Summary
Plants synthesize an amazing diversity of volatile organic compounds (VOCs) that facilitate interactions with their environment, from attracting pollinators and seed dispersers to protecting themselves from pathogens, parasites and herbivores. Recent progress in -omics technologies resulted in the isolation of genes encoding enzymes responsible for the biosynthesis of many volatiles and contributed to our understanding of regulatory mechanisms involved in VOC formation. In this review, we largely focus on the biosynthesis and regulation of plant volatiles, the involvement of floral volatiles in plant reproduction as well as their contribution to plant biodiversity and applications in agriculture via crop–pollinator interactions. In addition, metabolic engineering approaches for both the improvement of plant defense and pollinator attraction are discussed in light of methodological constraints and ecological complications that limit the transition of crops with modified volatile profiles from research laboratories to real-world implementation.
phenylpropanoids/benzenoids, fatty acid derivatives and amino acid derivatives in addition to a few species-/genus-specific compounds not represented in those major classes.

1. Biosynthesis of terpenoids

Terpenoids constitute the largest and most diverse class of secondary metabolites with many volatile constituents, which are derived from two common five-carbon precursors, isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl diphosphate (DMAPP) (McGarvey & Croteau, 1995). In plants, two independent, compartmentally separated pathways – the mevalonic acid (MVA) and methylerythritol phosphate (MEP) – are responsible for the formation of these C5-isoprene building units (Fig. 2). The MVA pathway gives rise to volatile sesquiterpenes (C15), while the MEP pathway provides precursors to volatile hemiterpenes (C5), monoterpenes (C10) and diterpenes (C20). The MEP pathway is considered to be exclusively plastidic, as a full set of the corresponding enzymes exists only in plastids based on experimental evidence and predictions of their subcellular localization (Hsieh et al., 2008). By contrast, subcellular localization of the MVA pathway is not as clear. Historically, this pathway was referred to as being cytosolic; however, new evidence suggests that the MVA pathway is distributed between the cytosol, endoplasmic reticulum and peroxisomes (Simkin et al., 2011; Pulido et al., 2012).

The MVA pathway consists of six enzymatic reactions and is initiated by a stepwise condensation of three molecules of acetyl-CoA to 3-hydroxy-3-methylglutaryl-CoA, which undergoes reduction to MVA followed by two subsequent phosphorylations and a decarboxylation/elimination step with formation of IPP as the final product (Fig. 2) (Lange et al., 2000). To date, it is still unclear which subcellular pool of acetyl-CoA is used for terpenoid biosynthesis, as acetyl-CoA cannot readily cross membranes, and pools exist in chloroplasts, peroxisomes, mitochondria, cytosol and nucleus (Oliver et al., 2009). The Arabidopsis genome contains two genes encoding acetoacetyl-CoA thiolase (AACT), one of which (AACT2) catalyzes the first step in the MVA pathway (Ahumada et al., 2008) and, based on proteome analysis, is localized in peroxisomes (Reumann et al., 2007). The MEP pathway involves seven enzymatic steps and begins with the condensation of d-glyceraldehyde 3-phosphate (GAP) and pyruvate (Pyr) to produce 1-deoxy-d-xylulose 5-phosphate, which is then subjected to isomerization/reduction with formation of the pathway’s characteristic intermediate, MEP (Fig. 2). Five consecutive steps are required to convert MEP to IPP and DMAPP. The MEP pathway relies on primary metabolism for the supply of Pyr and GAP, with the latter derived from both glycolysis and the pentose phosphate pathway (PPP). To date, the origin of Pyr in the chloroplasts is not fully understood, as plastids have low activities of the key glycolytic enzymes, phosphoglycerate mutase and enolase (Andriotis et al., 2010; Joyard et al., 2010; Bayer et al., 2011), and might not be able to sustain high Pyr demand for isoprenoid biosynthesis. Indeed, plastid-localized IPP biosynthesis was affected in Arabidopsis thaliana mutants lacking a Pyr transporter, which supplies cytosolic Pyr to the MEP pathway (Furumoto et al., 2011).

Both IPP and DMAPP are substrates for short-chain prenyltransferases, which produce prenyl diphosphate precursors, geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP), for a large family of terpene synthases/cyclases (TPSs) (Fig. 2) (Cane, 1999; Wise & Croteau, 1999). While the MVA pathway produces only IPP, the MEP pathway results in the synthesis of both IPP and DMAPP at a 6:1 ratio (Rohdich et al., 2003). Thus, both pathways rely on...
isopentenyl diphosphate isomerase (IDI), which reversibly converts IPP to DMAPP (Nakamura et al., 2001) and controls the equilibrium between them. Recently, in *Catharanthus roseus* and *Arabidopsis thaliana* genomes, a single and two distinct IDI genes were identified, respectively, which are transcribed as splice variants. In both plants, the ‘long’ proteins are transported into mitochondria and/or chloroplasts whereas the ‘short’ proteins, lacking the targeting signal, are located in the peroxisomes (Guirimand et al., 2012), highlighting the involvement of different subcellular compartments in isoprenoid biosynthesis in plants.

IPP, DMAPP, and short prenyl diphosphates (GPP and FPP) facilitate the metabolic crosstalk between the compartmentally separated MVA and MEP pathways by acting as connecting metabolites (Nabeta et al., 1997; Adam et al., 1999; Hemmerlin et al., 2003; Wu et al., 2006; Orlova et al., 2009). Trafficking of these compounds across the inner envelope membrane of plastids is mediated by an unidentified metabolite transporter (Bick & Lange, 2003; Flügge & Gao, 2005). Such connectivity of the isoprenoid biosynthetic pathways allows the MEP pathway, often with a higher carbon flux than the MVA route, to support biosynthesis of terpenoids in the cytosol (Laule et al., 2003; Dudareva et al., 2005; Ward et al., 2011). The contribution of these pathways to terpenoid biosynthesis is species- and/or organ-specific. While in snapdragon flowers, for example, the MEP pathway provides precursors for cytosolic sesquiterpene formation (Dudareva et al., 2005), in carrot leaves and roots, sesquiterpenes are derived from both MEP and MVA pathways (Hampel et al., 2005).

