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The role of volatiles in plant communication

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SUMMARY

Volatiles mediate the interaction of plants with pollinators, herbivores and their natural enemies, other plants and micro-organisms. With increasing knowledge about these interactions the underlying mechanisms turn out to be increasingly complex. The mechanisms of biosynthesis and perception of volatiles are slowly being uncovered. The increasing scientific knowledge can be used to design and apply volatile-based agricultural strategies.

Keywords: terpenoids, phenylpropanoids, volatiles, plant–insect/microbe/plant interactions, pollination, biosynthesis, regulation, agriculture.

INTRODUCTION/BACKGROUND

Through volatile organic compounds (VOCs), plants are in constant dialogue with the organisms in their environment. This communication is of great importance as it allows plants and the organisms they interact with to tune their growth, development, defence, propagation and life cycle to achieve maximal fitness. Plants themselves but also the organisms in their environment produce VOCs, which belong to various chemical classes, such as the terpenoids, the benzenoids and phenylpropanoids, fatty acid-derived molecules including the green leaf volatiles (GLVs) and minor classes such as nitriles, (ald)oximes and sulfides (Figure 1). Here we review the recent literature on volatile communication between plants and other organisms as well as the biosynthesis, transport, perception in the receiver organisms, and potential importance for agriculture of these VOCs.

ANALYSIS AND INFORMATION CONTENT OF VOCs

Volatile organic compounds mediate the interaction between plants and mutualists, pests and pathogenic antagonists and, although not addressed much yet and experimentally challenging, also multi-trophic interactions. Semiochemicals that function in these interactions can be produced constitutively or in response to outside interactions and stimuli, and this occurs above as well as below ground (Massalha et al., 2017). Although there are indications that above- and below-ground semiochemical responses are integrated (Danner et al., 2015; Van Dam et al., 2018), details on what drives this remain to be elucidated. Although semiochemicals can travel long distances, plant–plant and plant–microbe communication usually take place at relatively short distances, while plant volatiles with a role in plant–insect interaction are perceived at distances of several hundred metres. The resulting extreme dilution, and the large variation in chemical structures and properties of the VOCs pose a challenge to the analysis of the volatiles and their precursors (Fu et al., 2017). Another major challenge is to interpret VOC information content, especially across larger spatial scales in which the volatile blend carrying the information becomes increasingly complex through environmental effects (including noise) and community dynamics in an ecosystem (Aartsma et al., 2017; Kessler and Kalske, 2018). Comparison of volatile profiles is complicated due to their complexity, but also because the presence/absence and quantity of compounds are not independent measures in case of a shared biosynthetic origin. An interesting approach to deal with this is a so-called biosynthetically informed pairwise distance measure, calculating compound similarity based on the proportion of shared biosynthetic enzymes (Junker, 2018). In the
coming years we will see a strong progress in the interpretation of the effect of volatile blends on biological processes. This development will be fuelled by the development of new high-throughput behavioural bioassays in combination with high-throughput VOC analyses and increasing sensitivity of GC-MS. Upcoming tools such as network analysis, large-scale data integration, and machine learning are going to provide a breakthrough in the interpretation of the biological meaning of the complex VOC blends (Cuperlovic-Culf, 2018).

THE ROLE OF VOCS IN POLLINATION

One of the major functions of floral volatiles is the attraction of pollinators, either through triggering innate behavioural responses or by providing a stimulus that can be associated with the presence of rewards (Schiestl and Johnson, 2013; Haverkamp et al., 2016a). Pollinator attraction is often surprisingly specific, which can be beneficial as specificity increases the efficiency of pollen transfer between conspecific stigmas. Specificity in pollination can be achieved with floral filters (Raguso, 2008), for example through specialized morphology that prevents access to rewards for most animals, or signals that are only detectable or of interest to few potential pollen vectors. Among signals, colour is rarely highly specific, because most animals have limited abilities to discriminate subtle colour nuances. Therefore, floral scent is typically more important in encoding highly specific signals, because of its chemical complexity, and the high number of different olfactory neurons usually used by insects to detect it (Haverkamp et al., 2018). The chemical complexity in floral scent entails interactions between neurons detecting individual chemical components, together producing the sensation of a bouquet, which is different from the sum of the individual components (Strutz et al., 2014; Schiestl, 2015). An example for this is scent compounds that modify the attractive function of other molecules, as was shown, for example, in scent bouquets of orchids attracting fragrance-gathering male euglossine bees (Milet-Pinheiro et al., 2018). Floral scent is mostly detected by insects with neurons located...
on the antennae, but recently olfactory neurons on the tip of the proboscis have also been characterized (Haverkamp et al., 2016b).

Some of the most specific chemical signals are those that mimic non-floral items of interest to potential pollinators, such as oviposition substrates or mating partners (Johnson and Schiestl, 2016). Such signals are usually not attractive or not even detectable to ‘normal’ floral visitors that visit flowers for nectar and/or pollen, and therefore filter out a large number of potential visitors. Even more so, scent bouquets emitted by mimetic flowers are sometimes more specific than the volatiles emitted by the model they mimic. One curious example comes from the flowers of Rafflesia cantleyi (Wee et al., 2018) that emit a smell mimicking rotten meat and attracting flies that normally oviposit in such substrates, as pollinators. In the field, where this Rafflesia species flowers, rotting meat was found to attract nine species of flies, whereas only five species were found on Rafflesia flowers, and of those, only a single species, the blowfly Chrysomya chani, comprised 97% of all flower visitors and was the only species carrying pollen. On traps with rotting meat, C. chani represented only 25% of all flies caught, suggesting that floral volatiles of Rafflesia cantleyi effectively filter the available carrion-fly pollinator community (Van der Niet et al., 2011). The two main compounds of Rafflesia’s floral bouquet are dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS), products of bacterial breakdown of methionine and cysteine in meat, that are typically found in the floral emission of floral carrion mimics (Martin et al., 2017; Du Plessis et al., 2018; Wee et al., 2018). Many species of blowflies are attracted to these volatiles, but Rafflesia flowers also produce terpenoids, benzenoids and fatty acid derivatives, which may have a modulating function leading to higher specificity of the whole bouquet.

