Surface-to-air signals

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Powerful volatile regulators of gene expression, pheromones and other airborne signals are of great interest in biology. Plants are masters of volatile production and release, not just from flowers and fruits, but also from vegetative tissues. The controlled release of bouquets of volatiles from leaves during attack by herbivores helps plants to deter herbivores or attract their predators, but volatiles have other roles in development and in the control of defence gene expression. Some of these roles may include long-distance signalling within and perhaps between plants.

The plant kingdom abounds with natural chemicals, many of which are volatile. These molecules are chemically diverse, being represented by fatty acid derivatives, terpenes, indole and molecules from other chemical families. Ethylene[1], a molecule implicated in both development and defence, was the first gaseous hormone discovered in nature. It now seems likely that new and fundamental insights could emerge from the study of other plant volatiles that can act as signals within the plant, or can be exported selectively, changing the immediate environment of the producer, its neighbours and attackers.

Far from being passive in the face of attack, plants use many remarkable strategies to increase their chances of survival[2]. Some of the most elegant of these strategies involve the release of specific blends of volatiles. In response to herbivores damaging a single leaf, whole plants release a complex array of volatiles. Maize leaves, for example, release a mixture containing several terpenes, including linalool, in response to elicitors present in the regurgitant of beet armyworm larvae[3]. A powerful elicitor of volatile release, volicitin, has been purified from this secretion and identified as a conjugate of 17-hydrolinolenic acid and L-glutamine[4]. The bouquets of volatiles released in response to attack can have several effects that, in the field, have great importance for plant survival[5]. Chief among these is attraction of predators to the feeding herbivore, but other strategies are being discovered. At night, tobacco plants release a characteristic bouquet in response to the feeding larvae of nocturnal moths[6]. The bouquet repels further egg-ladened female moths from ovipositing on the tobacco leaves. These, and many other studies[7,8], underlie the tremendous importance volatiles play in signalling to insects, but can volatiles also affect neighbouring plants?

Volatile signalling within and between plants

A remarkable example of plant-to-plant signalling, probably unrelated to a role in defence, comes from tobacco plants in which dominant ethylene insensitivity has been engineered through introduction of the *Arabidopsis etr1-1* allele[9]. Normally, the leaves of wild-type tobacco plants tend to stop growing as they approach neighbouring tobacco plants — this may stop them wasting energy producing leaves that would be shaded from useful light. But the transgenic tobacco plants lacked normal social behaviour and their leaves grew over and among the leaves of their neighbours. This is a good indication of plant-to-plant signalling under laboratory conditions, but whether ethylene is a social signal in the field is as yet unclear, as is any role for ethylene in interplant defence signalling. A second example of intraspecific signalling in tobacco involves defence and methyl salicylate. This compound is released from wild-type tobacco leaves infected with tobacco mosaic virus (TMV)[10], a virus that causes necrotic lesion formation in the genotype of tobacco used for these laboratory experiments. Methyl salicylate produced by infected plants both increases the resistance of neighbouring uninfected tobacco plants to TMV infection and also induces the expression of the defence gene *PR1* in uninfected plants. Similar to the development of visual necrotic symptoms, release of methyl salicylate is blocked when infected plants are incubated at 32 °C, and commences when the temperature is lowered to 24 °C. This allows elegant control of volatile release in this laboratory system, although so far there is no evidence for a role of methyl salicylate as a plant-to-plant signal in nature.

One of the best studied volatile signals in plants is the fragrant compound methyl jasmonate (MJ), which has been studied as a volatile signal *in planta* and also in laboratory and field experiments in plant-to-plant signalling. The compound is the methyl ester of 3*R*,7*S*-jasmonic acid (Fig. 1), and both are potently active as regulators of gene expression[11,12]. Production of methyl jasmonate can be manipulated *in vivo* using an *Arabidopsis* gene encoding jasmonic acid carboxyl methyltransferase (JMT)[13], which methylates non-volatile jasmonic acid to produce volatile methyl jasmonate. *JMT* has been expressed constitutively in *Arabidopsis* under the control of a powerful viral promoter. The *JMT* transgenic plants had threefold-increased levels of methyl jasmonate compared with wild-type plants, although levels of jasmonic acid were unchanged. The plants were resistant to a fungal pathogen and showed constitutive expression of an inducible defence gene (*PDF1.2*) encoding an antimicrobial peptide. The study raises tractable questions, such as whether or not the conversion of jasmonic acid to methyl jasmonate is a control point *in vivo*. This might be addressed by strategies aimed at lowering *JMT* expression in *Arabidopsis* and other species. It is clear that volatile regulators of gene expression, acting as hormones, can affect the individual that produces them, but it is also possible that atmospheric transport of a signal from its source to distal parts of the same individual could occur (the term 'automone' might be suitable for such compounds).