Despite the presence of IPP and DMAPP in several compartments, biosynthesis of prenyl diphosphate intermediates is compartment-specific and depends on subcellular localization of corresponding short-chain prenyltransferases. In the cytosol, the sequential head-to-tail condensation of two IPP molecules with one molecule of DMAPP catalyzed by FPP synthase gives rise to FPP, the precursor of volatile sesquiterpenes. In plastids GPP and GGPP synthases are responsible for the head-to-tail condensation of one DMAPP molecule with one or three IPP molecules to form GPP and GGPP, respectively, the corresponding precursors of mono- and diterpenes.
The tremendous diversity of volatile terpenoids in plants is generated through the action of TPSs (Fig. 2), many of which have the distinctive ability to synthesize multiple products from a single prenyl diphosphate substrate (Degenhardt et al., 2009b). Indeed, in Arabidopsis two sesquiterpene synthases, TPS21 and TPS11, account for the biosynthesis of nearly all 20 sesquiterpenes found in the floral volatile blend (Tholl et al., 2005). In addition, many TPSs accept more than one substrate (Tholl, 2006; Bleeker et al., 2011), which expands the diversity of produced terpenoids by directing bifunctional enzymes to different compartments with a varying range of available substrates (Aharoni et al., 2004; Nagegowda et al., 2008; Huang et al., 2012; Gutensohn et al., 2013). Moreover, it has recently been shown that tomato monoterpene and sesquiterpene synthases can use prenyl diphosphates with a cis configuration, such as neryl diphosphate (NDP) and Z,Z-FPP, respectively, instead of the usual GPP and E,E-FPP, to form new products (Sallaud et al., 2009; Schilmiller et al., 2009; Bleeker et al., 2011). However, it still remains to be determined if this is a general property of TPSs.

To date, the TPS gene family consists of >100 members characterized from many plant species, with about one-third isolated from flowers and fruits. This gene family has been divided into seven subfamilies (designated TPS-a through TPS-g) based on sequence relatedness, functional assessment, and gene architecture (Bohlmann et al., 1998; Aubourg et al., 2002). Interestingly, TPSs from related plant species tend to cluster together more than enzymes of similar function, thus challenging substrate/product predictions based on sequence similarities (Bohlmann et al., 1998).

In addition to a wide range of volatile terpenoids formed directly by TPSs, terpenoid diversity is further increased by other enzymes that are capable of modifying the TPS products via hydroxylation, dehydrogenation, acylation, or other reactions, thus increasing their volatility and altering their olfactory properties (Dudareva et al., 2004). Plants also produce irregular volatile terpenoids with carbon skeletons ranging from C8 to C18, which originate from carotenoids via three step modifications, including an initial dioxygenase cleavage followed by enzymatic transformation and acid-catalyzed conversion to volatile compounds (Fig. 2) (Winterhalter & Rouseff, 2001). In some cases, including Arabidopsis, tomato, petunia and melon, the dioxygenase cleavage step itself can yield a volatile product, such as α- and β-ionone, geranylacetone, and pseudioionone, from an array of carotenoid pigments (Simkin et al., 2004; Ibda et al., 2006). The irregular acyclic C11- and C16-homoterpenes, 4,8-dimethylnona-1,3,7-triene (DMNT) and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), are derived from FPP and GGPP, respectively (Fig. 2). Their biosynthesis proceeds via two enzymatic steps, the formation of tertiary C15- and C20-alcohol precursors, (E)-nerolidol and (E,E)-geranylnalool, respectively, followed by an oxidative degradation catalyzed by a cytochrome P450 monoxygenase (Tholl et al., 2011).

2. Biosynthesis of phenylpropanoid/benzenoid compounds

The second largest class of plant VOCs comprises phenylpropanoid and benzenoid compounds (Knudsen et al., 2006), which originate from the aromatic amino acid phenylalanine (Phe) (Fig. 3). Seven enzymatic reactions of the shikimate pathway and three of the arogenate pathway connect central carbon metabolism to Phe (Tzin & Galili, 2010; Maeda & Dudareva, 2012). The immediate precursors of the shikimate pathway, phosphoenolpyruvate (PEP) and d-erythrose 4-phosphate (E4P), derive from glycolysis and the PPP, respectively. The same pathways provide precursors for the MEP pathway, and thus the latter has to compete for carbon allocation with the shikimate/phenylpropanoid pathway, especially as 30% of photosynthetically fixed carbon is directed to Phe, largely to make lignin (Razal et al., 1996). The first gene in the shikimate pathway, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHP synthase), plays a key role in controlling carbon flux into the pathway (Tzin et al., 2012). However, the molecular mechanisms involved in this regulation remain largely unknown in plants (Maeda & Dudareva, 2012).

While Phe biosynthesis takes place in plastids (Maeda & Dudareva, 2012), its further conversion to volatile compounds occurs outside this organelle. The first committed step in the biosynthesis of the majority of phenylpropanoids/benzenoids is catalyzed by a well-known and widely distributed enzyme, l-phenylalanine ammonia lyase (PAL), which deaminates Phe to trans-cinnamic acid (CA) (Fig. 3). Formation of the benzenoids (C6-C9) from cinnamic acid requires shortening of the propyl side chain by two carbons and has been shown to proceed via a β-oxidative pathway, a non-β-oxidative pathway, or a combination of these pathways (Fig. 3) (Boaright et al., 2004; Orlova et al., 2006). The β-oxidative route was only recently fully elucidated and appears to be analogous to that operating in catabolism of fatty acids and certain branched-chain amino acids. This pathway begins with activation of CA to its CoA thioester, which undergoes hydration, oxidation and cleavage of the β-keto thioester, resulting in the formation of benzyol-CoA (Van Moerkercke et al., 2009; Klemplien et al., 2012; Qualley et al., 2012). The peroxisomal localization of the β-oxidative pathway raises the question about the export of benzyol-CoA to the cytosol for benzylbenzoate and phenylethylbenzoate biosynthesis.

