

# The formation and function of plant volatiles: perfumes for pollinator attraction and defense

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Plants synthesize and emit a large variety of volatile organic compounds with terpenoids and fatty-acid derivatives the dominant classes. Whereas some volatiles are probably common to almost all plants, others are specific to only one or a few related taxa. The rapid progress in elucidating the biosynthetic pathways, enzymes, and genes involved in the formation of plant volatiles allows their physiology and function to be rigorously investigated at the molecular and biochemical levels. Floral volatiles serve as attractants for species-specific pollinators, whereas the volatiles emitted from vegetative parts, especially those released after herbivory, appear to protect plants by deterring herbivores and by attracting the enemies of herbivores.

## Addresses

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## Abbreviation

BAMT benzoic acid carboxyl methyl transferase

## Introduction

When the 1998 Nobel Prize in Physiology or Medicine was awarded for the discovery that nitric oxide acts as a signal molecule in the cardiovascular system of animals, the Nobel Assembly proclaimed that “signal transmission by a gas that is produced by one cell, penetrates through membranes and regulates the function of another cell represents an entirely new principle for signaling in biological systems”. In plants, however, cell-to-cell signal transmission by gaseous substances was recognized much earlier [1], as was gaseous signaling in a broader sense. The gaseous plant hormone ethylene was described in 1934, and it has been realized since antiquity that plants emit a variety of other volatile compounds from flowers and vegetative parts that exert effects on the behavior of other organisms. Nevertheless, with the exception of ethylene, little was known about the physiology and metabolism of plant volatiles until recently.

In the past few years, enormous strides have been made in understanding the biosynthetic routes to plant volatiles and the molecular mechanisms that regulate their formation. In addition, many plant volatiles have been implicated in

defensive and attractive roles. This progress is due both to general advances in biochemical and molecular techniques and to the development of new instrumentation for the collection and analysis of volatile substances [2]. Here, we summarize some of the highlights of plant volatile research published during the past few years, emphasizing the diverse assemblage of metabolites that are emitted from flowers and vegetative parts. We ignore ubiquitous substances such as oxygen, carbon dioxide, and the hormone ethylene, which has recently been covered elsewhere (e.g. in [3]). All of the compounds discussed are known to be emitted as volatiles from plants (as evidenced by their detection in headspace collections) even though most are liquids, rather than gases, at room temperature.

## Volatiles from flowers

The myriad of fatty-acid derivatives, benzenoids, terpenoids, nitrogen-containing compounds and other scented substances emitted from flowers [4] must surely rank as one of nature’s best-loved treasures. Although it seems self-evident that flowers emit scent to attract pollinators, there has been little experimental work to demonstrate the attractiveness of individual scent components to specific pollinators. Such proof requires detailed knowledge of the chemistry of floral fragrances coupled with sensitive behavioral assays on pollinators. An outstanding recent contribution in this area concerns the spectacular flowers of certain orchid species that are visited by male solitary bees who appear to mistake them for the body of a female bee (Figure 1). While trying to copulate with the flower, the males pick up pollen, which they transmit to the next flower they visit. It had been recognized previously that floral scent is an important factor in deceiving the bees. Until recently, however, the particular scent components responsible for the deception could not be conclusively identified.

Improved techniques for the collection of volatiles and for their gas chromatography (GC)- electroantennographic detection (EAD) have made it possible to show that the chemicals that elicit the ‘pseudocopulatory’ behavior of *Andrena nigroaenea* males towards flowers of the orchid *Ophrys sphegodes* are simple C<sub>21</sub>-C<sub>29</sub> straight-chain alkanes and alkenes [5]. These compounds are found in both receptive female *A. nigroaenea* and plant cuticles, but they are present in the scent of *O. sphegodes* in the ratios found in female bees rather than in those typical of plant cuticles. In another exciting chapter of this story [6\*], after pollination, *O. sphegodes* flowers have been found to emit farnesyl hexanoate, a compound that is usually released by non-receptive female *A. nigroaenea* to inhibit copulation. Thus, these orchids emit odors that are characteristic of either receptive or non-receptive female bees depending on the

