Chapter 20
Aromatic Volatiles and Their Involvement in Plant Defense

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More than one percent of secondary metabolites is represented by volatile compounds which are involved in plant reproduction and defense. About 20% of this volatile subset are aromatic constituents consisting of phenylpropanoids, benzenoids, phenylpropenes, and nitrogen-containing aromatics. Despite the wide diversity of aromatic compounds only a small number are released, often at low levels, in response to and anticipation of attacks by herbivorous insect species. Scattered amounts of information show that aromatic volatiles have roles in repulsion and intoxication of attacking organisms as well as in the attraction of beneficial insect species that control pest insect populations through predation and parasitization. In this chapter, we overview the current body of knowledge regarding biosynthesis of aromatic volatiles emitted from plants as well as their roles in plant-insect and plant–plant interactions and highlight the major unsolved questions awaiting further exploration.

20.1 Introduction

Volatile compounds represent approximately one percent of plant secondary metabolites whose primary functions include plant defense against herbivores and pathogens. These volatiles, when emitted from plant tissues as part of a defense system, can directly repel (De Moraes et al. 2001; Kessler and Baldwin 2001) or intoxicate (Vancanneyt et al. 2001) attacking organisms, and even attract natural enemies of herbivores, indirectly protecting the plant via tritrophic interactions (Mercke et al. 2004; Arimura et al. 2004; Degen et al. 2004). By releasing volatiles, an herbivore-damaged plant can simultaneously reduce the number of attackers (Kessler and Baldwin 2001) while alerting neighboring plants of impending danger (Shulaev et al. 1997). In neighboring plants, volatile warning signs induce the expression of defense genes and emission of volatiles (Arimura et al. 2000; Birkett

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et al. 2000; Ruther and Kleier 2005; Farag et al. 2005) and also, in some cases, prime nearby plants to respond faster to future herbivore attack (Engelberth et al. 2004; Kessler et al. 2006). Plant volatiles are mainly represented by terpenoids, fatty acid derivatives, and amino acid derivatives, including those from L-phenylalanine (Phe), the phenylpropanoids, and benzenoids (Dudareva et al. 2006). Although the number of volatile phenylpropanoids and benzenoids identified from plants is comparable to the volatile terpenoids, progress in research regarding their involvement in plant-plant and plant-insect interactions has been far from equal. In the last ten years some progress has been made elucidating the biosynthesis of volatile aromatic compounds, however, still very little is known about their contribution to plant defense. Our focus is on the biosynthesis of volatile aromatics and their involvement in plant defense.

20.2 Biosynthesis of Volatile Aromatic Compounds

Aromatic metabolites are recognized as an important class of widespread secondary metabolites possessing antiherbivory properties that actively participate in plant direct defense. Their structural diversity ranges from hydroxycinnamic acids to flavonoids and stilbenes (Dixon et al. 2002). These compounds are mainly derived from Phe with more complex metabolites (flavonoids, isoflavonoids, and stilbenes) formed by condensation of a phenylpropane unit with a unit derived from acetate via malonyl coenzyme A. While most of these aromatic compounds are usually non-volatile, the volatile subset is represented by benzenoid (C_6–C_1), phenylpropanoid (C_6–C_3) and phenylpropanoid-related compounds (C_6–C_2) and by products synthesized directly from the shikimic acid pathway (e.g., indole or methyl anthranilate; Fig. 20.1). Although some of these compounds are already fairly volatile (aldehydes, alcohols, alkanes/alkenes, ethers, and esters), further modifications such as hydroxylation, acylation and methylation often enhance their volatilities as well as those of non-volatile compounds (Dudareva et al. 2004).

20.2.1 Aromatic Volatiles Derived from Phenylalanine

The first committed step in the biosynthesis of the majority of phenylpropanoid and benzenoid compounds is catalyzed by a well-known and widely distributed enzyme, L-phenylalanine ammonia-lyase (PAL). PAL catalyzes the deamination of Phe to produce *trans*-cinnamic acid. Formation of the benzenoids (C_6–C_1) from cinnamic acid requires shortening of the side chain by a C_2 unit, for which several routes have been proposed (Fig. 20.1). This process could occur via a CoA-dependent – β-oxidative pathway, a CoA-independent –non-β-oxidative pathway, or a combination of both routes. The β-oxidative branchway is analogous to that underlying β-oxidation of fatty acids and proceeds through the formation of four CoA-ester intermediates. Shortening of the *trans*-cinnamic acid side-chain by
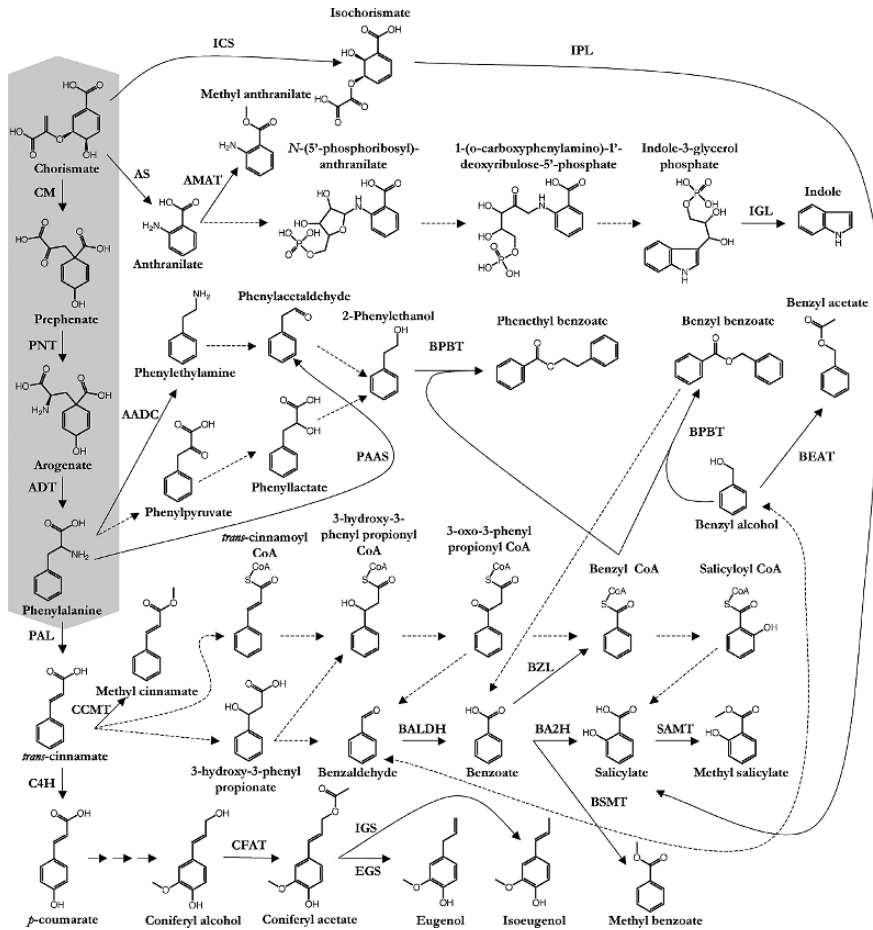
Fig. 20.1 Proposed biosynthetic pathways leading to biosynthesis of volatile aromatic compounds in plants. Solid arrows indicate established biochemical reactions, whereas broken arrows indicate possible steps not yet identified. AADC, amino-acid decarboxylase; ADT, arogenate dehydratase; AMAT, anthraniloyl-coenzyme A (CoA):methanol acyltransferase; AS, anthranilate synthase; BA2H, benzoic acid 2-hydroxylase; BALDH, benzaldehyde dehydrogenase; BEAT, acetyl-CoA:benzyl alcohol acetyltransferase; BPBT, benzoyl-CoA:benzyl alcohol/phenylethanol benzoyltransferase; BSMT, *S*-adenosyl-l-Met:benzoic acid/salicylic acid carboxyl methyltransferase; BZL, benzoate:CoA ligase; C4H, cinnamate 4-hydroxylase; CCMT, *S*-adenosyl-L-Met:coumarate/cinnamate carboxyl methyltransferase; CFAT, acetyl-CoA:coniferyl alcohol acetyltransferase; CM, chorismate mutase; EGS, eugenol synthase; ICS, isochorismate synthase; IGL, indole:glycerol-3-phosphate lyase; IGS, isoeugenol synthase; IPL, isochorismate pyruvate lyase; PAL, L-phenylalanine ammonia lyase; PAAS, phenylacetaldehyde synthase; PNT, prephenate aminotransferase; SAMT, *S*-adenosyl-L-Met:salicylate carboxyl methyltransferase