A similar case has recently been recorded in Ceropogia mixta, which is mostly pollinated by the house fly Musca domestica (Du Plessis et al., 2018). M. domestica is usually not strongly associated with carrion but also oviposits in dung, suggesting that C. mixta uses mixed carrion/faeces mimicry (Jürgens et al., 2013). The floral scent of this plant is also dominated by DMDS and DMTS, in combination with a complex blend of other compounds. A fascinating twist is the fact that even though vision models suggest that for flies the colour of the flowers is indistinguishable from the background, bioassays suggest that there is a visual component of attraction. Whereas odour alone was sufficient to attract flies, combined olfactory and visual signals attracted more flies. This finding is paralleled by data from the Rafflesia system, in which floral scent alone was insufficient to trigger landing behaviour of the pollinators in wind tunnel bioassays, suggesting visual signals are needed to guide insects to carrion flowers (Du Plessis et al., 2018).

A well known, and highly specific example of floral mimicry is sexual mimicry, in which flowers imitate mating signals and are pollinated by sexually aroused male insects during a mating attempt (Johnson and Schiestl, 2016). This type of pollination system is most commonly found among Australian orchids, in which a minimum of 11 genera are (at least partly) pollinated in this way. In some of these groups, exciting progress has been made in recent years in terms of identifying the pollinator-attracting floral scent compounds (i.e. the mimicry of the sex pheromone), and several unusual compounds have been identified. For example, (methylthio)phenols, up to now only known as natural products produced by bacteria, were identified as pollinator-attracting signals in the orchid genus Caladenia (Bohman et al., 2018b). Caladenia crebra produces four types of (methylthio)phenols, and a 10:1 blend of two of these compounds attracts and releases copulation attempts in its legitimate pollinator, a thynnine wasp. Interestingly, a 1:1 blend of the same compounds attracts another, as yet undescribed, thynnine wasp (Campylolithynus sp. A), which is the pollinator of Caladenia attingens subsp. attingens, demonstrating the importance of specific ratios of active volatiles for the specificity of pollinator attraction in sexual mimicry.

In another paper, Bohman and colleagues show a different mechanism of specificity, in which pollinators of sexual mimics strongly discriminate between structural isomers of active volatiles (Bohman et al., 2018a). This was shown to be the case for Caladenia plicata, which employs two unusual and biosynthetically unrelated volatiles as pollinator attractants, (S)-β-citronellol and 2-hydroxy-6-methylacetophenone. When (S)-β-citronellol was replaced by its enantiomer (R)-β-citronellol or 2-hydroxy-6-methylacetophenone by one of its regio-isomers in bioassays with synthetic volatiles, the volatile blend lost almost all of its attractiveness (Figure 2a). In a generalist pollinator searching for food on flowers, namely honey bees, a different result was found, as worker bees only poorly discriminated between isomers of different volatiles, even when they were paired with aversive stimuli like bitter taste (Aguilar et al., 2018). A fascinating aspect of specific floral signals is the potential of generalist predators to eavesdrop on such signals to optimize prey catching. On a Balearic island, for example, lizards use carcasses, but also the carrion-mimicking flowers of Helicodorus muscivorus, as a lurking site to catch flies (Perez-Cembranos et al., 2018). A very similar example, but without involving mimetic flowers, is represented by crab spiders that wait for their prey at flowers (Knauer et al., 2018). Both lizards and crab spiders are attracted by floral scent, which makes sense because a ‘sit and wait’ predator is likely to be more successful when using the same signals as its prey to identify potential hunting sites. Both lizards and crab spiders eat or repel pollinators and therefore have a negative effect on
the pollination success of the plant. In both cases, however, there is also a positive effect on plant fitness, as lizards act as fruit dispersers of *Helicodorus* and crab spiders also feed on florivores. This potential fitness increase counterbalances selection on plants to avoid eavesdropping predators and are likely to stabilize these associations. Another, often highly specific and scent-driven, pollination system is nursery pollination, in which pollinators oviposit into flowers and the pollinators’ larvae consume floral tissue of developing seeds. Jackfruit (*Artocarpus heterophyllus*) is a monoeocious crop pollinated by gall midges that visit male and female inflorescences, but oviposit only into male, fungus-infected inflorescences (Gardner et al., 2018). Male inflorescences are typically infested by a fungus after blooming. Female inflorescences are deceptive to pollinators as they do not provide reward, even though they produce more volatiles than the male flowers. Floral scent of both male and female inflorescences is dominated by three methyl esters that release responses in the midges’ olfactory neurons and also occur in the fruits. Highly specific, unusual scent compounds – such as (E, E)-α-farnesene-2(3), 9(10)-diepoxyde and 3-methylen-2-(pent-2(2)-enyl)-cyclopentanol – were found in three *Carludovicoidae* plant species with nursery pollination (Teichert et al., 2018) (Figure 2b). The floral staminoids of these plant species produce scent and elevated temperature to attract flower weevils that oviposit into the staminoids. In summary, highly specific associations between flowers and insects are typically mediated by chemical signals, that act as floral filters together with visual signals, floral morphology and specific types of reward.