Figure 1

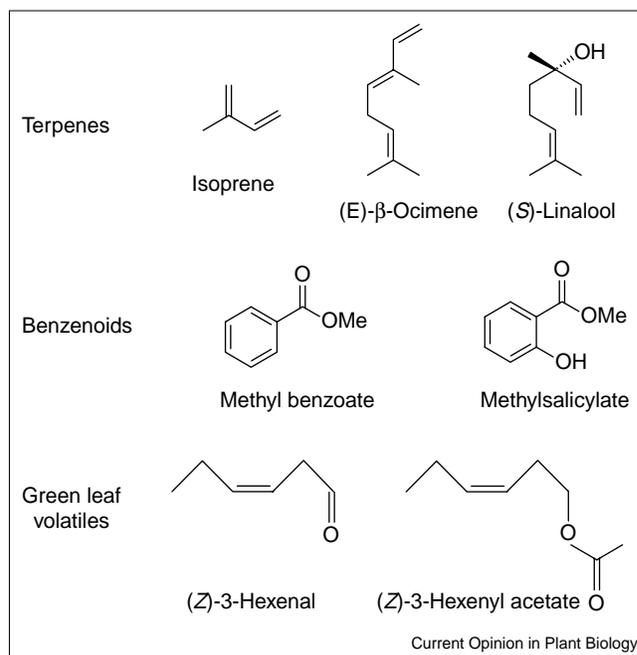


Flower volatiles may induce pollination by deceit. A flower of the orchid *Ophrys sphegodes* with its pollinator, a male of the solitary bee species *Andrena nigroaenea*. The flower mimics the appearance and odors of a female *A. nigroaenea*, inducing the male to attempt copulation, which results in pollination [5]. Copyright F Schiestl, with permission.

stage of floral development. Deterring floral visitors after pollination may help to minimize damage to the developing seed and direct pollinators to adjacent unpollinated flowers.

The role of individual volatiles in pollinator attraction can be elegantly tested by genetic manipulation of floral emission using appropriate mutants and transformants. An essential prerequisite for this work is knowledge of the biosynthesis of floral volatiles, a field that has enjoyed rapid progress in recent years. The initial breakthrough came when (*S*)-linalool synthase was purified from flowers of *Clarkia breweri* [7,8]. This was the first enzyme of floral volatile formation to be purified, and provided information on amino-acid sequence that facilitated the isolation of the corresponding gene, which is expressed solely in the flowers [9]. The characterization of additional genes that are involved in the biosynthesis of floral volatiles in *C. breweri* followed, including those encoding enzymes for the synthesis of the phenylpropanoids methyl eugenol and methyl isoeugenol [10], benzyl acetate [11], and methyl salicylate [12] (Figure 2 shows the structures of some of these compounds). These investigations revealed that scent formation is regulated principally by transcriptional control of biosynthetic gene expression at the site of