The alternative non-β-oxidative pathway proceeds via benzoyledehyde as a key intermediate followed by its oxidation to benzoic acid (Fig. 3). While NAD+-dependent benzoyledehyde dehydrogenase converting benzoyledehyde to benzoic acid was isolated and characterized (Long et al., 2009), biochemical steps leading to benzoyledehyde formation are still in question. During the last decade, significant progress has been made in the discovery of enzymes and genes involved in the final steps of benzenoid volatile formation. Two enzyme superfamilies, BAHD superfamily of acyltransferases (D’Auria, 2006) and the SABATH family of methyltransferases (D’Auria et al., 2003), were found to greatly contribute to the final biosynthetic steps of volatile benzenoids.

The volatile phenylpropanoids (C6-C9), such as eugenol, isoeugenol, methylbenzene, isomethylbenzene, chavicol, and methylchavicol, share the initial biosynthetic steps with the lignin biochemical pathway up to the phenylpropanol (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). Coniferyl alcohol is first converted to coniferyl acetate by coniferyl alcohol acetyltransferase (Dexter et al., 2007) before its reduction
to eugenol and isoeugenol by eugenol synthase or isoeugenol synthase, respectively (Fig. 3) (Koeduka et al., 2006, 2008). Similar to the role of coniferyl acetate in eugenol and isoeugenol formation, coumaryl acetate serves as the biosynthetic precursor of chavicol in basil (Vassao et al., 2006). Often, eugenol, isoeugenol, and chavicol undergo further methylation and require O-methyltransferases for the downstream production of methyl eugenol, isomethyl eugenol and methyl chavicol (Gang et al., 2002).

In contrast to benzenoids and phenylpropanoids, the biosynthesis of volatile phenylpropanoid-related (C6-C2) compounds, such as phenylacetaldehyde and 2-phenylethanol, does not occur via CA and competes with PAL for Phe utilization (Fig. 3) (Kaminaga et al., 2006; Tieman et al., 2007). In petunia and rose petals, phenylacetaldehyde is produced directly from Phe via an unusual combined decarboxylation-amine oxidation reaction catalyzed by phenylacetaldehyde synthase (Kaminaga et al., 2006; Farhi et al., 2010), while in tomato its biosynthesis occurs via two separate steps. Phe is first converted to phenylethylamine by an aromatic amino acid decarboxylase and requires the action of a hypothesized amine oxidase, dehydrogenase, or transaminase for phenylacetaldehyde formation (Tieman et al., 2006). Recently, the third enzymatic route was discovered in melon fruit (Cucumis melo L.), in which Phe is first transaminated to its corresponding \( \alpha \)-ketoacid, phenylpyruvate, followed by subsequent decarboxylation to phenylacetaldehyde (Gonda et al., 2010).

3. Biosynthesis of volatile fatty acid derivatives

Another class of plant VOCs comprises fatty acid derivatives such as 1-hexanal, \( \text{cis}-3 \)-hexenol, nonanal and methyl jasmonate, which...
arise from C18 unsaturated fatty acids, linoleic or linolenic (Fig. 4). Biosynthesis of these fatty acids relies on a plastidic pool of acetyl-CoA generated from Pyr, the final product of glycolysis. After entering the ‘lipoxygenase (LOX) pathway’, unsaturated fatty acids undergo stereospecific oxygenation to form 9-hydroperoxy and 13-hydroperoxy intermediates (Fig. 4) (Feussner & Wasternack, 2002), which are further metabolized via the two branches of the LOX pathway yielding volatile compounds. The allene oxide synthase branch uses only the 13-hydroperoxy intermediate and leads to the formation of jasmonic acid (JA), which in turn is converted to methyl jasmonate by JA carboxyl methyl transferase (Song et al., 2005). By contrast, the hydroperoxide lyase branch converts both types of hydroperoxide fatty acid derivatives into C6 and C9 aldehydes, which are often reduced to alcohols by alcohol dehydrogenases (Gigot et al., 2010), followed by further conversion to their esters (Fig. 4) (D’Auria et al., 2007). These saturated and unsaturated C6/C9 aldehydes and alcohols commonly referred to as green leaf volatiles, are usually synthesized in green organs of plants in response to wounding, but also provide fruits and vegetables with their characteristic ‘fresh green’ aroma.

4. Biosynthesis of volatiles derived from branched-chain amino acids

Numerous volatile compounds, especially those highly abundant in floral scents and fruit aromas, are derived from amino acids such as alanine, valine, leucine, isoleucine, and methionine, or intermediates in their biosynthesis, and contain nitrogen and sulfur (Fig. 5) (Knudsen et al., 2006). The biosynthesis of these amino acid-derived volatiles in plants is believed to proceed in a similar way to that found in bacteria or yeast (Dickinson et al., 2000; Beck et al., 2002; Tavaria et al., 2002), where these pathways have been studied more extensively. As in microorganisms, the amino acids undergo an initial deamination or transamination catalyzed by aminotransferases, leading to the formation of the corresponding α-ketoacid (Fig. 5) (Gonda et al., 2010). These α-ketoacids can be further subjected to decarboxylation, followed by reductions, oxidations and/or esterifications, forming aldehydes, acids, alcohols and esters (Fig. 5) (Reineccius, 2006). Amino acids can also be the precursors of acyl-CoAs, which are used in alcohol esterification reactions catalyzed by alcohol acyltransferases (AATs) (Beekwilder et al., 2004; Gonzalez et al., 2009).