β-oxidation has been shown in cucumber (*Cucumis sativus*) and *Nicotiana atten-uata* in feeding experiments using stable isotope-labeled (^{2}H$_6$, ^{18}O) 3-hydroxy-3-phenylpropionic acid, which resulted in labeling of benzoic and salicylic acids but not benzaldehyde, a key intermediate in the non-β-oxidative pathway (Fig. 20.1; Jarvis et al. 2000).

The CoA-independent – non-β-oxidative pathway involves hydration of free *trans*-cinnamic acid to 3-hydroxy-3-phenylpropionic acid and side-chain degradation via a reverse aldol cleavage, leading to the formation of benzaldehyde which is oxidized to benzoic acid by an NADP$^+$- dependent enzyme, benzaldehyde dehydrogenase (BALDH). A non-β-oxidative mechanism for the formation of *p*-hydroxybenzoic acid was demonstrated using in vitro studies carried out with cell-suspension cultures of carrot (Schnitzler et al. 1992), cell-free extracts of *Lithospermum erythrorhizon* (Yazaki et al. 1991), and potato (French et al. 1976). This pathway is characterized by the presence of *p*-hydroxybenzaldehyde as an important metabolic intermedi-ate formed prior to oxidation to *p*-hydroxybenzoic acid, and requires an aldehyde dehydrogenase for the conversion of an aldehyde to its corresponding carboxylic acid. A non-oxidative conversion of *trans*-cinnamic acid to benzaldehyde followed by oxidation to benzoic acid was also shown in *Hypericum androsaemum* (Hyperi-caceae) cell culture using pathway-specific radiolabeled precursors (Abd El-Mawla et al. 2001). Interestingly, this pathway was not found in *Centaurium erythraea* (Gen-tianaceae) cell culture wherein 3-hydroxybenzoic acid appears to originate directly from the shikimate pathway (Abd El-Mawla et al. 2001). The contribution of both β-oxidative and non-β-oxidative pathways towards the formation of benzenoid com-pounds was recently shown in petunia (*Petunia hybrida*) flowers using in vivo stable isotope labeling and computer-assisted metabolic flux analysis (Boatright et al. 2004; Orlova et al. 2006). In addition, a recent discovery in the biosynthesis of salicylic acid suggests that it may be formed directly from isochorismate during the *Ara-bidopsis thaliana* pathogen response, raising the possibility of benzenoid biosyn-thesis from shikimate/chorismate pathway intermediates (Wildermuth et al. 2001). Overall, these results show that different routes to benzenoid compounds exist in different plant taxa and even in conspecifics depending on physiological conditions. The mechanism of side chain shortening remains an important unsolved question and surprisingly little is known to date about the enzymes and genes responsible for biosynthesis of phenylpropanoid/benzenoid compounds. However, significant progress has been made in the discovery of enzymes and genes involved in the fi-nal steps of benzenoid volatile formation. Two enzyme superfamilies were found to greatly contribute to the final biosynthetic steps of volatile benzenoids. They include the BAHD superfamily of acyltransferases named according to the first four bio-chemically characterized enzymes of this family, BEAT (acetyl-CoA:benzyl alcohol acetyltransferase), AHCT (anthocyanin *O*-hydroxycinnamoyltransferase), HCBT (anthranilate *N*-hydroxycinnamoyl/ benzoyltransferase) and DAT (deacetylvindo-line 4-*O*-acetyltransferase; D'Auria, 2006) and the SABATH family of methyltrans-ferases, also named based on the first identified genes belonging to this family, SAMT (SAM:salicylic acid carboxyl methyltransferase), BAMT (SAM:benzoic acid car-boxyl methyltransferase), and theobromine synthase (D'Auria et al. 2003).

Acyltransferases belonging to BAHD superfamily catalyze the most common modification of secondary metabolites using alcohols and acyl CoAs as substrates to form acyl esters, many of which are volatile. Biosynthetic enzymes forming benzyl acetate (Dudareva et al. 1998), benzyl benzoate (D'Auria et al. 2002; Boatright et al. 2004), phenylethyl benzoate (Boatright et al. 2004) and cinnamyl acetate (Beekwilder et al. 2004) have been isolated and characterized from different plant species including *Clarkia breweri*, petunia and banana (*Musa* sp.).