Figure 2



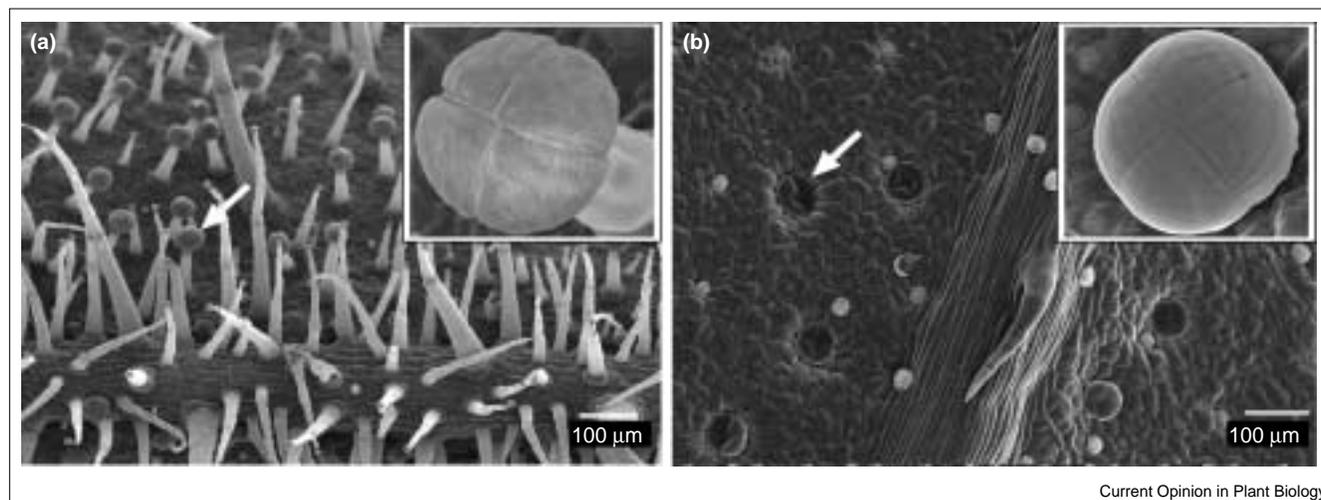
Structures of some representative plant volatiles that are discussed in the text.

emission, that is, in the epidermal cells of various floral parts, especially the petals.

Even more recently, a wealth of information on the biosynthesis of floral volatiles has come from studies of the common garden snapdragon, *Antirrhinum majus*. A gene encoding the enzyme benzoic acid carboxyl methyl transferase (BAMT), which produces one of the principal floral volatiles of this species, methyl benzoate, was isolated and shown to be flower-specific [13,14]. The expression of BAMT, which belongs to the same family as salicylic acid carboxyl methyl transferase (SAMT), is tightly correlated with the synthesis of methyl benzoate [13,15••]. Likewise, the expression of (*S*)-linalool synthase is closely correlated with the synthesis of (*S*)-linalool in *C. breweri*. The gene encoding BAMT is expressed exclusively in epidermal cells of the petals, some of which have a conical shape that increases their surface area [15••]. Interestingly, there is a higher concentration of the enzyme in parts of the petal that are closer to the path that bees take to reach the nectar, including the 'hairs' (i.e. unicellular glands) found in the center of the basal petal [15••]. Hence, the scent may serve as guide for bees to find their way inside the flower.

Snapdragon flowers emit methyl benzoate at a much higher rate during the day than during the night, and this oscillation is controlled by a circadian clock mechanism [16•]. However, the level of BAMT does not appear to be a rate-limiting factor in methyl benzoate emission. The supply of the substrate, benzoic acid, is much more tightly correlated with emission than is BAMT

Figure 3



Volatiles in vegetative plant tissues are often stored in surface glands that rupture on contact with herbivores. (a) Leaves of a wild tomato (*Lycopersicon hirsutum*) and (b) leaves of sweet basil (*Ocimum basilicum*) have several types of glands. The 'peltate' glands, identified in both pictures by white arrows and shown in the insets, contain most

of the volatile compounds, which are stored as liquids. The *L. hirsutum* peltate gland consists of four cells mounted on a long stock of 2–3 cells. The basil peltate gland also consists of four cells, is attached to the leaf surface by a single, short stock cell, and is recessed into the leaf surface. Pictures courtesy of (a) J Wang and (b) D Gang.

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activity. Therefore, control of the circadian rhythm of emission must reside in an earlier step of the biosynthetic pathway [16•].

The identification of genes for the production of floral scent opens up new opportunities to alter floral scent for research or commercial purposes. For example, the (*S*)-linalool synthase gene from *C. breweri* was recently fused to a 35S promoter and transferred into petunia [17•]. Although the gene was expressed everywhere in the plant, and linalool was made in many tissues, no linalool was emitted from the transformed plants. In fact, all of the linalool was found to be present as a non-volatile conjugate, linalool- $\beta$ -D-glucopyranoside. Clearly, the production of volatiles may depend not only on the availability of substrate and the requisite biosynthetic enzymes but also on the absence of further metabolism. In addition, volatile emission may require an active excretion process, but nothing is yet known about the actual mechanism of release.