Fig. 4 Synthesis of fatty acid-derived volatile organic compounds (VOCs) and green leaf volatiles (GLVs). Linoleic and linolenic acid serve as the precursors for a variety of fatty acid-derived VOCs. These precursors enter the lipoxygenase (LOX) pathway by an oxidation yielding 9-hydroperoxy and 13-hydroperoxy intermediates that are further converted to volatiles by hydroperoxide lyases and alcohol dehydrogenases. Stacked arrows illustrate the involvement of multiple enzymatic reactions. Volatile compounds are highlighted with a colored background, with green leaf volatiles shown in green. Abbreviations: AAT, alcohol acyltransferase; ADH, alcohol dehydrogenase; AOC, allene oxide cyclase; AOS, allene oxide synthase; 9-HPL, 9-hydroperoxide lyase; 13-HPL, 13-hydroperoxide lyase; ISO, isomerase; 9-LOX, 9-lipoxygenase; 13-LOX, 13-lipoxygenase; OPR, 12-oxophytodienoate reductase.
II. Regulation of volatile emission in plants

In contrast to the phenylpropanoids/benzenoids, even less is known about transcriptional regulation of terpenoid biosynthesis in plants. MYC2, a basic helix–loop–helix TF, was shown to activate expression of sesquiterpene synthase genes \( TPS21 \) and \( TPS11 \) in Arabidopsis inflorescence by integrating both gibberellic acid (GA) and JA signals into transcriptional regulation of volatile sesquiterpene production (Hong et al., 2012). MYB14 is the first MYB TF directly linked to the control of isoprenoid pathways. It was proposed that MYB14 contributes to the regulation of the volatile terpenoid biosynthesis preferentially via the MVA pathway, as well as JA metabolism during plant defense responses in conifer trees (Bedon et al., 2010). Despite the identification of several TFs for individual pathways, master regulators, which orchestrate formation of diverse volatile blends and act upstream of multiple metabolic pathways, are yet to be discovered.

Transcriptional regulation plays an important role in controlling VOC biosynthesis, but it is not the only mechanism involved. While little is known about post-transcriptional regulation of VOC formation, recent comprehensive reviews provide a thorough discussion of the regulation of the shikimate pathway (Maeda & Dudareva, 2012), as well as the MVA and MEP pathways (Hemmerlin et al., 2012).

The rate of biosynthesis of any particular VOC is not only limited by the activity of enzymes responsible for the final step of its formation, but is rather controlled by the amount of available substrate, especially for enzymes with broad substrate specificities (e.g. some carboxyl methyltransferases and acyltransferases, carotenoid cleavage dioxygenase 1, short-chain dehydrogenase/
reductases) (Effmert et al., 2005; Guterman et al., 2006; Vogel et al., 2008). Precursor availability was also shown to play an important role in the regulation of rhythmic emission of VOCs (Kolosova et al., 2001; Colquhoun et al., 2010; Maeda et al., 2010), as plants often release volatiles with distinct diurnal or nocturnal patterns (Lerdau & Gray, 2003; Martin et al., 2003; van Doorn & Woltering, 2008). However, the actual molecular mechanisms responsible for rhythmic emission of VOCs, frequently controlled by circadian clock (Kolosova et al., 2001; Lu et al., 2002), remain unknown.

Environmental factors such as light intensity, atmospheric CO₂ concentration, temperature, relative humidity, and nutrient status can greatly influence VOC emission (Staudt & Bertin, 1998; Gouinguene & Turlings, 2002). Additionally, successful pollination/fertilization triggers a decrease in overall scent emission mediated by the phytohormone ethylene (Negre et al., 2003; Underwood et al., 2005) and ethylene down-regulates scent biosynthetic gene expression before floral senescence as well (Colquhoun et al., 2010). Signal transduction cascades involved in defense-inducible emission are currently under extensive investigation (summarized in Arimura et al., 2005; Dudareva et al., 2006; Zebelo & Maffei, 2012; Zebelo et al., 2012); however, signaling mechanisms regulating fluctuations in VOCs in response to environmental or physiological factors still await further investigation.

III. Functions of plant volatile organic compounds

Plant VOCs define the chemical landscape of numerous ecosystems wherein they mediate intra- and interspecific interactions. They are notably involved in the attraction of pollinators (Raguso, 2008) and seed dispersers, above- and below-ground defense against herbivores (Degenhardt et al., 2009a; Unsicker et al., 2009; Ali et al., 2012; Hiltpold & Turlings, 2012), protection against pathogens (Huang et al., 2012), and plant–plant signaling (Baldwin et al., 2006). In addition, they protect plants against abiotic stresses such as high light, temperature or oxidative stress (Dudareva et al., 2006; Vickers et al., 2009). The defensive roles of vegetative VOCs are exhaustively discussed in most contemporary reviews (e.g. Unsicker et al., 2009; Mumm & Dicke, 2010; Hare, 2011), and thus here we will focus mainly on functions of floral volatiles in natural ecosystems and their application in agriculture, which are vastly understood in comparison to their vegetative counterparts. It should be noted that the contribution of VOCs to plant defense and reproduction is species-specific and, in the case of floral volatiles, depends on plant mating system and self-compatibility.