THE ROLE OF VOCS IN THE INTERACTION OF PLANTS WITH HERBIVORE INSECTS

The unique constitutive volatile blend produced by plants is used by mutualists such as pollinators, but also by antagonists such as herbivorous insects who use volatiles to identify and home-in on a host. The host blend consists of major and minor constituents, but the most abundant compounds do not automatically determine the response to the blend as a whole (McCormick et al., 2012). The olfactory system of insect antennae can discriminate patterns and use this to determine biological relevance, that is host suitability or unsuitability. This odour-mediated behaviour appears to be innate in insects (Hatano et al., 2015). Within a plant community, the chemical diversity in constitutive volatiles between individuals can be large enough to drive herbivore preference-behaviour (Clancy et al., 2018).

Herbivore-induced plant volatiles (HIPVs) are blends of volatiles – mostly GLVs, terpenes and aromatic compounds – that a plant produces in response to herbivory (McCormick et al., 2012). HIPVs are released after tissue disruption; however, albeit at low levels, some are also emitted constitutively into the plants headspace (Holopainen and Gershenzon, 2010). HIPVs serve as signals to attract natural enemies of herbivores such as parasitoids and predators (Aartsma et al., 2017; Gasmi et al., 2018; Turlings and Erb, 2018). Cockchafer larvae feeding on roots of *Populus* spp. induced the emission of a number of monoterpenes including 1,8-cineole, which had an inhibitory effect on *Phytophthora cactorum* (Lackus et al., 2018) (Figure 2c). Turlings and Erb (2018) question the common notion that HIPVs are signals produced to attract the pest enemy or as ‘cry for help’, as there is no evidence for directed evolution or a fitness benefit (Turlings and Erb, 2018). The signal itself that is produced in direct response to the attacker is associated with prey so accurately that parasitoids and predators have evolved to rely on the volatile cues to such an extent that they, for example, have even lost vision.

THE ROLE OF VOCS IN THE INTERACTION OF PLANTS WITH MICRO-ORGANISMS

Not only plants produce volatiles in response to interspecies interaction, but also micro-organisms associated with plants are a source of volatile (and non-volatile) compounds that can alter plant physiological and metabolic responses (Piechulla et al., 2017; Wenke et al., 2019). *Bacillus, Pseudomonas, Arthrobacter, Fusarium* and *Alternaria* rhizobacteria, for example, can stimulate root growth through the production of volatiles such as 3-hydroxy-2-butanone (acetoin), 2,3-butanediol and 2-pentylfuran (Fincheira and Quiroz, 2018) (Figure 2d). Others can induce resistance and tolerance in plants such as acetoin, dimethyl disulfide, 3-pentanol and 6-pentyl-α-pyrone (Fincheira and Quiroz, 2018) (Figure 2d). This shows that plants do not only produce volatiles, but also perceive them, and respond to them with altered growth or defence signalling (Schenkel et al., 2018). This is further discussed below under ‘Molecular mechanisms underlying VOC perception’. Microbe-induced plant volatiles can inhibit microbial growth and act as local bacteriostatic agents (Sharifi et al., 2018) as discussed for 1,8-cineole and *P. cactorum* above (Lackus et al., 2018). Indoles and green leaf volatiles (GLVs) can affect membrane integrity of bacteria on the plant surface and interfere with bacterial quorum sensing, thereby deregulating bacterial virulence (Joshi et al., 2016). In response to pathogen attack, plants do not only activate their own defence response, but the infection can also induce volatile emission into the headspace that can lead to an immune response in neighbouring plants (Riedlmeier et al., 2017) (Figure 2e). However, when multiple volatile signals have to be integrated, the immune (or defence) response can also be reduced. Hormonal cross-talk has a role in balancing trade-offs, which can also result in the suppression of volatile emission (Martínez-Medina et al., 2017; Erb, 2018). Therefore, plants can produce specific volatiles upon microbial interactions but, in turn, microbes
have been shown to be able to suppress VOC biosynthesis through the use of effectors (Sharifi et al., 2018). Microbes, in combination with their host plant, can also mediate tri-trophic interactions (Shikano et al., 2017). For example, viruses that depend on a (mobile) vector, may alter plant volatile biosynthesis in order to recruit the vector insect. The 2b protein of cucumber mosaic virus manipulates aphid behaviour by modifying the host volatile profile (Tungadi et al., 2017; Wu et al., 2017). Even further complicating the story, herbivorous insects carry facultative endosymbionts that can attenuate the production of predator-attracting volatiles emitted by the plant under attack (Frago et al., 2017). The role of micro-organisms in the volatile communication of plants also extends to flower volatiles. Microbes can colonize the nectar and change the volatile blend of a flower, which may alter floral visitation and hence plant reproduction (Vannette and Fukami, 2016; Rering et al., 2018). In conclusion, the dialogue between plants and microbes is complex and deciphering the information exchange between plants and beneficial as well
as pathogenic microbes remains of unaltered interest for application in (crop)plant production, as further discussed below.