Members of SABATH family of methyltransferases form the volatile methyl esters of salicylate, benzoate, cinnamate and *p*-coumarate. These enzymes use *S*-adenosyl-L-methionine to methylate the carboxyl groups of small molecules (Effmert et al. 2005). Eleven such carboxyl methyltransferases have been isolated and characterized at the molecular, biochemical and structural levels within the past decade. Two types of enzymes were distinguished based on their substrate preferences: the SAMT-type enzymes isolated from *C. breweri, Stephanotis floribunda, Antirrhinum majus, Hoya carnosa,* and petunia, which have higher catalytic efficiency and preference for salicylic acid, and the BAMT-type enzymes from *A. majus*, *Arabidopsis thaliana*, *Arabidopsis lyrata,* and *Nicotiana suaveolens* which prefer benzoic acid (reviewed in Effmert et al. 2005). Recently, a carboxyl methyltransferase that can methylate *trans*-cinnamate and *p*-coumarate with high efficiency was isolated from basil (*Ocimum basilicum*; Kapteyn et al. 2007). Surprisingly, this newly isolated carboxyl methyltransferase does not cluster with SAMT- and BAMT-type enzymes, having instead *Arabidopsis* indole-3-acetic acid carboxyl methyltransferase as its closest relative of known function (Kapteyn et al. 2007).

To date, none of the genes encoding the array of enzymes within the benzenoid network that lead to the formation of volatile compound precursors have been isolated. However, the activities of two enzymes, benzoyl-coenzyme A ligase (BZL) and benzoic acid 2-hydroxylase (BA2H), responsible for the formation of benzoyl-CoA and salicylic acid, have been shown in *C. breweri* and tobacco, respectively (Beuerle and Pichersky 2002; Leon et al. 1995).

Formation of phenylpropanoid-related (C_6–C_2) compounds such as phenylacetaldehyde and 2-phenylethanol from Phe does not occur via *trans*-cinnamic acid and competes with PAL for Phe utilization (Boatright et al. 2004; Kaminaga et al. 2006; Tieman et al. 2006). Moreover, a quantitative explanation of the labeling kinetics of phenylacetaldehyde and 2-phenylethanol from deuterium-labeled Phe suggest that phenylacetaldehyde in petunia flowers is not the only precursor of 2-phenylethanol and that the major flux to the latter goes through a different route (Orlova et al. 2006), possibly through phenylpyruvate and phenyllactic acid as recently reported in rose flowers (Watanabe et al. 2002). In contrast, neither phenylpyruvate nor phenyllactate were detected in tomato (*Solanum lycopersicum*) fruits implying that in this system, 2-phenylethanol is mainly formed from phenylacetaldehyde via the action of recently identified phenylacetaldehyde reductases (Tieman et al. 2006, 2007). Biosynthesis of phenylacetaldehyde from Phe requires the removal of both the carboxyl and amino groups, which in petunia occurs by the action of phenylacetaldehyde synthase (PAAS; Kaminaga et al. 2006). In tomato, Phe is first converted to phenylethylamine by aromatic amino acid decarboxylase

(AADC) and requires the action of a hypothesized amine oxidase, dehydrogenase, or transaminase for phenylacetaldehyde formation (Tieman et al. 2006).

The volatile phenylpropenes (C_6–C_3), such as eugenol, isoeugenol, methyleugenol, isomethyleugenol, chavicol, and methylchavicol share the initial biosynthetic steps with the lignin biochemical pathway up to the phenylpropenol (monolignol) stage and then require two enzymatic reactions to eliminate the oxygen functionality at C-9 position (Koeduka et al. 2006; Dexter et al. 2007). Phenylpropene-forming enzymes have been isolated and characterized from basil, petunia and *C. breweri* (Koeduka et al. 2006, 2007). In these species, coniferyl alcohol is first converted to coniferyl acetate by coniferyl alcohol acetyltransferase (CFAT; Dexter et al. 2007; D.R. Gang, personal communication) prior to its reduction by eugenol synthase or isoeugenol synthase (EGS or IGS) to eugenol and isoeugenol, respectively. However, CFAT and its homologs have been identified only in basil and petunia thus far. Although eugenol and isoeugenol differ exclusively by the position of the double bond in the propene side chain, in petunia their formation is mediated by two different and highly diverged NADPH-dependent reductases (Koeduka et al. 2007). In contrast, *C. breweri* contains three distinct NADPH-dependent reductases, two of which are responsible for eugenol formation and the third possessing isoeugenol synthase activity. While one eugenol synthase (EGS) isoform is closely related to isoeugenol synthase (IGS), the other is highly diverged (Koeduka et al. 2007). Similar to the role of coniferyl acetate in eugenol and isoeugenol formation in petunia, coumaryl acetate serves as the biosynthetic precursor of chavicol in basil (Vassao et al. 2006). Often eugenol, isoeugenol, and chavicol undergo further methylation and *O*-methyltransferases responsible for the downstream production of methyl eugenol, isomethyl eugenol and methyl chavicol have been identified and characterized from *C. breweri* and basil plants (Wang et al. 1997; Gang et al. 2002).

20.2.2 Aromatic Volatiles Derived from Chorismate

One volatile benzenoid compound formed independently from Phe is methyl anthranilate, whose precursor anthranilate is synthesized from chorismate (Radwanski and Last 1995). While anthranilate synthase has been isolated and characterized from several plant species (reviewed in Radwanski and Last 1995), an enzyme responsible for anthranilate carboxyl methylation has not yet been identified. It is possible that a carboxyl methyltransferase belonging to SABATH family of methyltransferases is responsible for this process in some species, however a member of the BAHD superfamily of acyltransferases, anthraniloyl-coenzyme A:methanol acyltransferase (AMAT), was found to be responsible for the formation of methyl anthranilate in Concord grape (*Vitis labrusca*; Wang and De Luca 2005). Anthraniloyl-CoA substrate used by AMAT could be synthesized by a BZL analog as was demonstrated for partially purified BZL from *C. breweri*, which possessed anthraniloyl-CoA ligase activity (Beuerle and Pichersky 2002).