1. Involvement of floral VOCs in plant reproduction

Of all plant organs, flowers generally emit the highest amounts and most diverse blends of VOCs (Knudsen et al., 2006), which function as olfactory cues for the attraction of insect (Dobson, 1994) and bat (van Helversen et al., 2000) pollinators to ensure plant reproductive success. Until recently, the involvement of VOCs in ornithophily remained unclear, as many bird-pollinated flowers emit negligible amounts of VOCs (Knudsen et al., 2004) and birds likely possess a reduced sense of smell (Bang & Cobb, 1968). However, the exposure of hummingbirds to different volatiles using artificial nectars or transgenic tobacco flowers revealed behaviors reflecting deterrent or attracting effects of scent compounds (Kessler & Baldwin, 2007; Kessler et al., 2008). VOCs act both as long- and short-distance attractants (Dobson, 1994) and provide pollinators with fine-scale spatial information about landing and food opportunities. This fine-scale spatial information relies on tissue-specific and developmentally regulated scent production and emission within the flowers (Dotterl & Jurgens, 2005; Kessler & Baldwin, 2007).

Floral volatile profiles are species-specific and tend to reflect the type of pollinator (Dobson, 2006). For example, plant species pollinated by moths emit high amounts of benzenoids and, to a lesser extent, terpenoids and nitrogen-containing compounds (Dobson, 2006), while bat-pollinated flowers predominantly release sulfur-containing volatiles (von Helversen et al., 2000). Moreover, the emission of VOCs often correlates with the foraging activity of associated pollinators and displays diurnal or nocturnal patterns (Kolosova et al., 2001). Pollinators, on the other hand, are capable of discriminating between complex multicomponent volatile profiles with unique ratios and intensities of compounds (Wright et al., 2005) and therefore develop preferences towards specific profiles (Ashman, 2000). These preferences modulate foraging behavior and promote flower constancy (i.e. the tendency to repeatedly visit flowers with a specific volatile profile), thereby increasing pollen transfer to conspecific plants and preventing stigma clogging with heterospecific pollen (Gruter & Ratnieks, 2011).

Volatile organic compounds emitted from flowers have roles beyond pollinator attraction. Many flower VOCs have antimicrobial or antifungal activities (Hammer et al., 2003; Huang et al., 2012), or function as deterrents against florivores, thus protecting valuable reproductive plant organs (Junker et al., 2011). Moreover, VOCs emitted from pollinated flowers can also repel pollinators and direct them to as yet unpollinated counterparts (Schiestl & Ayasse, 2001). Interestingly, the same VOCs that lure pollinators sometimes serve as signals for floral antagonists. Silene latifolia flower VOCs, for example, attract the nursery pollinator Hadena bicurvis, the larvae of which feeds on and destroys approximately one-quarter of developing seeds, causing a detrimental effect on plant fitness (Wolfe, 2002; Dotterl et al., 2006). In this plant species, a drastic reduction in emission of the key volatile advertising host location occurs after pollination to minimize further parasitism (Dotterl et al., 2005; Muhlemann et al., 2006). In contrast to S. latifolia, where pollinators also act as antagonists, the pollinators and florivores visiting Cirium arvense flowers belong to different insect species (Theis, 2006). In the case of C. arvense, diel emission of VOCs correlates with activity of pollinators and is low when florivores are active (Theis et al., 2007). Petunia flowers face the same defense/apparency dilemma as S. latifolia and C. arvense. To resolve the function of single volatile compounds in deterrence and attraction, five transgenic Petunia hybrida RNAi lines silencing individual scent biosynthetic genes were used in field and glasshouse experiments (Kessler et al., 2012).
Rather than reducing scent emission to avoid florivory, petunia flowers specifically emit deterrent compounds such as isoeugenol and benzyl benzoate. Thus, the scent emission profile in the described species is likely defined by opposite selective pressures arising from the necessity to maximize pollinator attraction while avoiding damage by florivores (Muhlemann et al., 2006; Theis et al., 2007; Schiestl et al., 2011). Moreover, recent studies have demonstrated that leaf herbivory alters floral VOC emissions, usually with negative consequences for pollinator attraction (Kessler et al., 2011; Pareja et al., 2012). In wild tomato, this effect is jasmonate-mediated, and therefore floral VOCs are mechanistically tied to phytohormonal pathways, triggering resistance responses (Kessler et al., 2011). This introduces additional challenges for attraction of pollinators while maintaining intrinsic plant defense traits.

Volatile organic compounds released from fruits and seed/spores attract various animal dispersers and are essential for the spatial dynamics of plant populations within ecosystems. In contrast to floral VOCs, a direct involvement of chemical cues in seed–disperser interaction has only been shown in a few instances. A blend of VOCs emitted by seeds of the epiphyte Peperomia macrostachyum attracts ant-garden ants Camponotus femoratus in the Amazonian rainforest and triggers their seed-collecting behavior (Youngsteadt et al., 2008). By storing seeds in ant gardens where they later germinate, ant-garden ants contribute to seed dispersal. Similarly, fruit-emitted VOCs allow bats to locate ripe fruits (Hodgkison et al., 2007). In the bat-dispersed fig species Ficus hispida, volatile profiles are subjected to substantial reconfiguration between the pre-pollination and seed dispersal phases, further supporting the role of VOCs in mammalian-mediated seed dispersal interactions (Borges et al., 2008). Even in nonvascular plants, VOCs can contribute to spore dispersal. In several entomophilic dung mosaics, which disseminate their spores by flies, sporophytes produce species-specific odors that attract flies as dispersal agents (Marino et al., 2009).