### Volatiles from vegetative parts

A walk through a conifer forest or a Mediterranean shrubland on a warm day provides convincing evidence that plants release substantial quantities of volatiles from vegetative as well as floral organs. However, the study of the physiology and function of these non-floral perfumes is still in its infancy.

Among the most widespread non-floral volatiles is isoprene (Figure 2), a simple C<sub>5</sub> terpene that is released from the foliage of many species, especially trees. Several recent contributions have added significantly to our knowledge of isoprene function. By blocking isoprene formation with

fosmidomycin, an inhibitor of the non-mevalonate pathway, Sharkey and coworkers [18] sharply suppressed the rate of photosynthesis of kudzu and red oak leaves at elevated temperatures. Rather than simply increasing the general thermal tolerance of photosynthesis, they suggest that isoprene helps the photosynthetic apparatus to recover from brief episodes of temperatures over 40°C, which are often experienced, even in temperate environments, by sunlit leaves at the tops of trees. Isoprene may act by physically stabilizing the thylakoid membranes at high temperatures [18] or by quenching reactive oxygen species, such as ozone, which can lead to membrane damage [19]. The recent cloning of a gene for isoprene synthase (a member of the terpene synthase family), which catalyzes the formation of isoprene from dimethylallyl diphosphate [20••], now makes it possible to manipulate isoprene production more carefully in order to study the role and mode of action of this compound.

The emission of other volatiles from foliage is thought to help protect plants against herbivores. For example, two species of trees belonging to the Brazil nut family (Lecythidaceae) that emit high levels of *S*-methylmethionine are colonized by wood-boring beetles at much lower frequency than other tree species in the same family that emit only minute amounts of *S*-methylmethionine [21], an interesting correlation that requires further investigation. Plant volatiles can also promote indirect defenses, such as those that help to protect the African tree *Leonardoxa africana* from herbivores. Ants of the species *Petalomyrmex phylax* patrol young but not old leaves of this tree and attack any phytophagous insects that they encounter. As the young leaves, but not the old

leaves, emit high levels of methyl salicylate, the authors hypothesize that the ants are attracted by methyl salicylate and make use of it either as a pheromone or as an antiseptic in their nests [22].

### Volatiles released after herbivory: function

Most reports on the emission of volatiles from plant foliage involve emission from herbivore-damaged plants. A wealth of terpenoids, fatty-acid derivatives and other substances, such as indole and methyl salicylate, are released from plants after bouts of herbivory. In some cases, these compounds are released when feeding ruptures pre-existing internal or external secretory structures in which the volatiles are synthesized and stored (Figure 3; [23•]). In other cases, volatiles are formed at the moment of damage. For example, the C<sub>6</sub> aldehydes, alcohols and esters known as 'green-leaf volatiles', because they embody the typical odor of damaged leaves, are derived via lipoxygenase cleavage of fatty acids within seconds of injury. Other volatiles emitted from herbivore-damaged plants are synthesized *de novo* several hours or even days after the onset of herbivore feeding [24].

Following the pioneering efforts of Marcel Dicke, Ted Turlings, Jim Tumlinson and others (for reviews, see [25–27]), it has been established that herbivore-induced volatiles often serve as indirect defenses, attracting insects and mites that prey upon or parasitize herbivores, and thereby reduce further damage to the plant. Three recent contributions now implicate herbivore-induced volatiles in direct defense as well. First, the green-leaf volatile (*Z*)-3-hexenyl acetate (Figure 2) and other aliphatic esters of (*Z*)-3-hexen-1-ol that are emitted by tobacco after damage were found to deter female *Heliothis virescens* moths from laying eggs on injured plants [28•]. Interestingly, the profile of emitted tobacco volatiles is different at night than during the day, and it was the nocturnal blend of volatiles, which is rich in (*Z*)-3-hexen-1-ol esters, that was most repellent to the night-active *H. virescens*.