Interspecific airborne signalling (Fig. 2) involving methyl jasmonate is also known and may relate to defence against wounding organisms. Many and perhaps most plants do not release significant levels of the compound. But it is produced by some flowers, including scented jasmine, as well as in the leaves of several species of *Artemisia*, most

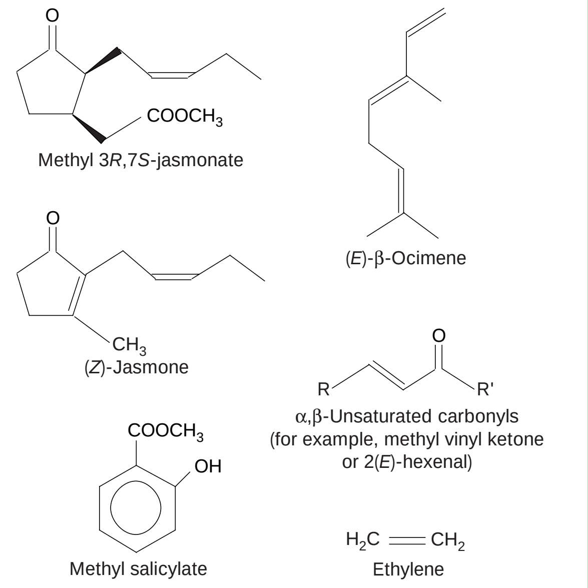

Figure 1 Volatile regulators implicated in plant defence and plant-to-plant information transfer. For α,β-unsaturated carbonyl-containing molecules, R and R′ are small substituents such as protons, methyl groups or alka(e)nyl groups; (*Z*)-jasmone would also fall into this category. In the laboratory, exposure to volatile regulators activates defence gene expression, but very low levels of the compounds, as might occur in nature, could possibly prime or sensitize the defence system of receiver plants, perhaps allowing them to respond faster to future attack.

notably big sagebrush (*A. tridentata*), a plant that dominates large areas of the Great Basin in the western United States. Placing tomato plants and sagebrush branches together for two days in a closed container resulted in the strong accumulation of defence-related proteinase inhibitors in the leaves of tomato. This was found to be due to the release of methyl jasmonate from the sagebrush[14].

This experiment inevitably led researchers to question whether long-distance methyl jasmonate signalling could take place in the field and, if so, what is its significance?[15,16] Karban *et al.*[17] have now addressed the first of these questions. In their experiment, tomato was replaced by a wild tobacco species, *Nicotiana attenuata*, which can grow in the presence of sagebrush in nature. Airborne levels of methyl jasmonate at a distance 3 m from sagebrush plants were found to be below the limit of detection. Next, ~10% of sagebrush leaves on a plant were removed or damaged and a gas trap was fit onto a damaged branch. Within one hour, a 6.5-fold increase of 3*R*,7*S*-methyl jasmonate was registered, proving damage-induced release of a biologically active enantiomer of methyl jasmonate in the field. As a presumed consequence of its release, natural herbivore damage to *N. attenuata* was reduced significantly in tobacco plants growing within 15 cm of the clipped sagebrush. Blocking air contact between sagebrush and tobacco prevented these effects. There is a complementary and perhaps simpler explanation for methyl jasmonate release by sagebrush, a role in plant–plant competition. Because methyl jasmonate is so physiologically active, it is possible that release of the compound could interfere with the growth of neighbouring plants — this aspect deserves much more attention.

Links between plant–insect and plant–plant signalling

Yet more candidate airborne plant-to-plant signals have been reported for bean plants (*Phaseolus lunatus*) infested with herbivorous spider mites (*Tetranychus urticae*). In response to attack, infested leaves release volatiles that can increase the resistance of uninfested leaves to attack by spider mites[18,19], as well as inducing the expression of several defence-related genes in neighbouring uninfected lima bean leaves. β-Ocimene (Fig. 1) and two other related terpenoids are thought to be responsible for this effect[19]. These compounds were shown recently to activate the expression of a number of defence genes in detached bean leaves, but it will be important to test whether the compounds upregulate gene expression in intact bean plants[8]. Potentially related to this work is the interesting biology of (*Z*)-jasmone, a product of jasmonic acid metabolism[20]. (*Z*)-Jasmone released by plants was found to be electrophysiologically active in insects, both herbivores and their predators[21]. Treatment of healthy bean (*Vicia faba*) plants with (*Z*)-jasmone induced both (*E*)-β-ocimene release and also α-tubulin gene expression[21]. One could speculate that, in bean, (*Z*)-jasmone treatment causes release of β-ocimene, which itself activates gene expression, but at present the evidence is lacking. The physiological relevance of α-tubulin gene expression is not yet known, but it is clear that because the expression of other, as yet uncharacterized genes might be affected, the α-tubulin gene serves as a good marker. What is evident from these and other studies is that some compounds, like (*E*)-β-ocimene and (*Z*)-jasmone, can affect both insect behaviour and gene expression in plants, and whenever a molecule is implicated in plant–plant signalling its relevance in plant–insect signalling should be investigated.

Interplant communication: universality or opportunism?