THE ROLE OF VOCs IN PLANT – PLANT INTERACTION

An intriguing function of volatiles is their signaling role in and between plants, a phenomenon first described already more than 35 years ago (Baldwin and Schultz, 1983; Rhoades, 1983). When attacked by herbivorous arthropods, plants often emit a different blend of volatiles, both qualitatively and quantitatively and this blend of volatiles can initiate responses in systemic leaves and neighbouring plants. As such, plants are also indicated as having a certain dialect or chemotype (Karban et al., 2014a, 2016). Many studies have shown that this volatile blend can activate or prime defence responses, for reviews see for example Heil and Karban (2010) and Ameye et al. (2018). A recent meta-analysis showed that adjacent plants exposed to volatiles from damaged neighbours become more resistant to herbivores (Karban et al., 2014b). However, tomato plants infested by whiteflies (Bemisia tabaci) produce a volatile blend that makes neighbouring plants more susceptible to whiteflies (Zhang et al., 2019). Whether this is an adaptive response, as it might make the plants more resistant to the viruses transmitted by the whiteflies, remains to be seen, but it indicates that not all HIPV blends result in more resistance to herbivores in the neighbours.

MOLECULAR MECHANISMS UNDERLYING VOC PERCEPTION

An intriguing function of volatiles is their signaling role in and between plants, and particularly how plants can perceive these signals (Cofer et al., 2018b; Erb, 2018). For examples of this, authors normally refer to the gaseous hormone ethylene and its receptor (Schaller and Bleecker, 1995) and suggest that other volatiles may also be detected by receptors in plants. However, receptors for other volatiles have remained elusive so far. Still, plants show an often surprisingly specific response to different volatiles. Therefore perhaps one has to look at alternative scenarios of which one example is the perception of CO2 by plants in guard cells. These cells in Arabidopsis have an S-type anion channel, SLOW ANION CHANNEL1 (SLAC1) that has bicarbonate-interaction sites (Yamamoto et al., 2016; Zhang et al., 2018a). It is conceivable that other ion channels have interaction sites for particular volatiles as well that can change their activity just as CO2/HCO3− can change SLAC1 activity and stomatal movement. Indeed, the first response to volatiles that can be measured in plants is a change in plasma membrane potential and cytosolic calcium flux (Zebelo et al., 2012). Very recently, the transcriptional co-repressor TOPLESS-like (TPL) protein was identified to bind volatile sesquiterpenes, that is caryophyllene analogues (Nagashima et al., 2018). TPL proteins are known to regulate various hormonal responses such as those induced by jasmonic acid (Pauwels et al., 2010). This suggests that nuclear proteins can be involved in volatile sensing, and opens up an interesting field of research while at the same time raising the question how these volatiles are transported to establish a relatively high concentration in the nucleus.

Also enzymes have been demonstrated to play a role in transport and perception of volatiles within or between plants. The most well known intracellular signalling volatile is methylsalicylate (MeSA) that is transported via the phloem from a tobacco (Nicotiana tabacum) leaf infected with tobacco mosaic virus (TMV) to a non-infected, systemic leaf to induce resistance (Park et al., 2007). Accumulation of high levels of salicylic acid (SA) in TMV-infected leaves results in the production of MeSA and the inhibition of a MeSA esterase in the local leaf (Figure 2e). In the systemic leaf, SA levels are low and the transported MeSA is converted to SA by the esterase resulting in SA production and resistance. In plant–plant communication it has recently become clear that in receiver tomato plants, the GLV (Z)-3-hexenol can be glycosylated to (Z)-3-hexenylvicianoside, which induces defence responses (Sugimoto et al., 2014) (Figure 2e). Intriguingly this particular glycosylation seems very plant species specific, as it does not occur in Arabidopsis, even though the latter also glycosylates many different volatile alcohols – GLVs, phenylpropanoids, terpenes – upon exposure (Sugimoto et al., 2015).

Plants show remarkably specific responses to different volatiles (Cofer et al., 2018b; Erb, 2018). Recent findings show that dual-volatile exposed plants respond differently than plants exposed to single volatiles. Maize (Zea mays) plants respond with stronger defences to exposure with both indole and the GLV (Z)-3-hexenyl acetate than to the single volatiles (Hu et al., 2018b) (Figure 2e). This adds another layer of complexity and might explain some of the very specific responses that have been observed. For instance, plant–plant communication in the shrub Baccharis salicifolia is specific for a particular aphid species: induced resistance in neighbours is only obtained against the same aphid (Moreira et al., 2018). This specificity can be assigned to the different volatile blends induced by the different aphids. Similarly, as in maize, a blend of synthetic volatiles mimicked this response better than the individual compounds. This seems to indicate that plants have multiple mechanisms to detect volatiles as already suggested by Cofer et al. (2018b).

Many new aspects of responses to volatiles and volatile signal transduction in plants are being discovered. GLVs, for example, can protect maize seedlings against cold stress (Cofer et al., 2018a) and particular GLVs can induce stomatal closure thereby enhancing resistance against Pseudomonas syringae pv. tomato infection (López-Gresa
et al., 2018). This GLV-induced priming comes at a cost for the plants as it reduced growth rates (Engelberth and Engelberth, 2019). Other interesting findings are that touch-induced volatiles can synchronize defences in neighbouring plants (Markovic et al., 2018) and that goldenrod (Solidago altissima) responds to (E,S)-conophthorin, a volatile produced by a specialist herbivore, the goldenrod gall fly (Eurosta solidaginis) (Helms et al., 2017). This brings another dimension to the field of plant–herbivore interactions. Regarding signalling, an advanced publication (Ye et al., 2018) indicates a role for mitogen-activated kinases and WRKY transcription factors in indole-mediated priming, similar to what has been shown for GLV signaling in Arabidopsis (Ameye et al., 2018), perhaps pointing to signalling commonalities. Finally, one very intriguing aspect is the response of plants to microbial volatiles (Ryu et al., 2003; Sharifi and Ryu, 2018). With, for instance, clear growth responses to specific volatiles as read out, genetic screens to identify potential receptors and signal transduction cascades should be more feasible than with volatiles in plant–plant communication (Scala et al., 2017).