Aromatic volatile plant defense compounds formed independently of Phe also include the nitrogen containing compound indole, a heterocyclic organic metabolite common to higher plants. Indole formation in plants occurs from chorismate via the well-characterized tryptophan biosynthesis pathway, preceding tryptophan formation (Radwanski and Last 1995). The final step of indole biosynthesis is catalyzed by indole-3-glycerol phosphate lyase (IGL), which cleaves phosphorylated glycerol from indole-3-glycerol phosphate with concurrent formation of the volatile indole (Frey et al. 2004). Maize IGL was likely derived from tryptophan synthase whose alpha subunit is responsible for formation of indole that is channeled to a beta subunit, converting it to tryptophan. During evolution, divergence of IGL from tryptophan synthase has allowed it to function efficiently independent of the beta subunit allowing for the release of volatile indole, an event that does not occur in tryptophan biosynthesis. Moreover, the *IGL* gene is transcriptionally activated in maize in response to herbivory and exogenous application of methyl jasmonate or volicitin from pest regurgitant. Its induced expression pattern parallels the level of volatile indole emission (Frey et al. 2000).

20.3 Aromatic Volatiles and Plant Defense

In the past two decades it has been well documented that plants emit blends of volatile compounds from their tissues in response to herbivore damage. Odor blends emitted by attacked plants are diverse; they are composed of more than 200 different compounds often present as minor constituents (Dicke and van Loon 2000). In many cases these minor constituents are aromatic compounds. Volatiles emitted can directly affect herbivore physiology and behavior due to their potentially toxic, repellent, or deterrent properties (Bernasconi et al. 1998; De Moraes et al. 2001; Kessler and Baldwin 2001; Vancanneyt et al. 2001; Aharoni et al. 2003) and attract enemies of attacking herbivores such as parasitic wasps, flies or predatory mites, to protect the signaling plant from further damage (Dicke et al. 1990; Turlings et al. 1990; Vet and Dicke 1992; Paré and Tumlinson 1997; Drukker et al. 2000; Kessler and Baldwin 2001). Moreover, some volatile compounds can mediate both direct and indirect defenses, as is shown for methyl salicylate (see below).

20.3.1 Aromatic Volatiles in Plant Direct Defense

The involvement of volatile compounds in plant direct defense is widely accepted, though, to date, surprisingly little is known about the role of aromatic volatiles in the intoxication, repulsion, or deterrence of herbivores. Only a small portion of 329 known volatile phenylpropanoids (Knudsen and Gershenzon 2006) were found in the headspaces of the herbivore-damaged plants and those were released from a limited range of plant taxa and often at very low levels (Tables 20.1 and 20.2). Moreover, biological activity toward insects has been shown for even fewer aromatic

Table 20.1 Herbivore-induced volatile aromatics. Entries are grouped first by compound, then plant species, pest species, and publication date

Compound	Plant Species	Herbivore	Reference
Indol	*Arachis hypogaea*	*Spodoptera exigua*	Cardoza et al. (2002)
	Gossypium herbaceum	*Spodoptera littoralis*	Gouinguene et al. (2005)
	Gossypium hirsutum	*Spodoptera exigua*	Loughrin et al. (1994, 1995a); Röse et al. (1996); Paré and Tumlinson (1997, 1998) and Rodriguez-Saona et al. (2003)
	Phaseolus lunatus	*Spodoptera littoralis*	Mithöfer et al. (2005)
	Vigna unguiculata	*Spodoptera littoralis*	Gouinguene et al. (2005)
	Zea mays	*Rhopalosiphum maidis*	Bernasconi et al. (1998)
	Zea mays	*Spodoptera exigua*	Frey et al. (2000); Schmelz et al. (2003) and D'Alessandro et al. (2006)
	Zea mays	*Spodoptera littoralis*	D'Alessandro and Turlings (2005) and Gouinguene et al. (2005)
Methylsalicylate	*Capsicum annuum*	*Tetranychus urticae*	Van Den Boom et al. (2004)
	Datura stramonium	*Tetranychus urticae*	Van Den Boom et al. (2004)
	Glycine max	*Tetranychus urticae*	Van Den Boom et al. (2004)
	Humulus lupulus	*Tetranychus urticae*	Van Den Boom et al. (2004)
	Medicago truncatula	*Spodoptera littoralis*	Leitner et al. (2005)
	Robinia pseudoacacia	*Tetranychus urticae*	Van Den Boom et al. (2004)
	Vicia faba	*Aphis fabae*	Hardie et al. (1994)
	Vigna unguiculata	*Spodoptera littoralis*	Gouinguene et al. (2005)
	Vigna unguiculata	*Tetranychus urticae*	Van Den Boom et al. (2004)
	Vitis vinifera	*Tetranychus urticae*	Van Den Boom et al. (2004)
Methylanthranilate	*Gossypium herbaceum*	*Spodoptera littoralis*	Gouinguene et al. (2005)
	Zea mays	*Rhopalosiphum maidis*	Bernasconi et al. (1998)

Table 20.1 (continued)

Compound	Plant Species	Herbivore	Reference
	Zea mays	*Spodoptera exigua*	D'Alessandro et al. (2006)
	Zea mays	*Spodoptera littoralis*	Degen et al. (2004); D'Alessandro and Turlings (2005) and Gouinguene et al. (2005)
2-phenylethyl acetate	*Zea mays*	*Rhopalosiphum maidis*	Bernasconi et al. (1998)
	Zea mays	*Spodoptera exigua*	D'Alessandro et al. (2006)
	Zea mays	*Spodoptera littoralis*	Degen et al. (2004); D'Alessandro and Turlings (2005) and Gouinguene et al. (2005)
Benzyl acetate	*Zea mays*	*Rhopalosiphum maidis*	Bernasconi et al. (1998)
	Zea mays	*Spodoptera exigua*	D'Alessandro et al. (2006)
	Zea mays	*Spodoptera littoralis*	Degen et al. (2004) and Gouinguene et al. (2005)
Methylbenzoate	*Gossypium herbaceum*	*Spodoptera littoralis*	Gouinguene et al. (2005)
	Malus sp.	*Tetranychus urticae*	Takabayashi et al. (1991)
	Vigna un-guiculata	*Spodoptera littoralis*	Gouinguene et al. (2005)
	Zea mays	*Spodoptera littoralis*	Gouinguene et al. (2005)
2-phenylethanol	*Malus* sp.	*Popilla japonica*	Loughrin et al. (1995b)
	Robinia pseudo-acacia	*Tetranychus urticae*	Van Den Boom et al. (2004)
3-hexenyl benzoate	*Malus* sp.	*Panonychus ulmi*	Takabayashi et al. (1991)
	Malus sp.	*Tetranychus urticae*	Takabayashi et al. (1991)
Cresol	*Medicago truncat-ula*	*Tetranychus urticae*	Leitner et al. (2005)
	Phaseolus lunatus	*Tetranychus urticae*	Hopke et al. (1994)
Trimethylbenzene	*Medicago truncat-ula*	*Spodoptera littoralis*	Leitner et al. (2005)
	Medicago truncat-ula	*Tetranychus urticae*	Leitner et al. (2005)

