld JH (1997) Formation of organic aerosols from the oxidation of biogenic hydrocarbons. J Atmos Chem **26:** 189–212

Howe GA, Schilmiller AL (2002) Oxylipin metabolism in response to stress. Curr Opin Plant Biol **5:** 230–236

Jakobsen HB, Olsen CE (1994) Influence of climatic factors on emission of flower volatiles *in-situ.* Planta **192:** 365–371

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science **291:** 2141–2144

Knudsen JT, Tollsten L (1993) Trends in floral scent chemistry in pollination syndromes–floral scent composition in moth-pollinated taxa. Bot J Linn Soc **113:** 263–284

Knudsen JT, Tollsten L, Bergstrom G (1993) Floral scents – a checklist of volatile compounds isolated by head-space techniques. Phytochemistry **33:** 253–280

Kolosova N, Gorenstein N, Kish CM, Dudareva N (2001a) Regulation of circadian methylbenzoate emission in diurnally and nocturnally emitting plants. Plant Cell **13:** 2333–2347

Kolosova N, Sherman D, Karlson D, Dudareva N (2001b) Cellular and subcellular localization of *S*-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase, the enzyme responsible for biosynthesis of the volatile ester methylbenzoate in snapdragon flowers. Plant Physiol **126:** 956–964

Koyama T, Ogura K (1999) Isopentenyl diphosphate isomerase and prenyltransferases. *In* DE Cane, ed, Comprehensive Natural Product Chemistry. Isoprenoids Including Carotenoids and Steroids, Vol 2. Pergamon Press, Oxford, pp 69–96

Laule O, Fürholz A, Chang H-S, Zhu T, Wang X, Heifetz PB, Gruissem W, Lange M (2003) Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana.* Proc Natl Acad Sci USA **100:** 6866–6871

Lavid N, Wang J, Shalit M, Gutterman I, Bar E, Beuerle T, Weiss D, Vainstein A, Pichersky E, Lewinsohn E (2002) *O*-methyltransferases involved in the biosynthesis of volatile phenolic derivatives in rose petals. Plant Physiol **129:** 1899–1907

Lerdau M, Gray D (2003) Ecology and evolution of light-dependent and light-independent phytogenic volatile organic carbon. New Phytol **157:** 199–211

Lewinsohn E, Ziv-Raz I, Dudai N, Tadmor Y, Lastochkin E, Larkov O, Chaimovitsh D, Ravid U, Putievsky E, Pichersky E, et al (2000) Biosynthesis of estragole and methyl-eugenol in sweet basil (*Ocimum basilicum* L). Developmental and chemotypic association of allylphenyl *O*-methyltransferase activities. Plant Sci **160:** 27–35

Liang P-H, Ko T-P, Wang AH-J (2002) Structure, mechanism and function of prenyltransferases. Eur J Biochem **269:** 3339–3354

Loreto F, Ciccioli P, Brancaleoni E, Cecinato A, Frattoni M, Sharkey TD (1996) Different sources of reduced carbon contribute to form three classes of terpenoid emitted by *Quercus ilex* L leaves. Proc Natl Acad Sci USA **93:** 9966–9969

Loreto F, Pinelli P, Brancaleoni E, Ciccioli P (2004a) ^{13}C labeling reveals chloroplastic and extra-chloroplastic pools of dimethylallyl pyrophosphate and their contribution to isoprene formation. Plant Physiol **135:** 1903–1907

Loreto F, Pinelli P, Manes F, Kollist H (2004b) Impact of ozone on monoterpene emissions and evidence for an isoprene-like antioxidant action of monoterpenes emitted by *Quercus ilex* leaves. Tree Physiol **24:** 361–367

Loreto F, Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol **127:** 1781–1787

Loughrin JH, Manukian A, Heath RR, Turlings TCJ, Tumlinson JH (1994) Diurnal cycle of emission of induced volatile terpenoids herbivore-injured cotton plants. Proc Natl Acad Sci USA **91:** 11836–11840

