

Volatile signaling in plant–plant–herbivore interactions: what is real?

Ian T Baldwin*, André Kessler, Rayko Halitschke

Plants release volatiles after herbivore attack in a highly regulated fashion. These compounds attract natural enemies and function as indirect defenses. Whether neighboring plants ‘eavesdrop’ on these volatile signals and tailor their defenses accordingly remains controversial. Recent laboratory studies have identified transcriptional changes that occur in plants in response to certain volatiles. These changes occur under conditions that enhance the probability of signal perception and response. Field studies have demonstrated repeatable increases in the herbivore resistance of plants growing downwind of damaged plants.

Addresses

Max Planck Institute for Chemical Ecology, Department of Molecular Ecology, Winzerlaer Strasse 10, Jena 07745, Germany
*e-mail: baldwin@ice.mpg.de

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Abbreviations

FAC	fatty-acid–amino-acid conjugate
HI	herbivore-induced
JA	jasmonic acid
MeJA	jasmonic acid methyl ester
PAL	phenylalanine ammonia lyase
VOC	volatile organic compound

Introduction

Plants are masters of gas exchange, not only literally building forests from gases taken from the air but also releasing complex bouquets of volatile organic compounds (VOCs) back into the air. This remarkable ability fuels the expectation that plants communicate through volatile signals. Although ‘communication’ is a loaded term that means different things to different researchers, most would accept a definition with the minimal requirement that information be exchanged, regardless of ‘intent’ or fitness consequence for either party. Two decades ago, researchers reported that wounding or herbivore attack resulted in changes in the herbivore resistance, or to the secondary metabolites that mediate this resistance, not only of the attacked plants but also of plants growing nearby. In some experiments, aerial transfer of information was the most parsimonious way in which the results could be interpreted [1], causing the phenomena to be dubbed ‘talking trees’ by the popular press. Given that neighboring plants compete for resources and that selection is unlikely to favor plants that provide information to competitors, the phenomena should be more aptly called ‘eavesdropping elms’. Experiments published in the past two years have been highlighted in reviews [2–4] and have rekindled interest in these phenomena. Last year, evidence for inter-plant

communication was compiled in a special issue of *Biochemical Systematics and Ecology* [5••]. Here, we review the evidence concerning how emissions are controlled, the signals involved, and the responses of downwind plants. We also summarize the challenges for future research.

Regulation of the composition, and the temporal and spatial patterns, of VOC release

After herbivore attack, plants release complex bouquets of volatiles into the air from their vegetative tissues. The release of some constituents is likely a passive consequence of damage to the compartments (e.g. vacuoles or trichomes) in which VOCs (or their precursors) are stored. The release of other constituents has been demonstrated to result from *de novo* synthesis and is tightly controlled. Even metabolites that occur in substantial pools in undamaged leaves may be actively discharged. For example, mechanical damage to *Artemisia tridentata* leaves causes the release of large amounts of jasmonic acid methyl ester (MeJA) into the air under field conditions. When compared to the pools of MeJA found in the leaves, the epimeric composition of the released MeJA is highly enriched in the thermodynamically unstable and biologically active enantiomer (i.e. 3R,7S MeJA) [6•], suggesting that the released material is newly synthesized or somehow epimerized during release.

Flowers and other reproductive organs are known to discharge complex blends of VOCs with distinct temporal patterns, and evidence is emerging that herbivore-induced (HI) releases from vegetative tissue are similarly regulated. The mechanisms that control floral emissions are only just being examined [7•,8], and the molecular and physiological controls over HI releases are not understood. Most HI-VOCs can also be found in the floral headspace of some species and are derived from phenolic, terpenoid and fatty-acid metabolic pathways that utilize both stored reserves and recently fixed carbon. After herbivore attack, HI-VOCs are released both locally from damaged tissues and systemically from undamaged tissues in discrete temporal patterns. Some constituents are emitted at maximum levels during daylight hours and become undetectable at night [9,10•], others have nocturnal maxima [11]. With the recent development of instrumentation that allows the real-time analysis of emission patterns [12••], the temporal and spatial complexity of these patterns will be more readily characterized.