Table 20.1 (continued)

Compound	Plant Species	Herbivore	Reference
3,5 dimethoxytoluene	*Medicago truncatula*	*Spodoptera littoralis*	Leitner et al. (2005)
3,5 dimethylanisole	*Medicago truncatula*	*Tetranychus urticae*	Leitner et al. (2005)
Benzene acetonitrile	*Robinia pseudoacacia*	*Tetranychus urticae*	Van Den Boom et al. (2004)
Benzyl alcohol	*Phaseolus lunatus*	*Tetranychus urticae*	Hopke et al. (1994)
Ethyl benzoate	*Malus* sp.	*Tetranychus urticae*	Takabayashi et al. (1991)
Phenylacetonitrile	*Malus* sp.	*Popilla japonica*	Loughrin et al. (1995)

volatiles. The repellent properties of methyl salicylate toward several aphid species have been observed both in olfactometer and field experiments. In behavioral studies, methyl salicylate repelled the black bean aphid (*Aphis fabae*) and also inhibited its attraction to host-specific cues from broad bean (*Vicia faba*; Hardie et al. 1994). In the field, exogenous application of methyl salicylate delayed the immigration and setting of bird cherry oat aphids (*Rhopalosiphum padi*) on barley plants (Pettersson et al. 1994; Ninkovic et al. 2003).

Another aromatic compound possessing repellent activity is eugenol, a major constituent of cloves and some basil species. Eugenol is highly repellent to the four beetle species *Sitophilus granaries*, *Sitophilus zeamais*, *Tribolium castaneum,* and *Prostephanus truncates* and inhibits the development of *S*. *granaries* and *S. zeamais* eggs, larvae, and pupae inside grain kernels (Obeng-Ofori and Reichmuth 1997). In addition, insecticidal activities have been shown for a broad range of other aromatic compounds such as benzyl benzoate, benzyl salicylate, isoeugenol, methyl eugenol, methyl cinnamate, cinnamaldehyde, safrole, and isosafrole (Ngoh et al. 1998; Bin Jantan et al. 2005). While the emission of these compounds in response to herbivory has yet to be reported in plants, many other aromatic compounds (summarized in Table 20.1) are emitted from herbivore-attacked plants, but their biological activities towards pest and beneficial insects remain to be determined.

20.3.2 Aromatic Volatiles in Plant Indirect Defense

In addition to their direct defenses, herbivore-attacked plants defend themselves indirectly by releasing volatiles that attract predatory or parasitic anthropods, natural enemies of the attacking herbivores. The tritrophic plant-herbivore-predator/ parasitoid interactions, since first suggested in 1980 (Price et al. 1980), are widely

Table 20.2 Volatile aromatics involved in tritrophic interactions. Entries are grouped first by compound, then plant species, pest species, predator/parasitoid species, and publication date

Compound	Plant	Herbivore	Predator/Parasitoid	Reference
Methylsalicylate	*Arabidopsis thaliana*	*Pieris rapae*	*Cotesia rubecula*	Van Poecke et al. (2001)
	Cucumus sativus	*Tetranychus urticae*	*Phytoseiulus persimilis*	Takabayashi et al. (1994)
	Gerbera jamesonii	*Tetranychus urticae*	*Phytoseiulus persimilis*	Gols et al. (1999)
	Glycine max	*Aphis glycines*	*Coccinella septempuntata*	Zhu and Park (2005)
	Lotus japonica	*Tetranychus urticae*	*Phytoseiulus persimilis*	Ozawa et al. (2004)
	Nicotiana attenuata	*Manduca quinquemaculata*	*Geocoris pallens*	Kessler and Baldwin (2001)
	Nicotiana attenuata	*Dicyphus minimus*	*Geocoris pallens*	Kessler and Baldwin (2001, 2004)
	Nicotiana attenuata	*Epitrix hertipennis*	*Geocoris pallens*	Kessler and Baldwin (2001)
	Nicotiana attenuata	*Manduca sexta*	*Geocoris pallens*	Kessler and Baldwin (2001)
	Phaseolus lunatus	*Tetranychus urticae*	*Phytoseiulus persimilis*	Dicke et al. (1990, 1999); Takabayashi and Dicke (1996); De Boer et al. (2004) and De Boer and Dicke (2004a)
	Phaseolus lunatus	*Tetranychus urticae*	*Amblyseius potentillae*	Dicke et al. (1990)
	Solanum lycopersicum	*Tetranychus urticae*	*Phytoseiulus persimilis*	Kant et al. (2004)
Indole	*Gerbera jamesonii*	*Tetranychus urticae*	*Phytoseiulus persimilis*	Gols et al. (1999)
	Phaseolus lunatus	*Tetranychus urticae*	*Phytoseiulus persimilis*	Dicke et al. (1999)
	Zea mays	*Heliothis virescens*	*Cardiochiles nigriceps*	De Moraes et al. (1998)
	Zea mays	*Pseudaletia separata*	*Cotesia kariyai*	Takabayashi et al. (1995)
	Zea mays	*Spodoptera exigua*	*Cotesia marginiventris*	Turlings et al. (1990, 1991, 1993); Hoballah-Fritsche et al. (2002); D'Alessandro et al. (2006) and Ton et al. (2007)
	Zea mays	*Spodoptera exigua*	*Microplitis croceipes*	Turlings et al. (1993)
	Zea mays	*Spodoptera littoralis*	*Cotesia marginiventris*	Ton et al. (2007)

