REVIEW

Volatile Signaling in Plant-Plant Interactions: "Talking Trees" in the Genomics Era

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Plants may "eavesdrop" on volatile organic compounds (VOCs) released by herbivore-attacked neighbors to activate defenses before being attacked themselves. Transcriptome and signal cascade analyses of VOC-exposed plants suggest that plants eavesdrop to prime direct and indirect defenses and to hone competitive abilities. Advances in research on VOC biosynthesis and perception have facilitated the production of plants that are genetically "deaf" to particular VOCs or "mute" in elements of their volatile vocabulary. Such plants, together with advances in VOC analytical instrumentation, will allow researchers to determine whether fluency enhances the fitness of plants in natural communities.

Plants excel at gas exchange: They can literally build forests from CO_2 taken from the air at about 120 Pg C year^{-1}, half of which is respired back to the atmosphere. Up to 36% of the assimilated carbon is released as complex bouquets of VOCs (1). Although some of these VOCs may be mere waste, others mediate various pollination and defense mutualisms with animals. These VOC-mediated interactions of plants with organisms of higher trophic levels suggest that they communicate similarly with each other (2). Two decades ago, researchers serendipitously discovered changes in herbivore resistance and secondary metabolites in plants ("receivers") growing adjacently to herbivore-attacked plants ("emitters"). Because in some experiments results were best explained by the aerial transfer of information (3), the phenomenon was popularly dubbed "talking trees." This phrase seems unfortunate, because selection most likely favors plants that "eavesdrop" on VOCs released from neighbors and respond by tailoring their phenotypes to enhance their own fitness.

What Are Plants Talking About?

An obvious conversation topic concerns impending attack from mobile herbivores, and most VOC-elicited responses have been in-terpreted accordingly. Measures of herbivore performance have been broadened to include the elicitation of various direct plant defenses (e.g., phenolics, alkaloids, terpenes, and defense proteins). Indirect defenses have also attracted attention, including food rewards that increase predation pressure on herbivores (4) and VOCs that help predators or parasitoids locate feeding herbivores (5, 6). Moreover, the signal cascades that elicit direct and indirect defenses have been scrutinized (7, 8) as have transcriptional responses (9–12) (Fig. 1).

VOC exposure alone, without actual herbivore attack, may directly increase the production of defenses. Alternatively, VOC exposure may allow nearby plants to ready their defenses for immediate use once the herbivores move from the neighboring plant to attack the "listening" receiver. Exposure to volatiles from damaged sagebrush primes the elicitation of defensive proteinase inhibitors (PIs) in wild tobacco, and exposed plants subsequently receive less damage (13–15) (Fig. 2). Corn seedlings previously exposed to either individual components or to the entire blend of VOCs released from herbivore-attacked seedlings responded to simulated herbivory with increased VOC production and higher jasmonate (JA) accumulations compared with the responses of unexposed plants (8). Whether these enhanced VOC emissions protect corn seedlings remains to be determined. The priming of defense cascades may benefit plants that would incur fitness costs by activating defense responses (16), particularly in the absence of herbivore attack (17). If VOC exposure directly elicited defense responses, receiver plants would incur similar fitness costs without being damaged.

Hence, plants that avoided investing fitness-limiting resources in the production of costly defenses before an herbivore arrives, but were able to prime defense metabolism to launch defense responses when attacked, could realize a fitness benefit over plants that "ignored" the information coded in the VOCs emanating from their damaged neighbors.