Lu S, Xu R, Jia JW, Pang JH, Matsuda SPT, Chen XY (2002) Cloning and functional characterization of a beta-pinene synthase from *Artemisia annua* that shows a circadian pattern of expression. Plant Physiol **130:** 1335–1348

Lücker J, Bouwmeester HJ, Schwab W, Blaas J, Van der Plas LHW, Verhoeven HA (2001) Expression of *Clarkia S*-linalool synthase in transgenic petunia plants results in the accumulation of *S*-linalyl-beta-D-glucopyranosid. Plant J **27:** 315–324

Lücker J, Schwab W, Van Hautum B, Blaas J, Van der Plas LHW, Bouwmeester HJ, Verhoeven HA (2004) Increased and altered fragrance of tobacco plants after metabolic engineering using three monoterpene synthases from lemon. Plant Physiol **134:** 510–519

Lupien S, Karp F, Wildung M, Croteau R (1999) Regiospecific cytochrome P450 limonene hydroxylases from mint (Mentha) species: cDNA isolation, characterization, and functional expression of (-)-*4S*-limonene-3-hydroxylase and (-)-*4S*-limonene-6-hydroxylase. Arch Biochem Biophys **368:** 181–192

Mahmoud SS, Croteau RB (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. Proc Natl Acad Sci USA **98:** 8915–8920

Martin D, Fäldt J, Bohlmann J (2004) Functional characterization of nine Norway spruce *TPS* genes and evolution of gymnosperm terpene synthases of the *TPS-d* subfamily. Plant Physiol **135:** 1908–1927

Martin DM, Gershenzon J, Bohlmann J (2003) Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate in foliage of Norway spruce. Plant Physiol **132:** 1586–1599

McCaskill D, Gershenzon J, Croteau R (1992) Morphology and monoterpene biosynthetic capabilities of secretory-cell clusters isolated from glandular trichomes of peppermint (*Mentha-piperita* L.). Planta **187:** 445–454

McConkey ME, Gershenzon J, Croteau RB (2000) Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. Plant Physiol **122:** 215–223

Mercke P, Kappers IF, Verstappen FWA, Vorst O, Dicke M, Bouwmeester HJ (2004) Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants. Plant Physiol **135:** 2012–2024

Murfitt LM, Kolosova N, Mann CJ, Dudareva N (2000) Purification and characterization of *S*-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase, the enzyme responsible for biosynthesis of the volatile ester methylbenzoate in flowers of *Antirrhinum majus*. Arch Biochem Biophys **382:** 145–151

Negre F, Kish CM, Boatright J, Underwood B, Shibuya K, Wagner C, Clark DG, Dudareva N (2003) Regulation of methylbenzoate emission after pollination in snapdragon and petunia flowers. Plant Cell **15:** 2992–3006

Negre F, Kolosova N, Knoll J, Kish CM, Dudareva N (2002) Novel *S*-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, an enzyme responsible for biosynthesis of methylsalicylate and methylbenzoate, is not involved in floral scent production in snapdragon flowers. Arch Biochem Biophys **406:** 261–270

Noel JP, Dixon RA, Pichersky E, Zubieta C, Ferrer JL (2003) Structural, functional, and evolutionary basis for methylation of plant small molecules. *In* JT Romeo, ed, Recent Advances in Phytochemistry, Vol 37. Elsevier Science, Oxford, pp 37–58

Pare PW, Tumlinson JH (1997) *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol **114:** 1161–1167

Pare PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiol **121:** 325–331

Pichersky E, Gershenzon J (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr Opin Plant Biol **5:** 237–243

Pott MB, Hippauf F, Saschenbrecker S, Chen F, Ross J, Kiefer I, Slusarenko A, Noel JP, Pichersky E, Effmert U, et al (2004) Biochemical and structural characterization of benzenoid carboxyl methyltransferases involved in floral scent production in *Stephanotis floribunda* and *Nicotiana suaveolens*. Plant Physiol **135:** 1946–1955

Pott MB, Pichersky E, Piechulla B (2002) Evening-specific oscillation of scent emission, SAMT enzyme activity, and mRNA in flowers of *Stephanotis floribunda*. J Plant Physiol **159:** 925–934