Table 20.2 (continued)

Compound	Plant	Herbivore	Predator/Parasitoid	Reference
2-phenylethanol	*Glycine max*	*Aphis glycines*	*Chrysoperla carnea*	Zhu and Park (2005)
	Glycine max	*Aphis glycines*	*Chrysoperla carnea*	Zhu and Park (2005)
	Solanum tuberosum	*Leptinotarsa decemlineata*	*Perillus bioculatus*	Weissbecker et al. (1999)
2-phenylethyl acetate	*Zea mays*	*Pseudaletia separata*	*Cotesia kariyai*	Takabayashi et al. (1995)
	Zea mays	*Spodoptera littoralis*	*Cotesia marginiventris*	Hoballah-Fritsche et al. (2002)
	Zea mays	*Spodoptera littoralis*	*Cotesia marginiventris*	Ton et al. (2007)
Benzyl acetate	*Zea mays*	*Pseudaletia separata*	*Cotesia kariyai*	Takabayashi et al. (1995)
	Zea mays	*Spodoptera littoralis*	*Cotesia marginiventris*	Hoballah-Fritsche et al. (2002)
Benzyl cyanide	*Phaseolus lunatus*	*Tetranychus urticae*	*Phytoseiulus persimilis*	Dicke et al. (1999)

spread in the plant kingdom. To date, this phenomenon has been reported in more than 23 plant species with a diverse combination of plants, herbivores and natural enemies (Dicke 1999), and aromatic volatiles have been reported in more than half of these examples (Table 20.2). One of the best-studied tritrophic interactions is between lima bean plants (*Phaseolus lunatus*), herbivorous spider mites (*Tetranychus urticae*), and carnivorous mites (*Phytoseiulus persimilis*). Infestation of lima bean plants by spider mites triggers the release of volatiles that attract predatory mites that prey on the pests (Takabayashi and Dicke 1996). One component of the volatile blend released by *T. urticae*-infested lima bean leaves is methyl salicylate, an aromatic volatile commonly detected in the headspace of herbivore-infested plants (Table 20.2; Dicke and van Poecke 2002). Methyl salicylate was not detected from untreated or mechanically damaged lima bean plants, in contrast to some other volatile compounds (Dicke et al. 1990). In addition, a dense spider mite infestation (40/leaf) leads to a larger methyl salicylate emission than infestation by smaller pest populations (10/leaf; De Boer et al. 2004). Thus, the level of methyl salicylate released from infested lima beans appears to be positively correlated with the severity of *T. urticae* attack, allowing predator mites to select the plants hosting the largest numbers of their prey.

Methyl salicylate attracts predatory mites in a dose-dependent manner, but at biologically unrealistic amounts (200 μg) repels the carnivores (De Boer and Dicke 2004a). Predators reared on spider mites infesting cucumber, which emits only a trace amount of methyl salicylate (Takabayashi et al. 1994), chose prey-infested lima bean plants over cucumber when tested in an olfactometer. The addition of exogenous methyl salicylate to the cucumber blend made it attractive to the carnivorous mites (De Boer and Dicke 2004b). Moreover, when this single compound was offered as an alternative odor source in a dual-choice assays, the preference of the predators for a methyl salicylate-containing volatile blend from *T. urticae*-infested lima bean plants was lost suggesting an important role of this volatile in the foraging behavior of natural enemies of herbivorous arthropods (De Boer et al. 2004).

Interestingly, lima bean plants release unique volatile blends upon infestation by different pests, thus allowing natural enemies to avoid unproductive foraging if only non-prey species are present. When lima bean plants were infested by the prey herbivore *T. urticae* or nonprey caterpillar *Spodoptera exigua*, carnivorous mites *P. persimilis* were able to locate their prey by detecting differences in the volatile blends emitted in response to herbivory. Addition of methyl salicylate to the volatile blend released by plants infested with nonprey species eliminated the predatory mites' ability to make this distinction, suggesting a specific role for methyl salicylate in this interaction (De Boer et al. 2004). The opposite situation was found in another legume *Medicago truncatula*, in which spider mite (*T. urticae*) feeding failed to induce methyl salicylate emission whereas *Spodoptera littoralis* herbivory did, highlighting the flexible roles of this ester in plant defense (Leitner et al. 2005).

In addition to *P. lunatus* and *M. truncatula*, at least 12 other plant species show documented release of methyl salicylate upon herbivore damage, although

at different levels (Tables 20.1 and 20.2). Slight induction of methyl salicylate emission following herbivory has been found in cucumber (*Cucumus sativus*) and gerbera (*Gerbera jamesonii*), where the levels of methyl salicylate were low compared to the other emitted volatiles (Takabayashi et al. 1994; Gols et al. 1999). High levels of methyl salicylate were detected relative to 38 total compounds released from *Arabidopsis* leaves infested with cabbage white butterfly larvae (*Pieris rapae*) but not from artificially damaged or undamaged plants (Van Poecke et al. 2001). *Arabidopsis* is the only cruciferous plant to date shown to emit methyl salicylate in response to herbivore attack, with subsequent attraction of the larval parasitoid *Cotesia rubecula*. In dual-choice bioassays, *C. rubecula* prefers volatile blends containing methyl salicylate from infested *Arabidopsis* plants versus mixtures from artificially damaged or undamaged plants (Van Poecke et al. 2001). Interestingly, caterpillar induced methyl salicylate emission was correlated with an increased transcript abundance of *AtPAL1* suggesting that flux through the pathway towards this compound was induced by herbivore infestation (Van Poecke et al. 2001). In *N. attenuata* the level of methyl salicylate was significantly elevated in volatile blends emitted from plants attacked by three prominent pest species present during the field experiments: the caterpillars of *Manduca quinquemaculata* (Lepidoptera, Sphingidae), the leaf bug *Dicyphus minimus* (Heteroptera, Miridae), and the flea beetle *Epitrix hirtipennis* (Coleoptera, Chrysomelidae; Kessler and Baldwin 2001, 2004), thus attracting the generalist predator *Geocoris pallens* (Kessler and Baldwin 2004).