The use of microarrays that monitor a large fraction of the plant's transcriptome can free analysis from observer bias about plants' conversation topics and identify selective pressures other than impending attack from mobile herbivores, which volatile signaling could be used to anticipate. Herbivores frequently transmit pathogens, and the elicited responses may concern attack by impending pathogens more than attack by herbivores (18). The relentless competition with other plants for resources that cannot be readily hoarded (such as light and nutrients) is likely the most important selective force for plants. Plants are able to anticipate impending competition through far red (FR) light signals and changes in the photon flux of blue light transmitted through their neighbors' canopies. These light signals are perceived by photoreceptors (e.g., phytochrome B) and elicit a complex of traits known as the shade-avoidance syndrome (SAS) (19). Experiments with tobacco plants transformed with a mutant ethylene receptor (etr1-1), which inhibits ethylene perception, have demonstrated that ethylene-insensitive tobacco could not respond rapidly to FR signals and consequently was outcompeted by wild-type plants (20). At concentrations apparently possible in dense plant canopies, ethylene by itself elicits the SAS (21). Similarly, exposure to unidentified VOCs from barley cultivars changes the allocation of biomass between roots and shoots without influencing biomass production of receiver barley genotypes (22), a re-allocation that may influence competitive ability. Thus, responses to the most important environmental factors in a plant's life may be anticipated by signals from neighboring plants. Almost anything can be a signal as long as it can be perceived and provides reliable information.

What Does It Take to Be a Signal?

Four steps characterize the transfer of VOC signals between plants: the release of the signal by the emitter plant and its transport, absorption, and perception by the receiver plant (Fig. 1). All are influenced by the signal's properties and its biological context. Most research on signal release has focused on the activation of biosynthetic enzymes and their substrate supply. The biochemical control mechanisms for the major VOC constituents are rapidly being clarified (Fig. 1). However, the release of foliar VOCs is also controlled by their physico-chemical properties (23): Volatility is deter-

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mined by partitioning the compound between the ''liquid'' phase of the leaf and the atmosphere, whereas molecular size and stomatal aperture constrain diffusive transport from the leaf into the air surrounding the leaf, its headspace. Once released into the headspace of the emitter, the potential signal has to be transported to receivers. Direction and dynamics of this transport are dictated by temperature, convective transport, and wind for above-ground signaling or water for below-ground signaling. Small highly volatile compounds (e.g., ethylene, methanol, isoprene, acrolein, methacrolein, and some monoterpenes) diffuse rapidly into the headspace and are diluted in the atmosphere (Fig. 2). For such compounds, signaling function is likely limited to the foliage of the emitter (as a systemic within-plant signal) and of neighbors with intertwined canopies. Heavier compounds with less volatility, such as terpene alcohols, methyl jasmonate (MeJA), aromatic compounds including methyl salicylate (MeSA), and green-leaf volatiles (GLVs), are more likely to function as signals over longer distances, because their comparatively slower dispersal allows development of plumes of higher concentrations (24) that may be carried farther as intact parcels by turbulent flow (Fig. 2). During transport, some VOC species are oxidized or otherwise processed in the atmosphere (1), possibly causing dilution but also activation. The concentration gradients, which ultimately regulate the receiver's exposure, remain largely uncharacterized. An example of a characterized concentration gradient comes from a study of corn seedlings that release the volatile sesquiterpene (E)-β-caryophyllene into the soil from their roots, a below-ground plume used by entomopathogenic nematodes to locate root-attacking beetle larvae (25).

Signal volatility and diffusion rates, as well as the stomatal conductance of re-