**VOC BIOSYNTHESIS**

Intriguingly, the VOCs that mediate the communication of plants and other organisms belong to a range of different compound classes such as fatty acid-derived molecules (including GLVs), terpenoids, benzenoids, and phenylpropanoids, and minor classes such as nitriles, (ald)oximes and sulfides. Fatty acid-derived VOCs (including GLVs) are produced from the C18 unsaturated fatty acids, linoleic and linolenic acid. These are produced through the acetate pathway that gets its substrate, acetyl-CoA, from glycolysis (Fu et al., 2017) (Figure 1). Through a series of oxidation and reduction steps and acetylation these fatty acids are converted to aldehydes, alcohols and acetics, such as (Z)-3-hexenol and (Z)-3-hexenyl acetate (Figure 1). Methyl jasmonate biosynthetically originates from linolenic acid through multiple enzymatic steps (Li et al., 2005). Methyl jasmonate is formed through methylation of jasmonic acid by jasmonic acid carboxyl methyltransferase (Cheong and Choi, 2003).

Acetyl-CoA from glycolysis is also the substrate for the cytosolic mevalonic acid (MVA) pathway, while pyruvate from glycolysis is the substrate for the plastidic methyleneglutamyl-threitol phosphate (MEP) pathway (Beyraghdar Kashkooli et al., 2018; Pichersky and Raguso, 2018). These two pathways both produce the isoprenoid precursors, isopentenyl diphosphate (IDP) and its isomer, methyllalyl diphosphate (DMADP). IDP and DMADP are condensed by prenyl transferases, such as geranyl diphosphate (GDP) synthase, farnesyl diphosphate (FDP) synthase, and geranylgeranyl diphosphate (GGDP) synthase to produce GDP (C10), FDP (C15), and GGDP (C20), respectively. FDP from the MVA pathway is the precursor for cytosolic sesquiterpenoid biosynthesis catalyzed mainly by sesquiterpene synthases, cytochrome P450s, alcohol dehydrogenases and reductases. GDP from the MEP pathway is the precursor for plastidic monoterpenoid and carotenoid formation. Monoterpenoid formation is mostly catalyzed by monoterpene synthases, cytochrome P450s, alcohol dehydrogenases and reductases (Beyraghdar Kashkooli et al., 2018; Pichersky and Raguso, 2018). GGDP is the precursor for carotenoid formation through a series of enzymatic steps, involving a.o. phytane synthase and phytoene desaturase. Carotenoids such as β-carotene are precursors for the production of volatile apocarotenoids through oxidative cleavage, catalyzed by carotenoid cleavage dioxygenases (Liang et al., 2018) (Figure 1).

The benzenoids and phenylpropanoids are derived from the shikimate pathway, which starts with the condensation of phosphoenolpyruvic acid from glycolysis, and d-erythrose-4-phosphate, from the pentose phosphate cycle (Widhalm et al., 2015; Santos-Sánchez et al., 2019) (Figure 1). Then six more enzymatic steps result in the formation of the key branch-point compound, chorismic acid, the final product of the shikimate pathway and the common precursor for all (volatile) phenolics. The volatile phenolics are represented by benzenoids (C6–C1), such as benzaldehyde, methyl salicylate and benzyl benzoate, which are formed from cinnamic acid; C6–C2 compounds such as 2-phenylethanol and phenyl acetaldehyde which are produced from phenylalanine; and C6–C3 compounds such as eugenol, methyl eugenol, and chavicol which are produced from 4-coumaroyl-CoA (Widhalm et al., 2015) (Figure 1).

The (ald)oximes are amino acid-derived metabolites and include volatiles, such as 2- and 3-methylbutyraldoxime. The volatiles are herbivory-induced or flower volatile blend constituents. See (Sørensen et al., 2018) for a recent review. In addition to these canonical VOC biosynthetic pathways occasionally unexpected biosynthetic solutions appear in the literature, showing how immensely intricate volatile biosynthesis has evolved (Sun et al., 2016). The enzyme responsible for geraniol biosynthesis in rose, for example, turned out not to be a monoterpene synthase but a Nudix hydrolase (a diphosphohydrolase) (Magnard et al., 2015). More function-based overviews of volatile biosynthesis are given in papers on the role of volatiles in plant–herbivore interaction including the perception by plants of herbivory and downstream signalling (Stahl et al., 2018), the role of volatiles in plant–microbe interaction (Sharifi et al., 2018) and on flower volatile biosynthesis (Ramya et al., 2017; Wong et al., 2017). Ryu and co-workers review the induction of volatiles by micro-organisms and the effects that these volatiles have on plants, showing that microbes are true manipulators of their plant host (Sharifi et al., 2018).

With regard to progress in our understanding of the biosynthesis of herbivory-induced volatiles, Liu et al.
PhTE1 exerts control on the flux between aromatic acyl-CoA esters, the authors concluded that et al. example of a genomics approach is the work by Kumar cytosol. the peroxisomes and phenylpropanoid biosynthesis in the recent added another player, a peroxisomal thioesterase, for terpene synthases (Kumar et al., 2018) elucidated (Qualley, 2019). With its capability to hydrolyze aromatic acyl-CoA esters, the authors concluded that PhTE1 exerts control on the flux between β-oxidation in the peroxisomes and phenylpropanoid biosynthesis in the cytosol.