Prestage S, Linforth RST, Taylor AJ, Lee E, Speirs J, Schuch W (1999) Volatile production in tomato fruit with modified alcohol dehydrogenase activity. J Sci Food Agric **79:** 131–136

Raguso RA, Levin RA, Foose SE, Holmberg MW, McDade LA (2003) Fragrance chemistry, nocturnal rhythms and pollination ''syndromes'' in Nicotiana. Phytochemistry **63:** 265–284

Ralston L, Kwon ST, Schoenbeck M, Ralston J, Schenk DJ, Coates RM, Chappell J (2001) Cloning, heterologous expression, and functional characterization of 5-epi-aristolochene-1,3-dihydroxylase from tobacco (*Nicotiana tabacum*). Arch Biochem Biophys **393:** 222–235

Reinhard J, Srivivasan MV, Zhang S (2004) Scent-triggered navigation in honeybees. Nature **427:** 411

Rodriguez-Concepcion M, Boronat A (2002) Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. Plant Physiol **130:** 1079–1089

Ross JR, Nam KH, D'Auria JC, Pichersky E (1999) *S*-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, an enzyme involved in floral scent production and plant defense, represents a new class of plant methyltransferases. Arch Biochem Biophys **367:** 9–16

Scalliet G, Journot N, Jullien F, Baudino S, Magnard JL, Channeliere S, Vergne P, Dumas C, Bendahmane M, Cock JM, et al (2002) Biosynthesis of the major scent components 3,5-dimethoxytoluene and 1,3,5-trimethoxybenzene by novel rose *O*-methyltransferases. FEBS Lett **523:** 113–118

Schoch G, Goepfert S, Morant M, Hehn A, Meyer D, Ullmann P, Werck-Reichhart D (2001) CYP98A3 from *Arabidopsis thaliana* is a 3′-hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway. J Biol Chem **276:** 36566–36574

Schuhr CA, Radykewicz T, Sagner S, Latzel C, Zenk MH, Arigoni D, Bacher A, Rohdich F, Eisenreich W (2003) Quantitative assessment of crosstalk between the two isoprenoid biosynthesis pathways in plants by NMR spectroscopy. Phytochem Rev **2:** 3–16

Schuler MA (1996) Plant cytochrome P450 monooxygenases. Crit Rev Plant Sci **15:** 235–284

Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Lee JS, Choi YD (2001) Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. Proc Natl Acad Sci USA **98:** 4788–4793

Shalit M, Guterman I, Volpin H, Bar E, Tamari T, Menda N, Adam Z, Zamir D, Vainstein A, Weiss D, et al (2003) Volatile ester formation in roses: identification of an acetyl-CoA:geraniol acetyltransferase in developing rose petals. Plant Physiol **131:** 1868–1876

Sharkey TD, Chen XY, Yeh S (2001a) Isoprene increases thermotolerance of fosmidomycin-fed leaves. Plant Physiol **125:** 2001–2006

Sharkey TD, Yeh SS (2001b) Isoprene emission from plants. Annu Rev Plant Physiol **52:** 407–436

Starks CM, Back K, Chappell J, Noel JP (1997) Structural basis for cyclic terpene biosynthesis by tobacco 5-*epi*-aristolochene synthase. Science **277:** 1815–1820

Staudt M, Bertin N (1998) Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (*Quercus ilex* L.) leaves. Plant Cell Environ **21:** 385–395

Steeghs M, Bais HP, de Gouw J, Goldan P, Kuster W, Northway M, Fall R, Vivanco JM (2004) Proton-transfer-reaction mass spectrometry (PTR-MS) as a new tool for real time analysis of root-secreted volatile organic compounds (VOCs) in *Arabidopsis thaliana.* Plant Physiol **135:** 47–58

St-Pierre B, De Luca V (2000) Evolution of acyltransferase genes: origin and diversification of the BAHD superfamily of acyltransferases involved in secondary metabolism. *In* JT Romeo, R Ibrahim, L Varin, V De Luca, eds, Recent Advances in Phytochemistry Evolution of Metabolic Pathways, Vol 34. Elsevier Science, Oxford, pp 285–315