ceiver plants, define the last steps in the signal transfer process: adsorption at the plant surface and uptake into the leaf via stomatal openings or cuticle diffusion. The low concentration gradient between atmosphere and leaf during the adsorption step amplifies the effects of the signal's physicochemical properties. Transport into the receiver leaf is influenced by stomatal conductance. The limited air volume of a sealed chamber increases VOC concentrations and also reduces CO_2 once the chambers are illuminated because of photosynthetic carbon fixation. Under such conditions, plants increase the number of open stomata, enhancing exposure of mesophyll cells to the VOCs. Therefore, sealed chambers are likely to influence the responsiveness of receiver plants, and studies that use them are more likely to report ecologically insignificant results.
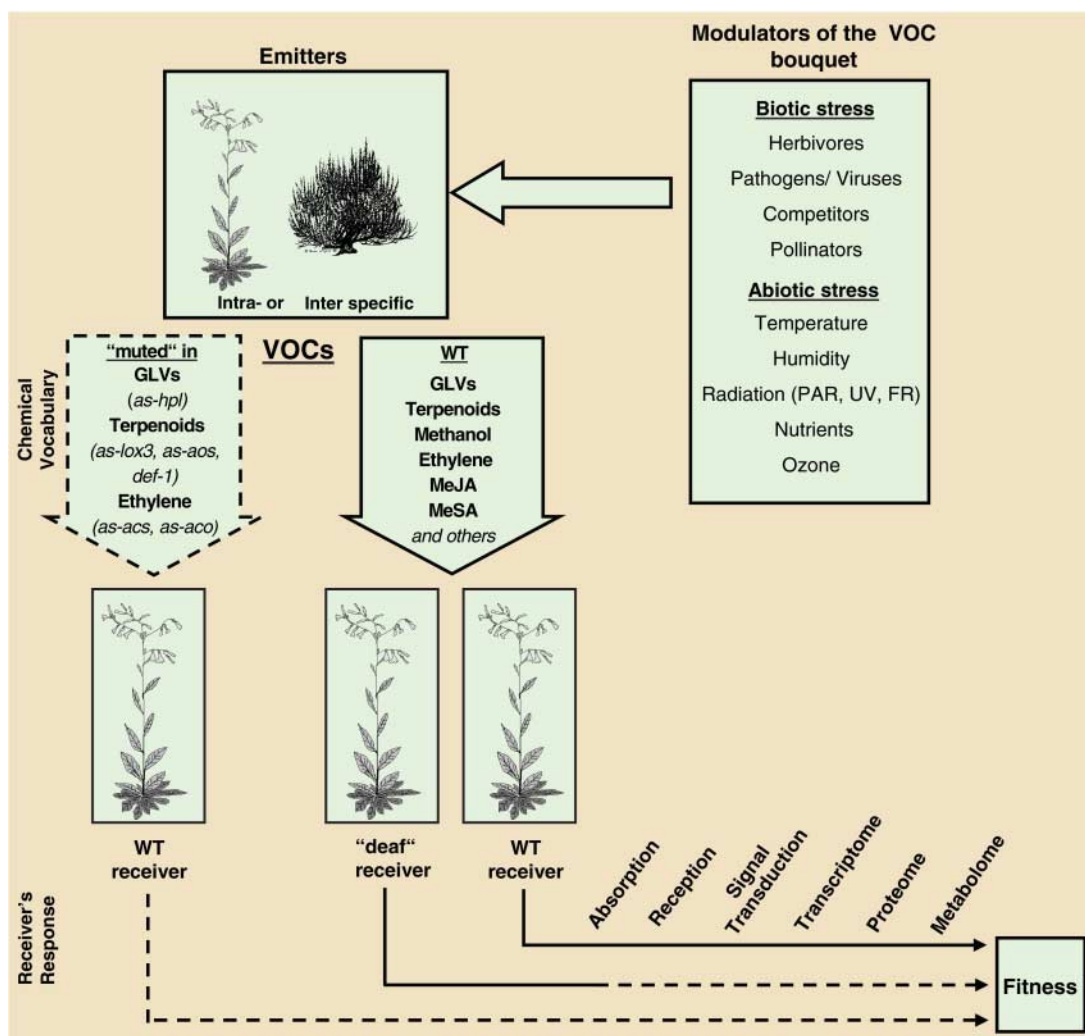
Fig. 1. Scheme of plant-plant interaction mediated by VOCs emphasizing the use of genetically manipulated plants to investigate the mechanisms underlying this process. Plants (e.g., wild tobacco) can be exposed to VOCs released from either conspecifics or from emitters of different species (e.g., sagebrush). The VOC bouquet of stressed plants consists of GLVs, terpenoids, MeJA, MeSA, methanol, ethylene, and other substances (32). Various biotic and abiotic stress factors modulate the chemical vocabulary emitted in quantity, quality, and timing. If the signal is recognized by the receiver plant, it may respond with changes in its signal transduction, transcriptome, proteome, and metabolome, which may or may not result in functionally significant changes in its fitness (→). Comparing responses to wild-type (WT) emitter plants with responses to mute emitters (--→) whose VOC bouquet is deficient in one or more VOCs allows researchers to identify compounds mediating the interaction between emitters and receivers. In addition to insertional mutants [e.g., def-1 (33)], various transgenic lines are generated by the expression of endogenous genes in antisense (as) orientations to silence enzymes necessary for eliciting or synthesizing VOCs, such as hydroperoxide lyase [HPL (34, 35)], lipoxygenase [LOX3 (36)], allene oxide synthase [AOS (35)], 1-aminocyclopropane-1-carboxylic acid synthase [ACS (37)], or 1-aminocyclopropane-1-carboxylic acid oxidase [ACO (38)]. These lines represent possible mute emitters. Deaf receiver plants, such as the etr1-1 line (39) impaired in functional VOC receptors for individual substances, could be used to verify each individual VOC's bioactivity.