For the identification of genes involved in volatile biosynthesis several different strategies are being used. An example of a genomics approach is the work by Kumar et al. (2018) who mined the genome sequence of Ocimum sanctum for terpene synthases (Kumar et al., 2018). They identified 47 putative terpene synthases. To get a better insight in their involvement in the biosynthesis of the O. sanctum terpenoids additional analyses will be necessary, such as transcriptomics preferably in combination with chemical analysis (and followed by functional characterization through heterologous expression). Transcriptome analysis through RNA sequencing is increasingly being used to identify genes involved in volatile biosynthesis, Fan et al. (2018), Huang et al. (2018) and Tian et al. (2018) used RNA-seq to try to pinpoint genes that are involved in flower volatile biosynthesis in Polianthes tuberosa, Freesia hybrida and wintersweet, respectively, that emit benzenoids and terpenoids, mostly terpenoids and terpenoids and benzenoids, respectively (Fan et al., 2018; Huang et al., 2018; Tian et al., 2018). All three base their selection of gene candidates on homology and expression patterns. Although in these studies chemical analysis was also carried out, these data were not used to the maximum to further narrow down and/or support their candidate genes and the studies therefore remain rather descriptive. A more advanced network (co-expression) analysis with RNA-seq and GC-MS results is very powerful in identifying promising candidate genes and will therefore shorten the gene discovery process substantially (He et al., 2018). A next step in this process will be the use of metabolomics as exemplified for strawberry (Haugeneder et al., 2018), which will allow correlation analysis also with non-volatile precursors and/or further functionalized non-volatile downstream products.

With these advanced approaches, the discovery process of biosynthetic genes will further speed up. The elucidation of biosynthetic pathways of VOCs – in combination with the possibility to genetically knock out or knock in the production of specific volatiles through (transient) transformation in combination with behavioural assays – will remain an important tool in elucidating the exact role of individual VOCs in the communication of plants with other organisms.

REGULATION OF VOC BIOSYNTHESIS

Perhaps even more than for the biosynthetic genes, we are just scratching the surface in which our understanding of the regulation of VOC production is concerned. VOC production in flowers and upon herbivory, for example, is tightly controlled and regulated, developmentally in the case of flower volatiles, biochemically in the case of herbivores. Plants recognize herbivory from damage by herbivore associated molecular patterns (HAMPS), present in the oral secretions of the herbivore, or damaged associated molecular patterns (DAMPS), endogenous plant signals (Duran-Flores and Heil, 2016). This leads to the production of the defence-regulating hormones, predominantly jasmonoyl isoleucine (JA-Ile), and the induction of volatile production, with other hormones also playing a

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role, while herbivores try to produce effector proteins to counteract this induction (Vos et al., 2013; Kant et al., 2015; Villarroel et al., 2016).

In flowers, volatile production transcription factors of especially the MYB family have been demonstrated to regulate biosynthesis, especially of the benzenoids/phenylpropanoids, as reviewed by Ramya et al. (2017). The importance of MYB TFs in regulating flower scent is supported by the discovery of a MYB TF, LhODO1, in lily which seems to regulate volatile benzenoid/phenylpropanoid production (Yoshida et al., 2018), LhODO1 showed a diurnal rhythm in tune with the expression of shikimate pathway and PAL genes. LhODO1 expression was high in the strongly scented oriental hybrids and very low in wild lilies. Although the role of the bHLH (basic helix–loop–helix) transcription factors in regulating plant-specialized metabolism has become quite clear (Goossens et al., 2017), recent papers also point to their role in regulating volatile sesquiterpene production in Arabidopsis (Hong et al., 2012) and in volatile mono- and sesquiterpene biosynthesis in the type VI glandular trichomes of tomato (Xu et al., 2018). By using stable transformation in maize and transient activation assays in citrus, a role for AP2/ERF transcription factors in regulating a terpene synthase was illustrated (Li et al., 2015, 2017). Similarly, in kiwi NAC transcription factors were shown to bind to the promoter of terpene synthase 1 (Nieuwenhuizen et al., 2015). To allow for the identification of TFs that regulate terpene biosynthesis in the traditional Chinese medicinal plant Amomum villosum, He et al. (2018) used a combination of RNA sequencing and volatile analysis after induction by methyl jasmonate (MeJA) (He et al., 2018). Network analysis revealed correlations between putative terpene synthase Unigenes, terpene abundance and candidate WRKY TFs. Further analyses will be needed to prove that these WRKYs indeed regulate terpene biosynthesis.

On top of regulation by classical TFs, specialized metabolite biosynthesis is likely to involve transcriptional and post-transcriptional silencing, that is regulation via endogenous small non-coding RNAs (sRNAs). Although a substantial number of sRNAs has been computationally predicted to target genes in specialized metabolic pathways, very few have so far been experimentally validated (Kortbeek et al., 2018). microRNAs (miRNAs), although quantitatively by far a minor type amongst the total of small RNAs encountered, have been studied most extensively. In relation to specialized metabolites, there appears to be a pivotal role for miRNA156b (Schwab et al., 2005; Gou et al., 2011; Yu et al., 2015). Yu et al. (2015) showed that miR156b targets an SPL9-type transcription factor that directly binds the promoter of TPS21, thereby affecting (E)-β-caryophyllene biosynthesis in Arabidopsis (Figure 1). With that finding and the insight in the role of the conserved miR156 in developmental processes, the authors revealed a link between development and volatile biosynthesis, a link that was confirmed by Singh and Sharma (2017) studying the role of miR156b in cucurmin biosynthesis and rhizome development (Singh and Sharma, 2017).