2. Floral VOCs as drivers of plant biodiversity

The emergence of insect pollination has been proposed to have driven angiosperm diversification in the early Cretaceous (Friis et al., 2006). While the exact mechanisms that led to speciation in early angiosperms remain elusive, evidence from recently evolved species suggests that plant speciation is predominantly the result of prezygotic isolation mechanisms, which prevent mating or fertilization between species (Rieseberg & Willis, 2007). Prezygotic barriers are manifold and include divergent habitat preferences, pollen competition, and pollinator-mediated isolation, the latter being promoted by flower morphology or pollinator behavior. Pollinator behavior, in turn, is pre-determined by the pollinator’s attraction to specific flower cues. Recent reports suggest that floral VOCs, in conjunction with other flower signals such as color, size and shape, are involved in the evolution and maintenance of reproductive isolation between plant species (Fig. 6). The properties of volatile blends, including intensity, ratios of constituents, and the presence of unique compounds, could contribute to the reproductive isolation of closely related plant species (Fig. 6). Sexually deceptive orchids represent a well-studied example of the role of floral VOCs in plant speciation. In this highly specialized system, flowers release VOCs mimicking the female pollinator’s sex pheromone, thereby deceitfully attracting male pollinators (Schiestl, 2005). Comparative analysis of floral VOCs emitted by different sexually deceptive orchids of the genera Chiloglottis and Ophrys revealed that volatiles play a key role in pollinator-mediated isolation. By emitting species-specific volatile profiles, each orchid species in these genera provides a unique attraction channel for a single pollinator species (reviewed in Ayasse et al., 2011). Differences in even a single VOC can strongly influence reproductive isolation between two plant species, as was shown for phenylacetaldehyde in the closely related Silene dioica and S. latifolia, which emit blends with similar constituents but in different relative amounts (Waelti et al., 2008).

The genetic basis underlying phenotypic VOC differences and ethological isolation between closely related species was recently investigated in different plant systems. Using Petunia exserta and Petunia axillaris, it was shown that a quantitative trait locus, identified as the MYB transcription factor ODO1, contributes to differences in the scent profile and reproductive isolation between these species (Klahre et al., 2011). In orchids, reproductive isolation among sexually deceptive species of the genus Ophrys was proposed to rely on species-specific alkene emission profiles that are defined by differences in enzyme activity and gene expression of a few stearoyl-acyl carrier protein desaturases (Schluter et al., 2011; Xu et al., 2012).

Fig. 6 Role of floral volatile organic compounds (VOCs) in reproductive isolation. In closely related plant species lacking postzygotic mechanisms for the maintenance of reproductive isolation, species integrity is retained by pre-mating isolation. Floral VOCs are an essential component in this isolation. Species-specific floral VOC profiles (illustrated here as differently sized VOC clouds) drive assortative visitation by pollinators, thus preventing gene flow between plant species.
3. Impact of floral VOCs on agriculture

Unlike predators and parasitic wasps whose functional roles could be substituted by chemical insecticides, pollinators cannot be readily replaced. While most crops (e.g., apple, blueberry, canola, cucurbits) benefit to varying degrees from insect pollinators (Losey & Vaughan, 2006), for dioecious crops with self-incompatible flowers it is vital. Thus, floral VOCs have been inadvertently selected to retain some baseline number of pollinator-attracting flowers it is vital. Thus, floral VOCs have been inadvertently selected to retain some baseline number of pollinator-attracting traits, without which the crop would simply fail to yield fruit. Indeed, comparative analysis of volatiles across several citrus species revealed that self-incompatible pummelo (Citrus grandis) blossoms release higher amounts of total VOCs than species (e.g. orange, grapefruit, lemon) with flowers containing both male and female organs (Jabalpurwala et al., 2009). This provides correlative evidence that extreme pollinator dependency has been a driving force to maintain floral VOC profiles in certain crops.

Only a few studies have experimentally evaluated the impact of floral odor on crop pollination (Fig. 7). In one of these studies, bee visitation (number of visits per unit time) was compared between wild-type and Bacillus thuringiensis (Bt)-expressing eggplant (Arpaia et al., 2011). Although the Bt genetic transformation (cry3Bb gene) was conducted purely for insect pest resistance, an unintended side-effect was the elevated emission of five (methyl salicylate, Z-jasmone, α-pinene, α-methyl styrene, δ-2-carene) out of 13 total floral compounds from transgenic flowers, all of which elicited positive electroantennogram responses from bee antennae. When both plant types were subjected to commercial bumblebees in a glasshouse, bees strongly preferred Bt-eggplant despite significantly smaller flowers and overall plant size, illustrating the dominance of floral scent in pollinator behavior. This study also uncovered an important issue that floral odors likely exist at suboptimal amounts for pollination in many crop species. In addition, it raises the important agricultural question as to whether these suboptimal amounts cause inefficient pollination and subsequent yield loss in commercial fields (Fig. 7).

To date, there are only two examples that demonstrate the link between floral VOC composition and crop pollination failure. Alfalfa seed production, for example, relies on pollination by honeybees, which routinely display low fidelity to alfalfa flowers and, instead, prefer other crops and weedy plants. Low visitation rates and inefficient pollen transfer by honeybees translate into low seed yield. Analysis of behavioral and electrophysiological responses to alfalfa floral VOC chemistry revealed that honeybees, while ignoring major scent compounds (e.g. ocimene), are highly responsive to minor constituents (e.g. linalool) that comprise < 1% of the volatile blend (Henning & Teuber, 1992). As alfalfa flowers also emit deterrents, a generation of new cultivars with elevated emission of attractants and simultaneously suppressed emission of repellants will enhance honeybee visitations to alfalfa. Another example includes glasshouse tomato production, where imported bumblebees are routinely used as pollinators, but preferentially forage on other flowering plants outside the glasshouse. This behavior was observed even when the tomato plants were in full bloom, resulting in poor fruit set and subsequent yield loss (Morse et al., 2012). Glasshouse tomato flowers primarily emit four monoterpens (β-phellandrene, α-pinene, p-cymene, (+)-2-carene), all of which are known as herbivore-induced leaf volatiles used in defense and possess toxic properties. Emission of these compounds was negatively correlated with bumblebee visitation, suggesting that this largely repellent-dominated floral VOC blend caused pollination failure in glasshouse tomatoes. Taken together, these examples further support the notion that numerous pollinator-dependent crops likely emit suboptimal VOCs (Fig. 7); however, to our knowledge, no breeding programs exist to select for floral fragrances that are ‘pollinator-friendly’.