Once a VOC enters the leaf, a response will only occur if the compound is "active," a poorly understood condition. Several proposed between-plant signals have hormone or hormone-like functions, including MeSA (26), GLVs (8, 9, 27), ethylene (28), and MeJA (29). However, proof that any of these are released and transported to receiver plants in quantities sufficient to elicit responses under natural conditions is either lacking or belies a signal function (10, 13, 30). Although most studies of bioactivity have examined whether the presence of a VOC elicits a response, removing certain components from a volatile bouquet can also elicit a response. The removal of GLVs from the wound-induced volatile blend by silencing hydroperoxide lyase strongly influenced the regulation of gene expression in neighboring conspecific tobacco plants (10). In other words, plants may respond to the "sounds of silence."

A class of electrophilic α,β-unsaturated carbonyl compounds represents potent regulators of gene expression (11). Although exposure to these highly volatile compounds increased the production of endogenous phytohormones, their activity was partially independent of the JA, SA, and ethylene signal cascades. A redox-based signal process, generated by the depletion of cellular reductants resulting from the electrophile reactivity of these compounds, suggests a mechanism for their activity that resembles the activation of the regulatory protein for pathogen defense, NPR1 (31). Similar processes may provide the basis of a general chemical "sense," which may have predated the evolution of receptors for particular volatiles.

Ecological Realism: "Deaf" and "Mute" Plants to the Rescue

Constitutive and herbivore-induced VOC emissions are influenced by a variety of abiotic factors [nutrient availability, temperature, wind, ultraviolet (UV) radiation and photosynthetically active radiation (PAR), and ozone exposure]. To lessen this variability, most studies of plant-plant signaling have been performed in the laboratory under experimental conditions (sealed or low air-flow chambers) that maximized the probability of detecting responses in receiver plants by increasing exposure [reviewed in (10)]. Although this work has shown that plants respond to being fumigated, its ecological relevance will remain unclear until the responses are verified in open-grown plants.