Infestation of soybean (*Glycine max*), tomato, and *Lotus japonicus* with the herbivorous mite *T. urticae* resulted in release of volatile blends which contained methyl salicylate and were more attractive to the predatory mite *P. persimilis* than volatiles from uninfested or artificially damaged plants (Ozawa et al. 2000; De Boer and Dicke 2004a, b; Kant et al. 2004; Van Den Boom et al. 2004). In soybean plants, methyl salicylate is also released after attack by soybean aphid (*Aphis glycines*), the single aphid species known to develop large colonies on soybean in North America. This is the only compound from the headspace of aphid-infested plants shown to elicit a significant gas chromatography-electroantennography (GC-EAG) response in lady beetle *Coccinella septempunctata*, a species known to attack soybean aphids in the field. In field tests, traps baited with methyl salicylate were highly attractive to *C. septempunctata*, but not to the other common lady beetle *Harmonia axyridis*, suggesting that the former may use this volatile ester as an olfactory cue for prey location (Zhu and Park 2005).

Infestation of cowpea (*Vigna unguiculata*) and hops (*Humulus lupulus*) with *T. urticae* also led to either a large increase or a novel release of methyl salicylate while in other species including black locust (*Robinia pseudo-acacia*), sweet pepper (*Capsicum annuum*), thorn apple (*Datura stramonium*), and grapevine (*Vitis vinifera*) its emission was induced to a lesser extent (Van Den Boom et al. 2004). When attractiveness of methyl salicylate was tested in field experiments, it was seen that the grape- and hop-yards containing sticky cards baited with this volatile ester captured significantly greater numbers of predatory insects including *Chrysopa nigricornis*, *Hemerobius* sp., *Deraeocoris brevis*, *Stethorus punctum picipes*, and

Orius tristicolor than the yards with unbaited sticky cards (James and Price 2004). Moreover, this increase of predatory insects coincided with a dramatic reduction in the population of spider mites, the major arthropod pest of hops (James and Price 2004).

Taken together, these results show that methyl salicylate can be a general indicator of herbivore damage in the context of the other volatiles also emitted following infestation. Surprisingly, in many of the tritrophic interactions described above, the herbivore-predator participants are the same while the attacked plants are different and they emit different volatile blends with the exception of methyl salicylate. Analysis of chemoreceptors from parasitoid wasps and predatory mites demonstrated that insects are able to detect this compound within the volatile blend (De Bruyne et al. 1991; Van Poecke and Dicke 2002). However, methyl salicylate is not emitted by all plant taxa and moreover, its emission decreases in maize leaves after infestation by *Pseudaletia separata* larvae (Takabayashi et al. 1995), suggesting niche roles for methyl salicylate in select tritrophic interactions.

Indole is another aromatic compound found in the volatile blends of some herbivore-damaged plants. Although the role of indole in tritrophic interactions lacks extensive study, induced indole emission subsequent to herbivory is well established and has been reported from many plant species including maize (Frey et al. 2000), cotton (*Gossypium birsutum*; Turlings et al. 1995; Paré and Tumlinson 1997), gerbera (Gols et al. 1999), lima bean (Mithöfer et al. 2005), and peanut (Cardoza et al. 2002). In maize, indole becomes a major constituent of the volatile spectrum released a few hours after feeding by the beet armyworm (*S. exigua*; Turlings et al. 1990). Its biosynthesis occurs de novo in response to insect damage (Paré and Tumlinson 1998) and positively correlates with the level of infestation (Schmelz et al. 2003). The level of indole emission is also affected by the developmental stage of the infesting herbivores, as was shown during infestation by *P. separata* larvae (Takabayashi et al. 1995), suggesting that it is more advantageous for the plant to attract parasitoids while the pest larvae are still small. Interestingly, a broad genetic variability was found with respect to herbivore-induced indole emission among 31 maize inbred lines ranging from trace to >70% of total volatile emissions (Degen et al. 2004).

The most prominent and well-studied tritrophic systems involving indole consist of maize plants, armyworms, and parasitic wasps of the genus *Cotesia*. The *Cotesia* sub-species *kariyai* (Takabayashi et al. 1995) and *marginiventris* (Turlings et al. 1991) are known to visit maize plants emitting indole in response to damage by *P. separata* larvae and *S. exigua* larvae, respectively. However, the emissions of indole and other herbivore-induced volatile compounds are not always pest-species specific. In maize, regurgitates from five different caterpillar species as well as grasshoppers induce the same set and relative ratio of volatile compounds including indole, but at different total amounts, indicating that these highly detectable cues may not always reliably signal the presence of a suitable host to foraging predators and parasitoids (Turlings et al. 1993). Surprisingly, attraction of *C. marginiventris* was not affected by the presence or absence of indole in studies where caterpillar-infested maize plants and those treated with glyphosate, an

inhibitor of the 5-enolpyruvylshikimate-3-phosphate, were studied together. Furthermore, indole seemed to repel rather than attract the parasitoid *Microplitis rufiventris* (D'Alessandro et al. 2006) suggesting that two parasitoids with a comparable biology may employ different strategies in their use of plant-provided cues to locate hosts and indole might play different roles in inducible defenses across plant and insect taxa.

Coincidence of indole emissions and *C. marginiventris* parasitoid activity has also been documented in cotton plants following damage by *S. littoralis* (Paré and Tumlinson 1997) or *S. exigua* (Röse et al. 1996; Loughrin et al. 1994, 1995a). Similar to the situation in maize, indole emissions following herbivory have been shown to differ greatly between cotton cultivars (Loughrin et al. 1995a). However, in contrast to maize, release of indole from cotton plants occurs only at the site of caterpillar damage and not systemically (Röse et al. 1996; Turlings and Tumlinson 1992). Interestingly, simultaneous infestation of cotton plants by *S. exigua* and the whitefly *Bemisia tabaci* led to a reduction of indole emission possibly weakening the attraction strengths between parasitoids and their hosts (Rodriguez-Saona et al. 2003).

When two types of attacking herbivores infested lima bean plants, only feeding by *S. exigua* induced indole emission but not *T. urticae* (De Boer et al. 2004). Even a high-density infestation by spider mites led to only low indole emission whereas feeding by moderate numbers of *S. exigua* larvae (two/leaf) resulted in drastic increases of indole release compared to control and spider mite test groups (De Boer et al. 2004). Neither artificial mechanical damage nor damage done by the snail *Cepaea hortensis* caused a release of indole (Mithöfer et al. 2005). Interestingly, only the jasmonic acid-related signaling pathway is involved in the production of caterpillar-induced volatiles in lima bean plants, while both the salicylic acid- and jasmonic acid-related signaling pathways are involved in the production of *T. urticae*-induced volatiles (Ozawa et al. 2000).