Co-transcription, another intriguing form of regulation – through alternative splicing – is described by Liu et al. (2018b) in tea (Liu et al., 2018b). The authors found that CsLIS/NES is transcribed into two splicing forms: CsLIS/NES-1 and CsLIS/NES-2 with the latter lacking a 305 bp fragment at the N-terminus and is why the encoded protein is targeted to the cytosol, while CsLIS/NES-1 is targeted to the plastids. Upon expression in E. coli, both splice forms produce nerolidol and linalool from FDP and GDP, respectively (Figure 1). Expression in tobacco and silencing in tea leaves suggested that CsLIS/NES-1 in vivo acts as linalool synthase and CsLIS/NES-2 as a nerolidol synthase, although the experimental evidence for the latter is not very strong, possibly due to conjugation of the produced nerolidol (Houshyani et al., 2013). Intriguingly, only one of the two splice forms, CsLIS/NES-1, was induced by MeJA (Liu et al., 2018b). It would be interesting to see if this matches with the induction of linalool but not nerolidol (or the derived DMNT) upon JA application.

Volatile formation in plants is also effected by abiotic factors. Abscisic acid (ABA) seems to play a role in this regulation. Upon application of exogenous ABA to tomato the level of volatile compounds such as 1-penten-3-one, β-damascenone and benzaldehyde increased just as the expression of genes possibly involved in the biosynthesis of these volatiles, such as a lipoxygenase, alcohol dehydrogenase, carotenoid cleavage dioxygenase and a hydroperoxide lyase (Wu et al., 2018) (Figure 1). Promoter analysis showed that cis-acting elements involved in ABA responsiveness (ABREs) exist in some of these genes. In a very different study, a similar phenomenon was demonstrated. Drought increased the level of ABA in Salvia dolomítica and increased the production of sesquiterpenes and the expression of FDP synthase that produces the substrate for sesquiterpene biosynthesis (Caser et al., 2019). In a study on the influence of salt on volatile emission from tomato leaves, however, the results are less clear (Zhang et al., 2018b). The production of some volatiles is altered, as is the expression of some genes involved in volatile biosynthesis, but the relationship between the two is unclear. Clearly, we are making ample progress in identifying the transcription factors regulating VOC biosynthetic pathways. The networks in which these TFs operate remain to be elucidated as well as the role of sRNAs and epigenetic regulation, especially in the developmental regulation of floral volatile biosynthesis.

EMISSION OF VOCs

Work on transport of volatiles intensified some 5 years ago when it was realized that volatiles, just as other lipophilic
secondary metabolites, are likely to need active transport to be emitted by plants (Widhalm et al., 2015). Although there are still many caveats in our knowledge on transport (Tissier et al., 2017), there is also progress with the identification of the role of ABC transporters and LTP proteins as important players (Wang et al., 2016; Adebisin et al., 2017). Direct proof for the involvement of an ABC transporter in volatile emission in Petunia hybrida came from the silencing of PhABCG1, which resulted in a decrease in volatile emission (Adebisin et al., 2017). For the further unravelling of transport, particularly transcriptomics studies will be instrumental (Tissier et al., 2017). Intriguingly, in such a transcriptomics study in Petunia axillaris, an ABC transporter, PaABCG1, was reported with high homology to PhABCG1 (Amano et al., 2018). The authors did not carry out any functional characterization but discussed the fact that these types of ABCG transporters are mostly involved in transport of suberin and cuticle components. This also holds to some extent for the lipid transfer proteins for which Van der Krol and co-workers show evidence that they also play a role—in concert with an ABC transporter for transport across the cell membrane—in transport of volatile and non-volatile sesquiterpenoids across the cell wall (Wang et al., 2016). To what extent ABC transporters and LTPs play a role in the secretion of volatiles form the glandular cells of a trichome to the extracellular cavity remains to be investigated. A recent, elegant, opinion paper describes various scenarios on how this can be achieved (Tissier et al., 2017), but little information is known about this. This also holds true for how these volatiles are retained in the extracellular cavity as emission is often limited. Clearly, several questions remain and further studies are indeed needed to completely resolve the mystery of volatile transport and storage in plants (Tissier et al., 2017).

**AGRICULTURAL IMPORTANCE OF VOCs**

The multitude of biological effects of VOCs makes them an attractive target to try to improve agriculture but the complexity of these interactions also make the application a large challenge (Beck et al., 2016; Adebesin et al., 2017). An intriguing example of the integration of semiochemicals in agriculture is the control of stem borers through a ‘push–pull’ strategy (Pickett and Khan, 2016). Here, cereals were offered protection from the stem borer moth, Busseola fusca, by the intercropped Desmodium spp. that provide ‘the push’ through producing volatiles that repel the moth. ‘The pull’ comes from (E)-β-caryophyllene emitted by Napier grass that attracts the moth and moreover produces a viscous substance that kills the larvae of the stem borer (Khan et al., 2007). A variation on the push–pull system described above is a semiochemical-based pest management strategy that targets insect behaviour by using pheromones in a lure application (Mafra-Neto et al., 2013). The authors developed a matrix that provide a steady release of a variety of volatile compounds including attractants such as sex pheromones in combination with insecticides, but also repellents. The system is targeted to disrupt mating, repel or attract-and-kill insects. The authors provide successful examples of the latter for serious crop-pest species such as the fall armyworm (Spodoptera frugiperda) and the tomato pest, Tuta absoluta, both of which are lured to formula-treated host plants by insect-specific pheromones where they are killed by the insecticide. A formulation that releases verbenone, a repellent of the pine bark beetle Dendroctonus ponderosae, was shown to effectively keep the tree free of this
bark beetle (Mafra-Neto et al., 2013). The same holds for dimethyl disulfide, a repellent of the Asian citrus psyllid (Diaphorina citri) that transmits the Greening disease Huanglongbing (HLB), which severely threatens citrus production around the world (Mafra-Neto et al., 2013). As described above, the ubiquitous volatile terpene (E)-β-caryophyllene repels a number of agriculturally important pests, including psyllids (Zhang et al., 2017). Intercropping (with the (E)-β-caryophyllene emitting guava) or possibly the application of (E)-β-caryophyllene dispensers may provide solutions for this enormous agricultural problem (Mafra-Neto et al., 2013; Alquézar et al., 2017) (Figure 2f).