One solution to the problems of ecological realism in between-plant signaling studies is to use mutants or transgenic plants whose ability to either release or perceive particular components of the wild-type volatile blend is deficient. The use of "mute" emitters (10) allows complex herbivore-induced VOC blends to be dissected (Fig. 1). Complementation studies, in which synthetic constituents supplement the volatile blend to determine whether the receivers' response is subsequently restored, confirm function. The biosynthetic pathways contributing constituents to the herbivore-induced volatile bouquet and their regulatory cascades represent possible genetic targets. Mutants whose
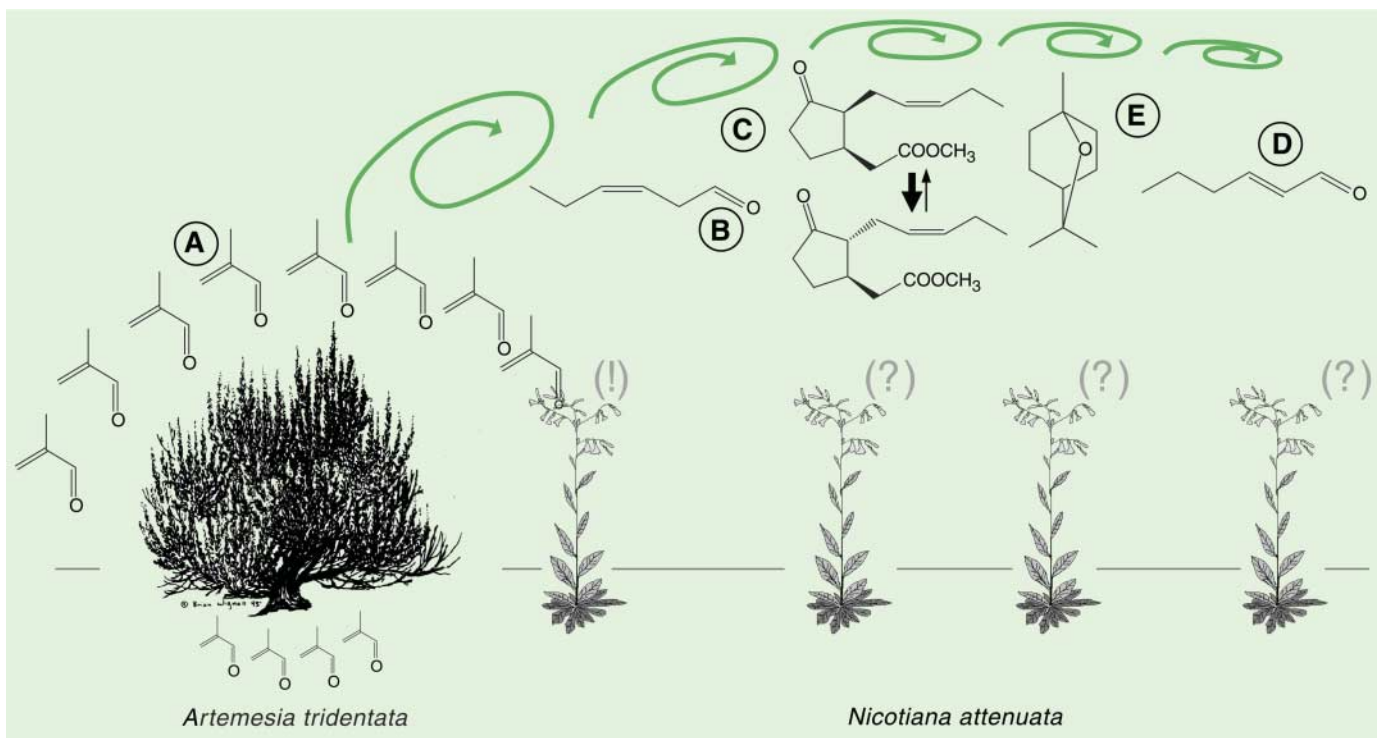
Fig. 2. Aerial interaction of the wild tobacco (*Nicotiana attenuata*) and sagebrush (*Artemisia tridentata tridentata*) (40) is the best-documented example of between-plant signaling via above-ground VOCs in nature (14, 15, 41). When transplanted to within 15 cm of clipped sagebrush, tobacco plants suffered less herbivory and produced more seed capsules than did plants transplanted adjacent to undamaged sagebrush. Damaged sagebrush releases a variety of VOCs, which are composed of highly volatile substances that disperse by diffusion, namely, methacrolein (A) and less volatile compounds such as GLVs [e.g., *cis*-3-hexenal (B) and *trans*-2-hexenal (D)], oxygenated monoterpenes [e.g., cineole (E), thujone, and camphor] and the epimers of MeJA (C), which are likely transported by turbulent flow in fragmented plumes. The plume from damaged sagebrush is highly enriched in the *cis* epimer of MeJA, which is thermodynamically unstable but putatively more biologically active than the *trans* epimer (14, 30, 42). Hence, MeJA was the most obvious candidate for the volatile signal mediating the response; subsequent studies were unable to confirm that either epimer of MeJA elicited known herbivore defenses when applied in quantities relevant to those released by damaged sagebrush (30, 42). Rather than directly eliciting defenses, exposure to volatiles from excised sagebrush foliage (and two constituents of its aromatic headspace: *trans*-2-hexenal and methacrolein) primes defense responses, so that plants increase the production of their defense protein, PI, faster when attacked (13). The progress in this system highlights the difficulty of predicting how plant-plant signaling functions from first principles.

herbivore- or wound-induced vocabularies have been modified by silencing genes involved in either the biosynthesis of particular volatiles or the oxylipin signal cascade represent potential mute emitter plants (Fig. 1).