Interplant communication involving airborne regulators of gene expression can occur in the laboratory and might also occur in the field. But it is too early to make generalizations about the frequency of this phenomenon in nature and, as the distances between plants increase, good evidence for plant-to-plant signalling becomes scarcer. Is the phenomenon likely to be universal or restricted to a few examples? An argument against universality concerns chance, opportunity and the rich diversity of molecules made in the plant kingdom. Even considering that the release of volatiles from flowers and leaves is highly controlled and sensitive to many environmental factors[22,23], a few molecules capable of eliciting gene expression will be released, here and there, in the plant kingdom. Therefore, it might be simply a question of putting the right plant species together in the laboratory to see an effect. Furthermore, if intraspecific plant–plant airborne signalling was widespread, there might be problems for the plants concerned. Volatile release might, in some cases, serve to inform congeners of impending attack, but it could be used opportunistically by neighbouring, competing species, which might gain a selective advantage if they could perceive this valuable information. To avoid this, one could imagine selection pressure to develop species-specific volatile signals, a process that could lead to signal diversification in nature. Alternatively, the plant might just cease releasing the volatile in question.

An argument for universality, at least in intraspecific systems, is the number of reports of plant-to-plant signalling where airborne

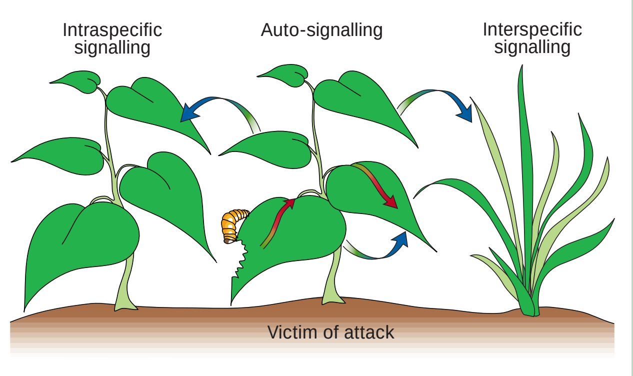

Figure 2 Communicating danger with airborne signals. Four modes of signalling from or within diseased or wounded plants are indicated: signalling to healthy congeners, signalling to members of other species, or auto-signalling either within (arrow in leaf) or outside the plant body. Good evidence exists for plant-to-plant airborne signalling in the laboratory, but field studies are limited.

signals are implicated but have yet to be characterized[7,24–29]. Recent work on European black alder trees (*Alnus glutinosa*) showed that intact alders close to manually defoliated individuals subsequently showed decreased herbivore damage compared with more distant individuals, with airborne signals from the damaged trees implicated[28]. It will take a sustained effort to bring systems such as these into the molecular arena where the signals produced in the field can be characterized. But it would be worth the effort. Perhaps dense swards of grass would also offer an attractive searching ground for new intra- and interspecific airborne signalling systems?

Volatile electrophiles

Some compounds may have escaped detailed attention with regard to activating gene expression in diseased tissues. These include the electrophile 2(*E*)-hexenal, which is produced by many trees and shrubs, particularly upon wounding, and also as an odour component of various fruit, including cucumber, banana and apple. 2(*E*)-Hexenal is a biocidal molecule that is produced in response to bacterial pathogenesis[30]. Although described many years ago as a widespread volatile antibiotic[31], the broader biological significance of 2(*E*)-hexenal production has received remarkably little attention during the past 20 years. Recently, this compound has been shown to induce the accumulation of sesquiterpenoid phytoalexins in wounded cotton[32] and, in common with a hexenyl acetate isomer(s), induced stress-related gene expression in *Arabidopsis*[33]. 2(*E*)-Hexenal (but not isomers of hexenyl acetate) contains an α,β-unsaturated carbonyl group, and it seems likely that this electrophilic feature in a molecule will confer the ability to induce stress and defence responses in plants[34]. This small reactivity feature alone, presented in volatile form as acrolein, leads to cell damage and to the powerful expression of the glutathione-*S*-transferase 1 (*GST1*) gene in *Arabidopsis*[34]. Electrophiles such as 2(*E*)-hexenal or acrolein are susceptible to nucleophilic attack, for example Michael addition, but it is not known whether they have to undergo chemical reactions in the plant cell in order to produce their effects.

Reactive electrophile species probably are crucial in microbial disease, perhaps even contributing to the damage of cells undergoing hypersensitive (programmed) cell death (ref. 34, and see review in this issue by Lam, Kato and Lawton, pages 848–853). Their volatile counterparts could also be considered in future studies as candidates in information transfer from plant to plant. However, because they are antibiotics, it is possible that the simple absorption of released electrophiles onto the leaves of a healthy plant could lead to increased 'resistance' to microbial pathogens. Some studies on volatile signalling in plants[27] need re-evaluating in this light.

Each of the many facets of research on volatiles — plant–insect signalling, intraplant signalling and plant–plant signalling — could have exciting applications. In the quest for new ways to control insect pests, the chemical induction of volatile release has great practical potential. This has been demonstrated with tomato plants that were induced (by spraying with jasmonic acid) to release a volatile blend attractive to parasitic wasps. Caterpillars feeding on these chemically induced plants were more often victims to the parasites than were caterpillars feeding on control plants[35]. Some volatiles can be remarkably powerful regulators of gene expression in plants and their exploitation in engineered plant defence strategies is now on the horizon, with candidate genes such as *JMT*[13] already in hand. □

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