St-Pierre B, Laflamme P, Alarco AM, De Luca V (1998) The terminal *O*-acetyltransferase involved in vindoline biosynthesis defines a new class of proteins responsible for coenzyme A-dependent acyl transfer. Plant J **14:** 703–713

Tholl D, Kish CM, Orlova I, Sherman D, Gershenzon J, Pichersky E, Dudareva N (2004) Formation of monoterpenes in *Antirrhinum majus* and *Clarkia breweri* flowers involves heterodimeric geranyl diphosphate synthases. Plant Cell **16:** 977–992

Uefuji H, Ogita S, Yamaguchi Y, Koizumi N, Sano H (2003) Molecular cloning and functional characterization of three distinct *N*-methyltransferases involved in the caffeine biosynthetic pathway in coffee plants. Plant Physiol **132:** 372–380

Vancanneyt G, Sanz C, Farmaki T, Paneque M, Ortego F, Castanera P, Sanchez-Serrano JJ (2001) Hydroperoxide lyase depletion in transgenic potato plants leads to an increase in aphid performance. Proc Natl Acad Sci USA **98:** 8139–8144

Van Poecke RMP, Posthumus MA, Dicke M (2001) Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. J Chem Ecol **27:** 1911–1928

Vuorinen T, Nerg A-M, Holopainen JK (2004a) Ozone exposure triggers the emission of herbivore-induced plant volatiles, but does not disturb tritrophic signalling. Environ Pollut **131:** 305–311

Vuorinen T, Nerg A-M, Ibrahim MA, Reddy GVP, Holopainen JK (2004b) Emission of *Plutella xylostella*-induced compounds from cabbages grown at elevated CO_2 and orientation behaviour of the natural enemies. Plant Physiol **135:** 1984–1992

Walker K, Croteau R (2000) Molecular cloning of a 10-deacetylbaccatin III-10-*O*-acetyl transferase cDNA from *Taxus* and functional expression in *Escherichia coli*. Proc Natl Acad Sci USA **97:** 583–587

Wang J, Dudareva N, Bhakta S, Raguso RA, Pichersky E (1997) Floral scent production in *Clarkia breweri* (Onagraceae). II. Localization and developmental modulation of the enzyme SAM:(Iso)Eugenol *O*-methyltransferase and phenylpropanoid emission. Plant Physiol **114:** 213–221

Wein M, Lavid N, Lunkenbein S, Lewinsohn E, Schwab W, Kaldenhoff R (2002) Isolation, cloning and expression of a multifunctional *O*-methyltransferase capable of forming 2,5-dimethyl-4-methoxy-3(2H)-furanone, one of the key aroma compounds in strawberry fruits. Plant J **31:** 755–765

Whittington DA, Wise ML, Urbansky M, Coates RM, Croteau RB, Christianson DW (2002) Bornyl diphosphate synthase: structure and strategy for carbocation manipulation by a terpenoid cyclases. Proc Natl Acad Sci USA **99:** 15375–15380

Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defense. Nature **414:** 562–565

Wise ML, Croteau R (1999) Monoterpene biosynthesis. *In* DE Cane, ed, Comprehensive Natural Products Chemistry. Isoprenoids Including Carotenoids and Steroids, Vol 2. Pergamon Press, Oxford, pp 97–153

Wolfertz M, Sharkey TD, Boland W, Kühnemann F (2004) Rapid regulation of the methylerythritol 4-phosphate pathway during isoprene synthesis. Plant Physiol **135:** 1939–1945

Wolfertz M, Sharkey TD, Boland W, Kühnemann F, Yeh S, Weise SE (2003) Biochemical regulation of isoprene emission. Plant Cell Environ **26:** 1357–1364

Wu SQ, Watanabe N, Mita S, Dohra H, Ueda Y, Shibuya M, Ebizuka Y (2004) The key role of phloroglucinol *O*-methyltransferase in the biosynthesis of *Rosa chinensis* volatile 1,3,5-trimethoxybenzene. Plant Physiol **135:** 95–102