Wounding plays an important role in eliciting the VOC release. In some plant species, mechanical damage can provoke releases of the same VOCs as are elicited by herbivory. In many plant species, however, the HI-VOC release differs from that elicited by mechanical wounding. Exogenous

jasmonic acid (JA) treatments can trigger a VOC release [13], but the exact ratios of constituents in such VOCs can sometimes differ from those in VOCs released after herbivore attack [9,10*,14]. When herbivores attack, they not only cause damage but also introduce saliva-derived compounds to the wound sites. Fatty-acid–amino-acid conjugates (FACs) in herbivore saliva have been shown to elicit both an endogenous JA burst as well as a HI-VOC release in native tobacco [15]. If these FACs are removed from the oral secretions, eliciting activity is lost, but is regained when synthetic FACs are added back [15]. These results demonstrate that FACs probably activate the endogenous jasmonate cascade in a manner that differs from that effected by wounding. A FAC containing a 17-hydroxy functionality (i.e. volicitin), which causes a HI-VOC release in corn but not in lima bean [16], was found to be just as effective or less effective in eliciting a HI-VOC release than exogenous applications of JA [10*]. Whether or not volicitin elicits a JA burst that is associated with the HI-VOC release in corn remains to be determined.

The study of HI-VOC releases has focused primarily on species other than *Arabidopsis*, largely because the small stature and low emission rate of *Arabidopsis* challenge the analytical sensitivity of volatile detection systems. A recent study solved this problem by using short-day conditions to prolong vegetative growth, thereby producing abnormally large *Arabidopsis* plants [17*]. This strategy allowed Van Poecke *et al.* to demonstrate that plants infested with *Pieris rapae* larvae attracted *Cotesia rubecula* parasitoid females, and that this attraction was correlated with greater emissions of methyl salicylate, myrcene and two nitriles. The attractiveness of the nitriles, which are likely derived from larval feces, is noteworthy as the recently cloned *TASTY* locus, which confers susceptibility to feeding by *Trichoplusia ni* larvae, encodes genes that are responsible for nitrile biosynthesis [18]. These results suggest that nitrile production may attract both herbivores and their predators. *Arabidopsis*' small stature makes it unlikely that the attraction of parasitoids or predators by volatile emissions is under current selection as an indirect defense in this species. Nevertheless, the powerful genomic tools available in *Arabidopsis* make it a valuable system for the identification of signal cascades and downstream genes that are involved in VOC release.

Are plants the receiver for VOC emissions?

Predators and parasitoids of insect herbivores are clearly attracted to HI-VOC releases [19], which have been shown to function as a powerful indirect defense for plants [14]. Whether or not other plants respond to these emissions remains controversial, largely because the experimental conditions under which 'communication' has been demonstrated are seldom found in nature. Receptors are known for the volatile intra-plant signal ethylene, but not for the larger molecular weight volatiles of the HI-VOC release. Without a receptor and signal transduction system to amplify the signal, information transfer from plant to plant would probably

depend on the diffusion and convection of the volatile signal between sender and receiver. It would therefore require a high release concentration or accumulation after exposure. Moreover, if large quantities of the signal must be released, plants must face the challenge of being insensitive at their release sites while remaining sufficiently sensitive to perceive the signal after the many-fold dilution that inevitably occurs during air transport.