IV. Metabolic engineering of plant volatiles

In natural ecosystems, pollinators often select for elevated floral VOCs (Kessler et al., 2008; Majetic et al., 2009; Parachnowitsch et al., 2012), suggesting that pollination services can be improved by producing more fragrant flowers (Fig. 8). Moreover, many modern crop cultivars are deficient in VOC production as a result of breeding for increased growth or yield which negatively affects secondary metabolism, including VOCs. Indeed, flowers produced by horticultural cultivars of cacao (Theobroma cacao) are small, barely fragrant, and only attract opportunistic flies that are inefficient pollinators, whereas wild Theobroma sp. possess large aromatic flowers that are predominantly bee-pollinated (Young & Severson, 1994). Similar trends were observed for vegetative VOCs (Fig. 8). Corn domestication resulted in varieties exhibiting a susceptibility to insect pests as a result of the lack of key odorants, primarily (E)-β-caryophyllene, that attract pest-killing parasitic wasps (Tamiru et al., 2011) and entomopathogenic nematodes (Degenhardt et al., 2009a). Even more recently domesticated crops display analogous growth-defense trade-offs. Cranberry, for example, is only one or two crosses away from its wild progenitor, Vaccinium sp. Yet high-yielding cranberry genotypes have suppressed herbivore-induced sesquiterpene emissions relative to lower-yielding ancestors (Rodriguez-Saona et al., 2011). Taken together, these examples show that crop VOCs are poorly adapted
to maintain beneficial insects in agricultural systems. Additionally, present-day agriculture, while expanding in both size and intensity, tends increasingly to rely on crop monocultures. This reduction in plant diversity translates into adverse effects on ecosystem services provided by the arthropod community, including beneficial insects controlling pests and pollinating crops (Gardiner et al., 2010). Thus, approaches have to be developed to overcome these problems, and metabolic engineering of both floral and defense-related VOCs is a promising tool for enrichment of plant chemodiversity as well as biodiversity of beneficial insect. In the past decade, numerous attempts have been made to modulate plant volatile profiles and to investigate the effect of changes in volatile emission on insect behavior (Fig. 8). Several strategies were used, including the introduction of new gene(s) or branchways in the host plant, and the modification of existing pathways via up-/down-regulation of biochemical step(s) or by blocking the competing pathways. Indeed, direct plant defense was improved by producing the volatile patchoulol along with additional sesquiterpene products in transgenic tobacco, over-expressing Pogostemon cablin patchoulol synthase (Wu et al., 2006). Introduced volatiles deterred tobacco hornworms, a majority of which had migrated away from transgenic to wild-type leaves, consuming 20–50% more of the control leaf material (Wu et al., 2006). In Arabidopsis, high concentrations of linalool, achieved by the overexpression of strawberry linalool/nerolidol synthase gene (FaNES1) targeted to chloroplasts, repelled the aphid Myzus persicae in dual-choice assays (Aharoni et al., 2003). Interestingly, elevated concentrations of linalool obtained in a similar way in potato attracted predatory mites and affected tritrophic interactions (Lucker et al., 2006). However, attraction of predatory mites in Arabidopsis was attained by producing (3S)-(E)-nerolidol and (E)-DMNT via ectopic expression of the same FaNES1 gene in mitochondria, which contains the sesquiterpene precursor FPP (Kappers et al., 2005). Furthermore, when behavioral responses of aphids, parasitoids and predators were compared in three Arabidopsis thaliana accessions constitutively expressing FaNES1 targeted to the mitochondria, different effects were observed depending on ecotypes (Kos et al., 2012). Ecotype-specific function of volatile compounds was also recently recorded for phenylacetaldehyde, which contributed to defense in the Columbia (Col-0) ecotype and was involved in pollinator attraction in Sei-0 and Di-G (Gutensohn et al., 2011). Different effects on plant defense were also observed when rice and oregano (E)-β-caryophyllene synthases were constitutively overexpressed in rice and maize, respectively (Cheng et al., 2007; Degenhardt et al., 2009a; Xiao et al., 2012). In rice, (E)-β-caryophyllene emission improved above-ground plant defense by attracting parasitoid wasps to transgenic plants. By contrast, in a maize line normally lacking (E)-β-caryophyllene and susceptible to western corn rootworm, restored emission of (E)-β-caryophyllene enhanced below-ground defense by providing a signal to entomopathogenic nematodes that infect and kill the root pest (Degenhardt et al., 2009a). These results show that natural defense of crop plants could be improved via metabolic engineering of VOCs, thereby providing an effective and sustainable alternative to current pest-management strategies. However, owing to the species-specific nature of volatile-based plant defenses, results obtained in model systems cannot be directly translated to crops. Moreover, the effect of modified VOC profiles on plant defense needs to be evaluated in an agricultural setting.