Mutants whose perception of specific VOCs is impaired ("deaf" plants) represent another tool for analyzing the consequences of VOC signaling as illustrated by the ethylene-insensitive tobacco plants, *etr1-1*. The produce industry long ago developed a sophisticated ethylene trapping and releasing technology, but the first clear demonstration of the functional significance of ethylene signaling in competitive interactions required plants that were "deaf" to this VOC (*20*). Receptors for most of the herbivore-induced VOCs remain to be discovered, but transcriptional responses to VOC exposure can be used in mutant screens to identify new VOC receptors. Identification of these genetic elements and the creation of VOC-reporter plants [with β-glucuronidase (GUS) or green fluorescent protein] will allow researchers to readily determine the quantity of signals that are perceived by receivers at different distances from an emitter. Combining deaf and mute plants with wild-type plants in natural settings will clarify the relevance of VOC signaling for a plant's performance and/or fitness in the real world. Because differences in performance among plants that are unable to produce or perceive certain volatiles are likely to be subtle, the analysis will likely require long-term studies in natural settings. The more deaf plants that are available to complement the growing list of available mute plants, the more tools researchers will have to fully evaluate the significance of volatile signaling among plants in natural settings. These experiments will determine whether being a native speaker enhances a plant's fitness in its community.

References and Notes

1. J. Kesselmeier et al., *Global Biogeochem. Cycles* **16**, 1126 (2002).
2. M. Dicke, A. A. Agrawal, J. Bruin, *Trends Plant Sci.* **8**, 403 (2003).
3. I. T. Baldwin, A. Kessler, R. Halitschke, *Curr. Opin. Plant Biol.* **5**, 351 (2002).
4. C. Kost, M. Heil, *Basic Appl. Ecol.* **6**, 237 (2005).
5. J. Ruther, S. Kleier, *J. Chem. Ecol.* **31**, 2217 (2005).
6. Y. Choh, T. Shimoda, R. Ozawa, M. Dicke, J. Takabayashi, *J. Chem. Ecol.* **30**, 1305 (2004).
7. G. Arimura et al., *Plant J.* **29**, 87 (2002).
8. J. Engelberth, H. T. Alborn, E. A. Schmelz, J. H. Tumlinson, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 1781 (2004).
9. N. J. Bate, S. J. Rothstein, *Plant J.* **16**, 561 (1998).
10. A. Paschold, R. Halitschke, I. T. Baldwin, *Plant J.,* in press.
11. E. Alméras et al., *Plant J.* **34**, 205 (2003).
12. G. Arimura et al., *Nature* **406**, 512 (2000).
13. A. Kessler, R. Halitschke, C. Diezel, I. T. Baldwin, *Oecologia,* in press.
14. R. Karban, I. T. Baldwin, K. J. Baxter, G. Laue, G. W. Felton, *Oecologia* **125**, 66 (2000).
15. R. Karban, J. Maron, *Ecology* **83**, 1209 (2002).
16. M. Heil, I. T. Baldwin, *Trends Plant Sci.* **7**, 61 (2002).
17. I. T. Baldwin, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 8113 (1998).
18. E. S. Jimenéz-Martinéz et al., *Environ. Entomol.* **33**, 1207 (2004).
19. C. L. Ballaré, *Trends Plant Sci.* **4**, 97 (1999).
20. R. Pierik, E. J. W. Visser, H. De Kroon, L. Voesenek, *Plant Cell Environ.* **26**, 1229 (2003).
21. R. Pierik, G. C. Whitelam, L. Voesenek, H. de Kroon, E. J. W. Visser, *Plant J.* **38**, 310 (2004).
22. V. Ninkovic, *J. Exp. Bot.* **54**, 1931 (2003).
23. U. Niinemets, F. Loreto, M. Reichstein, *Trends Plant Sci.* **9**, 180 (2004).
24. H. W. Thistle et al., *Forest Sci.* **50**, 610 (2004).
25. S. Rasmann et al., *Nature* **434**, 732 (2005).
26. V. Shulaev, P. Silverman, I. Raskin, *Nature* **385**, 718 (1997).
27. G. Arimura, R. Ozawa, J. Horiuchi, T. Nishioka, J. Takabayashi, *Biochem. Syst. Ecol.* **29**, 1049 (2001).
28. T. Tscharntke, S. Thiessen, R. Dolch, W. Boland, *Biochem. Syst. Ecol.* **29**, 1025 (2001).
29. E. E. Farmer, C. A. Ryan, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7713 (1990).
30. C. A. Preston, G. Laue, I. T. Baldwin, *Biochem. Syst. Ecol.* **29**, 1007 (2001).
31. X. Dong, *Curr. Opin. Plant Biol.* **7**, 547 (2004).
32. P. W. Paré, J. H. Tumlinson, *Plant Physiol.* **114**, 1161 (1997).
33. J. S. Thaler, M. A. Farag, P. W. Pare, M. Dicke, *Ecol. Lett.* **5**, 764 (2002).
34. G. Vancanneyt et al., *Proc. Natl. Acad. Sci. U.S.A.* **98**, 8139 (2001).
35. R. Halitschke, J. Ziegler, M. Keinanen, I. T. Baldwin, *Plant J.* **40**, 35 (2004).
36. R. Halitschke, I. T. Baldwin, *Plant J.* **36**, 794 (2003).
37. P. W. Oeller, L. M. Wong, L. P. Taylor, D. A. Pike, A. Theologis, *Science* **254**, 437 (1991).
38. A. J. Hamilton, G. W. Lycett, D. Grierson, *Nature* **346**, 284 (1990).
39. J. Hua, C. Chang, Q. Sun, E. M. Meyerowitz, *Science* **269**, 1712 (1995).
40. B. Wignall, in *Shrubs and Trees of the Southwest Deserts*, J. E. Bowers, Ed. (Western National Parks Association, Tucson, AZ, 1993), p. 19.
41. R. Karban, M. Huntzinger, A. C. McCall, *Ecology* **85**, 1846 (2004).
42. C. A. Preston, G. Laue, I. T. Baldwin, *J. Chem. Ecol.* **30**, 2193 (2004).
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REVIEW