Second, the volatiles of another tobacco species, *N. attenuata* of the Great Basin desert in the southwestern United States, were found to act as both direct and indirect defenses, deterring lepidopteran oviposition as well as attracting herbivore enemies [29•]. Significantly, this was the first demonstration that damage-induced volatiles have a defensive function in natural, non-agricultural settings. Third, the green-leaf volatiles, hexanal and (*Z*)-3-hexenal (Figure 2) were suggested to be involved in the resistance of potatoes to the aphid *Myzus persicae*. This finding was based on experiments with a transgenic potato line that contained reduced levels of these aldehydes owing to antisense suppression of a hydroperoxide lyase that participates in their biosynthesis [30•]. It is possible that in this case, as in others, the volatiles contribute to defense at least in part by their direct toxicity to insects.

Other important advances in research on herbivore-induced plant volatiles include the discovery that *Arabidopsis thaliana* also releases a mixture of substances when fed upon by herbivores. Feeding by *Pieris rapae* larvae induces *A. thaliana* to emit a blend of volatiles consisting principally of simple aliphatic compounds, terpenoids and methyl salicylate that is attractive to a larval parasitoid of *P. rapae* [31•]. Hence, the extensive array of genetic resources available for *A. thaliana* can now be employed to help elucidate the formation, signaling and ecological roles of induced volatiles.

Just as volatiles induced by the herbivory of insect larvae attract parasitoids of larvae, so volatiles that are induced by egg laying can attract parasitoids of the eggs [32]. Oviposition of the elm leaf beetle (*Xanthogaleruca luteola*) on the elm *Ulmus minor* triggers the release of a volatile blend consisting principally of terpenoids and green-leaf volatiles that attracts the hymenopteran egg parasitoid *Oomyzus gallerucae* [33].

Plant volatiles have the intriguing potential to influence not only animals but also other plants in the vicinity. Indeed, the notion that plants could have evolved the ability to perceive herbivore-induced volatiles that are emitted by a neighboring plant and to respond to this information by activating their own defenses seems eminently reasonable, given that the presence of herbivores on one plant increases the probability that nearby plants will be attacked. Nevertheless, experiments carried out over the past 20 years that attempted to demonstrate this phenomenon have been regarded skeptically [34•]. However, a recent field investigation showed convincingly that *N. attenuata* plants growing adjacent to artificially wounded *Artemisia tridentata* (sagebrush), with air but no soil contact between the plant species, suffered reduced levels of herbivore damage and exhibited increased levels of the defensive enzyme polyphenol oxidase compared to *N. attenuata* plants growing adjacent to undamaged sagebrush [35•]. Meanwhile, a laboratory study on *Phaseolus vulgaris* revealed that undamaged plants exposed to volatiles from a herbivore-infested neighbor (or to several pure terpene standards) had elevated expression of several genes involved in defense metabolism [36•]. Similarly, *Vicia faba* plants exposed to (*Z*)-jasmone, a volatile jasmonate metabolite emitted from flowers and damaged tissues, had elevated levels of the monoterpene  $\beta$ -ocimene and an increased steady-state level of mRNA for an  $\alpha$ -tubulin isoform [37]. These results should stimulate renewed curiosity about the possibilities of communication between damaged and undamaged plants.

Now that we have recognized that herbivore-induced plant volatiles appear to mediate both direct and indirect defenses, and even signal to nearby plants, interest in their evolutionary origin and value to the plant seems certain to increase. Whatever the original reasons for the induced emission of volatiles, if emission came to be closely associated with herbivore damage during the

Table 1

## Selection of biosynthetic steps in the formation of herbivore-induced volatiles for which genes have recently been isolated.\*