The aromatic volatiles benzylacetate, 2-phenethyl acetate, benzyl cyanide, and methyl anthranilate were also released following herbivore attack (Table 20.2). These compounds were emitted from maize after armyworm infestation with the exception of benzylcyanide and 2-phenylethanol, which were detected in lima beans and potato plants following spider mite and potato beetle damage, respectively (Takabayashi et al. 1995; Bernasconi et al. 1998; Hoballah-Fritsche et al. 2002; D'Alessandro and Turlings 2005; Dicke et al. 1999; Weissbecker et al. 1999). Moreover, it has been shown that 2-phenylethanol, emitted exclusively following potato beetle feeding, elicits very high GC-EAG responses from *Perillus bioculatus*, a predator of potato beetle (Weissbecker et al. 1999).

Release after herbivory of a majority of aromatic compounds at relatively low levels raises the question about their roles in the interactions between plants and arthropods. GC-EAG recordings show that the relative quantities of compounds within a volatile blend do not always correlate with their elicited EAG responses, indicating that minor compounds may provoke some of the strongest responses (Gouinguene et al. 2005; Zhu and Park 2005) and the key insect attractants remain to be determined.

20.4 Aromatic Volatiles and Plant–Plant Interactions

Volatiles released from herbivore-infested plants also mediate plant–plant interactions and may induce expression of defense genes and emission of volatiles in healthy leaves of the same plant or on neighboring, unattacked plants, thus increasing their attractiveness to carnivores and decreasing their susceptibility to the damaging herbivores (Dicke et al. 1990; Arimura et al. 2002, 2004; Ruther and Kleier 2005). Exposure of maize to the volatile blend emitted from *S. littoralis*-infested conspecifics reduces caterpillar feeding and development while significantly increasing attractiveness of the volatile-exposed plants to parasitic *C. marginiventris* wasps, thus boosting both their direct and indirect defenses (Ton et al. 2007). This effect could partly be due to the enhanced production of the aromatic compounds indole and 2-phenethyl acetate in plants following their exposure to caterpillar-induced volatiles. Also, neighboring lima bean plants became more attractive to predatory mites and less susceptible to spider mites when exposed to volatiles emitted from conspecific leaves infested with *T. urticae* but not volatiles emitted by artificially wounded leaves (Arimura et al. 2000). *T. urticae*-infestation induced transcript accumulation of six defense-related genes in lima bean leaves, while exposure of undamaged plants to volatiles from infested plants upregulated transcript levels of only five out of six genes investigated including *PAL*. Treatment of undamaged plants with gaseous methyl salicylate reproduced the expression patterns of six defense genes observed in the *T. urticae*-infested leaves, indicating the potential importance of this compound in plant–plant interactions (Arimura et al. 2000).

In addition to direct elicitation of defenses, exposure to volatile compounds from attacked plants may lead to priming of plant defensive responses in their neighbors. Priming by volatiles prepares the plant to respond more rapidly and intensely against subsequent attack by herbivorous insects (Engelberth et al. 2004; Kessler et al. 2006; Tumlinson and Engelberth this volume). Priming by volatile compounds provides a different way of responding to the threat of insect herbivory via the incomplete activation of defense-related processes, thus reducing biochemical investment towards defenses for the receiver plants until the onset of actual attack (Engelberth et al. 2004; Kessler et al. 2006). While priming by aromatic volatile compounds could be one of the mechanisms involved in plant–plant signaling in nature, the roles of the aromatic volatiles in and the underlying molecular mechanisms and ecological relevance of these particular interactions still remain to be determined.

20.5 Future Directions

To date, it is widely accepted that airborne volatile compounds play important roles in plant defense, however, the contribution of aromatics to these processes still requires further investigation. The most interesting unsolved question is why aromatic volatiles lack the broad representation seen with terpenoids in herbivore-induced volatile blends. The ability of plants to synthesize a wide spectrum of volatile aromatic compounds, often at high levels, has been shown in floral organs

(Verdonk et al. 2003; Boatright et al. 2004). However, in the limited number of plant species studied, aromatic volatile compounds are comparatively underrepresented in plant defense. This discrepancy will be clarified through investigation of plant-insect interactions occurring in a greater number of plants. Emission of lower levels of aromatic compounds may also indicate that their actions are synergistic and may incorporate a level of specificity to the airborne signal, possibilities requiring further exploration at the sensory levels and in field experiments.

The importance of aromatic volatiles should not be overlooked based on their subtle presences since insects have an astonishing ability to detect low levels of volatile compounds that may provoke some of the strongest responses. Previous attempts to determine the effects of individual aromatic volatiles on insect behavior were typically accomplished by the addition of exogenous compounds to existing odor mixtures. Recent breakthroughs in gene discovery and metabolic engineering now make it possible to manipulate the levels of specific volatiles *in planta*, either omitting or increasing their emission. Although the contributions of some volatile terpenoids to plant defense have been explored via this method (Aharoni et al. 2003; Kappers et al. 2005; Schnee et al. 2006), it has yet to be applied to volatile aromatics and will likely corroborate and clarify their previously conceived roles in plant-insect interactions. Of particular interest is how flux through the shikimate pathway, which provides precursors to aromatic volatiles, is regulated during plant defense with the goal of determining whether higher emission of aromatics would be beneficial for plant fitness and defense. Recent discovery of the MYB transcription factor AtMYB15, which activates the shikimate pathway in response to wounding (Chen et al. 2006), will enable us to solve this question via metabolic engineering.

Another important unsolved question concerns the factors determining specificity of plant response to a particular threat. An understanding of signal transduction pathways that lead to the herbivore-induced plant defenses will shed some light on possible mechanisms for the exclusive release of certain compounds in response to given stimuli. In addition, the use of -omics-based approaches applied to both plants and pests will be useful in elucidating general mechanisms involved in plant-insect and plant-plant interaction. Understanding of the mechanisms underlying plant-insect interaction and in particular the role of volatiles in biological control of pests will allow us in the future to supplement and optimize plant volatile signaling and generate crops with enhanced attractiveness to natural enemies during herbivory, providing a reduced need for chemical pesticide application in agriculture.

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