In contrast to metabolic engineering of defense-related volatiles, where the effect of altered VOC emissions on insect behavior was investigated, examination of the impact of modified floral scent on animal attraction is in its infancy (Fig. 7). Floral scent bouquet has been modified in several plant species, including petunia (via down-regulation of scent biosynthetic genes (Underwood et al., 2005;
Kaminaga et al., 2006; Orlova et al., 2006), up-regulation of Myb transcription factor PAP1 (Ben Zvi et al., 2008), heterologous expression of scent gene (Guterman et al., 2006) or redirection of carbon flux toward target compound (Koeduka et al., 2008), carnations (via heterologous expression of scent gene (Lavy et al., 2002) or diversion of carbon flux toward scent formation (Zuker et al., 2002)), and tobacco (via introduction of a novel branchway consisting of three genes (Lucker et al., 2004) or an increase in substrate level (Orlova et al., 2009)). However, the first investigation of the consequences of metabolic engineering of floral VOC on pollinator visitation was performed in transgenic tobacco Nicotiana attenuata plants with diminished concentrations of benzyl acetone (Kessler et al., 2008). Monitoring the activity of floral visitors in their native habitat revealed that plants lacking benzyl acetone attracted fewer hawkmoths and hummingbirds than flowers emitting this volatile. It was also shown that changes in more than one volatile compound can influence pollinator behavior. Indeed, remodeling of the scent bouquet in rose flowers overexpressing the Arabidopsis PAPI transcription factor was easily distinguished by honeybees (Zvi et al., 2012), the native pollinators of some wild rose species (Shalit et al., 2004). Attractive and repellent functions of different volatiles within the scent bouquet were recently analyzed in petunia flowers using five transgenic lines silencing biosynthetic genes responsible for individual scent compounds (Kessler et al., 2012). While the most highly abundant volatile methylbenzoate acted as both attractant and deterrent, isoeugenol and benzylbenzoate only exhibited the latter function.

Several attempts have been made to improve the flavor of tomato fruits (Fig. 8) (Lewinsohn et al., 2001; Davidovich-Rikanati et al., 2007, 2008). While humans were able to assess changes in fruit aroma, the effect of these genetic manipulations on fruit dispersers has not been investigated.

Overall, the studies described have demonstrated the potential of metabolic engineering of VOCs for both the improvement of plant defense and pollinator attraction (Fig. 8). Despite multiple successes, these results also revealed unexpected problems that can be encountered in VOC engineering: negative effects on plant growth and development as a result of limited carbon availability for essential metabolites or toxicity of the newly introduced compounds (Aharoni et al., 2003, 2006; Orlova et al., 2009); modification of the volatile compounds into nonvolatile forms, for example, by glycosylation (Lucker et al., 2001, 2006; Aharoni et al., 2003); masking by other volatiles (Togni et al., 2010); low to no yield of the desired compound resulting from an insufficient amount of precursors for its biosynthesis (Guterman et al., 2006); and formation of unpredicted compounds as a result of occult metabolic capacities of the host plant (Lewinsohn & Gitzen, 2009). Further, ecological complications will arise once these methodological constraints are lifted. In the wild gourd, Cucurbita pepo var. texana, for example, experimentally elevated floral emission of 1,4-dimethoxybenzene, considered the most attractive compound to specialist squash bee pollinators, decreased plant reproduction as a result of concomitant attraction of florivorous beetles (Theis & Adler, 2012). Given that C. pepo also contains cultivated squash and pumpkin, crops that rely heavily on managed pollinators, genetic enhancement of floral scent in cucurbits and related species for increased yield may prove difficult. A comprehensive understanding of plant metabolic networks and their regulation, as well as knowledge about key compounds involved in plant–insect interactions in the near future, will greatly advance metabolic engineering of VOCs and help to overcome these challenges.

V. Conclusions

Current advances in chemical ecology highlight the importance of plant VOCs in natural ecosystems as crucial signaling molecules in plant defense, pollination and plant–plant communication. In the past decade, plant volatile research has witnessed a shift from studying VOC composition and the role of individual synthetic compounds in insect behavior toward the elucidation of the intricate interactions between plant-produced volatiles and the native arthropod communities in realistic field settings. Recent breakthroughs in scent gene discovery and metabolic engineering make it possible to manipulate the amount of specific volatiles in planta, either omitting or increasing their emission, thus greatly facilitating the identification of key attractants for pollinators and natural enemies. In the future, this transgenic approach will also allow us to determine species-specific functions of different volatiles under varying natural conditions.

Unfortunately, our limited current understanding of the regulation of VOC biosynthetic pathways constrains the manipulation of VOC emission for enhanced pollination and sustainable pest management strategies based on biological control. The emergence of new approaches, however, such as kinetic modeling in conjunction with -omics technologies, will allow us to generate transgenic plants with more precise fine-tuned VOC emissions which will be synchronized with the activities of potential pollinators or herbivores. The identification of transcription factors that regulate the orchestrated emission of volatiles originating from different metabolic pathways will significantly improve our success. Nevertheless, future attempts to increase crop yield will necessitate collaborative efforts by ecologists, entomologists, agronomists, biochemists and plant metabolic engineers.

Another central factor limiting the transition of crops with modified volatile profiles from research laboratories to real-world implementation is the likelihood of nontarget effects. These effects may be unavoidable because most VOCs have multiple functions in plants. A good example is (E)-β-caryophyllene, whose function varies dramatically depending on environmental context; from serving as an antimicrobial agent in flowers to reduce bacterial growth (Huang et al., 2012) to acting as an inducible root volatile that attracts nematodes involved in tritrophic below-ground defense (Degenhardt et al., 2009a). To circumvent this barrier, novel creative solutions have to be employed to limit the unintended side-effects of VOCs. Application of -omics approaches, such as transcriptomics and metabolomics, to both plants and insects will undoubtedly promote our understanding of the mechanisms underlying plant–insect interactions and help to achieve desired effects such as maximizing pollination while minimizing pest attraction. Enhancing crop–pollinator interactions becomes even more critical for modern agriculture, which is
facing sudden declines in honeybee populations and is thus increasingly dependent on native bees. Proper selection of emission timing rather than emission quantity and mimicking natural plant ‘behaviors’ that have presumably been optimized via natural selection over many generations are crucial for obtaining beneficial effects of insects on plant fitness. The successful application of these approaches in agriculture will require additional investigations of basic ecological and evolutionary mechanisms of VOC-mediated interactions in natural plant systems.

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References


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