Insect control can potentially also be pursued using metabolic engineering of volatiles (Paul et al., 2016; Sun et al., 2016; Abbas et al., 2017; Tissier, 2018). In an attempt to change the resistance of chrysanthemum against aphids, Hu et al. (2018a) introduced chrysanthemol synthase that was isolated from pyrethrum and catalyzes the first committed step in pyrethrin biosynthesis, into chrysanthemum (Hu et al., 2018a) (Figure 1). Interestingly, this did not only result in the emission of the, expected, volatile chrysanthemol but also in accumulation of a chrysanthemol glycoside. Both compounds were shown to be produced through the transient expression of (E)-β-caryophyllene and sesquiterpene production increased. Moreover, the authors then tested overexpression of Arabidopsis 3-hydroxy-3-methylglutaryl-coenzyme A reductase, AtHMGR – considered the rate-limiting step in the mevalonate pathway – with MPD in tobacco and found a considerable further increase in both monoterpene and sesquiterpene production. Overexpression of these two genes in combination with the Santalum album santalene synthase resulted in almost 10-fold higher emission of α-santalene.

Zhang et al. (2018c) studied the effect of overexpression of two enzymes that catalyze an important, possibly rate-limiting, steps in the plastidic MEP pathway, 1-deoxy-d-xylulose-5-phosphate synthase (DXS) and 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR) (Zhang et al., 2018c). The encoding genes were isolated from Lilium ‘Siberia’ and heterologously overexpressed in tobacco of which the flowers produced more of the monoterpene, linalool (LiDXR overexpression line; as anticipated) but also of the sesquiterpene, caryophyllene (in both the LiDXR and LiDXS overexpression line), possibly as a result of increased IPP transport from the plastids to the cytosol as a result of the larger IPP pool produced in the transgenic lines (Figure 1).

In case the engineered volatiles needed to be stored in the plant rather than emitted, Delatte and colleagues invented an interesting engineering strategy in which oil bodies are heterologously introduced through transient expression of a diacylglycerol acyltransferase (DGAT), the transcription factor WRINK1 and oleosin (OLE1) in Nicotiana benthamiana (Delatte et al., 2018). This indeed resulted in the formation of oil bodies that were associated with the heterologous sesquiterpenes that were simultaneously produced through the transient expression of sesquiterpene synthases, together resulting in a 17-fold increased accumulation of volatile sesquiterpenes.

In conclusion, there are many promising scientific reports about the biological relevance of volatiles in the interaction of plants with other organisms. In these reports, the potential application of this knowledge in agriculture is usually mentioned. These possible applications include the use of volatiles in dispensers, or other matrices, the optimization of volatile production by plants through breeding or genetic modification and the application of selected microbes to change volatile emission by the plant. Particularly the use of natural enemies (that use volatile cues to find their prey), especially in greenhouses and the successful lure and kill strategies that employ attractive volatiles are examples that show that these approaches can work, despite the fact they have not really been optimized. The use of natural enemies for example has not, or hardly, been selected for despite the fact that scientists have shown there is genetic variation for HIPV induction in crops and that this results in difference in attractiveness of natural enemies (Kappers et al., 2011). Further research should show whether more intricate applications and optimization of the existing ones are possible such that they can be used to achieve a more sustainable agriculture.

CONCLUSIONS/FUTURE DIRECTIONS

The field of VOC research is going through an exciting phase. We are seeing intriguing new developments such as...
as the role of micro-organisms in VOC communication, producing volatiles themselves (Lemfack et al., 2017; Schenkkel et al., 2018), affecting the induction of HIPVs as endosymbionts of herbivorous insects (Frago et al., 2017) or changing the flower VOC blend because they colonize nectar (Vannette and Fukami, 2016; Rering et al., 2018). Also the elucidation of VOC transport, emission and accumulation and their perception in the receiver organism are hot topics in research and we are only just beginning to understand how specific volatiles and their blends convey information from one organism to the other and how flexible this process is. Advanced techniques such as genomics, transcriptomics and metabolomics will be associated with behavioural data using novel tools such as network analysis, large-scale data integration and machine learning approaches, facilitating the interpretation of the biological meaning of the complex VOC blends in various dimensions. With these advanced approaches, the discovery of biosynthetic genes will also further speed up, allowing – through genetic modification/metabolic engineering in combination with behavioural assays – to further underpin the role of individual VOCs in the communication of plants with other organisms. This also holds for the genes and regulatory mechanisms underlying the regulation of the – constitutive and induced – formation of VOCs and their transport and sequestration. Genetic screens using the response to specific VOCs will be used to identify receptors and signal transduction cascades (Scala et al., 2017). With regard to the use of scientific knowledge on VOCs in agriculture, the use of natural enemies in greenhouses and lure and kill strategies are examples that already prove that the biological effect of VOCs can be employed in agriculture. A further deepening of our knowledge on the mechanisms underlying plant communication with beneficial as well as harmful organisms will surely provide the basis for more intricate applications in a more sustainable agriculture.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**AUTHOR CONTRIBUTIONS**

All authors contributed equally to the writing and revising of the paper.

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