Plant Volatile Compounds: Sensory Cues for Health and Nutritional Value?

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Plants produce many volatile metabolites. A small subset of these compounds is sensed by animals and humans, and the volatile profiles are defining elements of the distinct flavors of individual foods. Flavor volatiles are derived from an array of nutrients, including amino acids, fatty acids, and carotenoids. In tomato, almost all of the important flavor-related volatiles are derived from essential nutrients. The predominance of volatiles derived from essential nutrients and health-promoting compounds suggests that these volatiles provide important information about the nutritional makeup of foods. Evidence supporting a relation between volatile perception and nutrient or health value will be reviewed.

Plants are capable of synthesizing tens to hundreds of thousands of primary and secondary metabolites with diverse biological properties and functions. Plant volatile organic compounds (defined hereafter as volatiles) generated from both primary and secondary metabolites are generally low molecular weight lipophilic compounds (*1*, *2*). More than 7000 flavor volatiles have been identified and cataloged from foods and beverages (*3*, *4*). Many volatiles are produced in plant tissues at specific developmental stages—for example, during flowering, ripening, or maturation. Although a single fruit or vegetable synthesizes several hundred volatiles, only a small subset generates the "flavor fingerprint" that helps animals and humans recognize appropriate foods and avoid poor or dangerous food choices.

Although perception of flavor is often described as a combination of taste and smell (*5*), appearance, texture, temperature, mouth feel, and past experience also play major roles in flavor perception, indicating that multiple distinct sensory inputs are processed to generate the overall sensation (Fig. 1). Integration of this sensory information in the brain ultimately results in a flavor preference or aversion with a strong influence on subsequent perception and behavior. Studies of flavor preferences and aversions suggest that flavor perception may be linked to the nutritional or health value associated with the perceived foods (*6–11*). For example, fatty acids that stimulate taste responses are essential long-chain cis-polyunsaturated fatty acids rather than nonessential saturated fatty acids (*11*). Flavor preferences begin to develop before birth and develop rapidly in the newborn