Most studies of plant–plant communication through volatiles have exposed plants to unrealistically high concentrations of putative volatile signals and have enclosed them in air-tight chambers without replenishing CO₂ for long periods of time. When plants that are below their CO₂ compensation points are fumigated, they are likely to receive abnormally high exposures to HI-VOCs. Moreover, many researchers have used excised leaves or shoots rather than intact plants in their assays. The use of excised leaves increases the probability of a response for two reasons. First, as has been demonstrated in corn [10*] and lima bean [20], excised leaves release more HI-VOCs than do intact plants. Second, excised leaves are more sensitive to HI-VOCs than are intact plants. Working with the lima bean system, Arimura and coworkers [20] demonstrated that excised leaves responded to HI-VOCs by increasing transcripts encoding the pathogenesis-related proteins β-1,3 glucanase (Pr-2) and chitinase (Pr-3), phenylalanine ammonia lyase (PAL), lipoxygenase (LOX) and farnesyl pyrophosphate synthetase (FPS). When intact plants were exposed to the same volatile treatments, however, transcription of the *LOX* and *FPS* genes did not increase [20]. This excellent study also highlights another trend that increases the chances of seeing a plant–plant response: the use of transcriptional changes as a response variable. Transcriptional changes are likely rapid indicators of a response that do not necessarily represent a full commitment to a change in defense phenotype. Nevertheless, they demonstrate that the plant has perceived the signal and provide valuable insight into the environmental signals that induce a response.

Although most studies of plant–plant communication sacrifice ecological realism to increase the likelihood of seeing a response, the results provide tantalizing hints as to how signals could be examined under more realistic conditions. The most sustained effort, from work by Takabayashi and colleagues with lima bean plants [21**], has demonstrated that intact plants that are exposed to HI-VOCs in sealed chambers increase the transcription of *Pr-2*, *Pr-3* and *PAL*, as well as of genes involved in ethylene biosynthesis (i.e. those encoding S-adenosylmethionine [SAM] synthase, 1-aminocyclopropane-1-carboxylic acid oxidase [ACO] and SAM decarboxylase [SAMDC]). When exposed to HI-VOCs in the cuvette of an open flow photoacoustic spectrophotometer, so as to permit the measurement of ethylene release in real time, plants emitted more ethylene [21**]. This is consistent with the transcriptional control of ethylene release in responses to HI-VOCs. Interestingly, the research by

Takabayashi and colleagues also demonstrated an important role for green leaf volatiles, that is C6-alcohols and aldehydes, in eliciting the PR-protein transcriptional responses in uninfested leaves [20]. These results are consistent with earlier findings that suggest an intra-plant signaling function for green leaf volatiles [22,23]. Clearly, more attention should be given to the potential signaling roles of compounds with α - β -unsaturated carbonyl groups [4].

Results from field studies raise fewer concerns about ecological realism, but the replication of results and identification of responsible mechanisms can be onerous in this research. Work on inter-plant signaling between native tobacco and sagebrush by Karban and colleagues [24–26] represents the best-replicated study to date. In five consecutive field seasons, the mechanical clipping of sagebrush increased the herbivore resistance of native tobacco plants that were transplanted to within 10–15 cm of the clipped sage relative to the resistance of tobacco plants transplanted to be adjacent to unclipped sage. Experiments from one field season strongly implicated aerial (as opposed to below-ground) transfer of information [24]. Clipping of sagebrush dramatically increased the release of the biologically active enantiomer (3R,7S) of MeJA [24], but ongoing research [6*] has not confirmed that this constituent is the biologically active signal.

Conclusions and future prospects

The increasing sensitivity of analytical instrumentation has recently allowed plant VOC emissions to be characterized in real time [12**,27]. Unless receptors for these putative volatile signals are discovered, however, similar advances will be required to characterize plant ‘immisions’: the signals entering a plant. If the ongoing transcriptional analyses identify genes that are strongly regulated by HI-VOCs, these will likely provide a source of promoters that could be fused to easily characterized reporters (e.g. β -glucuronidase [GUS] or green fluorescent protein [GFP]). Plants that have been transformed with such reporter genes could provide much needed information about how plants respond to volatile signals under natural conditions. While some researchers continue to look for evidence of aerial communication, others are examining communication below ground [28]. Below-ground signaling, in contrast to VOC signaling, limits the dialog to immediate neighbors and competitors, and is not affected by wind direction, but will require additional sophisticated approaches to disentangle it.

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