Enzyme	Species	Volatiles formed	Reference
<b>Terpenoid volatiles</b>			
(S)-Linalool synthase	<i>C. breweri</i>	(S)-Linalool	[9]
Myrcene/ocimene synthase	<i>A. thaliana</i>	Myrcene, Ocimene	[48]
(E)- $\beta$ -Farnesene synthase	<i>Mentha x piperita</i>	(E)- $\beta$ -Farnesene	[49]
	<i>Citrus junos</i>		[50]
(E)-Nerolidol synthase	<i>Cucumis sativus</i>	(E)-Nerolidol (intermediate to dimethyl-nonatriene)	[51]
(enzyme activity only, gene not yet isolated)	<i>Phaseolus lunatus</i>		[51]
	<i>Zea mays</i>		[52]
<b>Green leaf volatiles</b>			
Lipoxygenase	<i>Solanum tuberosum</i>	13-Hydroperoxy-linolenic acid (intermediate to green leaf volatiles)	[53]
Hydroperoxide lyase	<i>Cucumis melo</i>	9-Oxononanoate, (3Z)-Nonen-1-al	[54]
Hydroperoxide lyase	<i>S. tuberosum</i>	1-Hexenal, (3Z)-Hexen-1-al	[30*]
<b>Other types of volatiles</b>			
Indole-3-glycerol phosphate lyase	<i>Z. mays</i>	Indole	[43*]
Salicylate methyltransferase	<i>C. breweri</i>	Methyl salicylate	[12]
Jasmonate methyltransferase	<i>A. thaliana</i>	Methyl jasmonate	[55]

\*Although all of the genes listed are involved in producing volatiles, they do not necessarily produce herbivore-induced volatiles in the species from which they were isolated.

course of evolution, it would become a reliable cue for herbivore enemies (telling them where to find their prey), for herbivores (providing information about the potential presence of competitors or enemies, or about the reduction in the food quality of an attacked plant because of the induction of its direct defenses), and for neighboring plants (telling them when to expect herbivore attack). To demonstrate that induced volatiles are valuable to the plant, it is necessary to show that the defensive benefits they bestow cause a measurable increase in reproductive fitness. This task is especially critical when parasitoids are attracted to damaged plants by volatiles, because a parasitized insect is not immediately killed and may continue feeding. Two recent studies showed that parasitism of herbivores does indeed help the plant. Plants that were fed upon by parasitized lepidopteran larvae had significantly greater seed production than plants fed upon by unparasitized larvae [38,39], suggesting that volatiles that attract parasitoids do play a valuable role in plant defense.

### Volatiles released after herbivory: formation

Further studies on the value to plants of herbivore-induced emission of volatiles would be facilitated by the ability to manipulate precisely volatile quantity and composition through the altered expression of genes encoding steps in their biosynthesis. Many genes encoding specific steps in the biosynthesis of terpenoids, green-leaf volatiles and other types of volatiles that are induced by herbivory have recently been isolated (Table 1), and can be used for this purpose.

Another method of changing damage-induced emission is to manipulate the signal transduction pathway that is

initiated by herbivore feeding. Specific elicitors found in the oral regurgitant of lepidopteran herbivores have the ability to trigger volatile emission. Most attention has focused on volicitin, *N*-(17-hydroxylinolenoyl)-L-glutamine, an elicitor isolated from *Spodoptera exigua* larvae that have fed on maize [40,41], but other active fatty-acid conjugates have been isolated from other lepidopteran species [42]. Volicitin has been shown to stimulate the expression of genes involved in the biosynthesis of indole and sesquiterpenes in maize [43\*,44]. Jasmonic acid and related substances also trigger the formation of herbivore-induced volatiles in various species [45–47], suggesting the participation of an octadecanoid signaling cascade in this process.

### Conclusions and future prospects

For plants rooted in one place, the release of volatile compounds provides a way to communicate with organisms across distances. The enormous variety of metabolites emitted by plants suggests that volatile compounds may provide a detailed language for communication. We are just beginning to decipher the content and significance of these messages and identify the intended recipients. Recent advances in the biochemistry and molecular biology of plant volatile biosynthesis have provided new tools for evaluating the natural roles of these substances, and for investigating the mechanisms underlying their regulation and evolution.

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