

# Sweet smells prepare plants for future stress

## Airborne induction of plant disease immunity

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**P**lants require protection against a wide range of attackers such as insects and pathogens. The adequate plant defense responses are regulated via sophisticated signal cascades, which are activated following the perception of specific cues of the attackers. Plants might, however, gain a significant fitness advantage when pre-empting enemy attack before it actually occurs. Monitoring cues from attacked neighbors can permit plants to reach this goal. We have recently found airborne disease resistance against a bacterial pathogen in uninfected lima bean plants when these were located close to conspecific, resistance-expressing neighbors. The emitters could be chemically induced with benzothiadiazole or biologically with an avirulent pathogen. Unexpectedly, receiver plants, although expressing a functioning resistance, did not show reduced growth rates, which represent a common side-effect of directly induced pathogen resistance. Nonanal was identified as an active volatile and, rather than directly inducing full resistance, primed defense gene expression, which became fully activated only when the plants were subsequently challenged by a virulent pathogen. Priming by airborne signals allows for a more efficient and less costly preparation of plants for future attack and airborne signaling can affect resistance against both major groups of plant enemies: herbivores and pathogens.

Plants are continuously threatened by herbivores and pathogens and the occurrence

of these mobile enemies is difficult to anticipate. In order to maintain high fitness in the presence of enemies, plants express numerous defense traits, which are usually subject to phenotypic plasticity:<sup>1</sup> plants respond to attack by pathogens or herbivores with extensive changes in gene expression that lead to induced resistance phenomena.<sup>2</sup> Because any induced resistance expression suffers from the intrinsic problem of a time lag between attack and the full expression of a functioning resistance, plants might gain a significant fitness advantage when they can pre-empt future encounters with their enemies and prepare themselves before they are actually damaged. In fact, three phenomena indicate that plants can make use of cues that are indicative of future enemy attack: (i) resistance induction by egg deposition, (ii) plant-plant signalling and (iii) priming of resistance expression.

A concrete 'sign of danger' is the deposition of insect eggs onto the plant surface, since herbivorous larvae will most likely emerge from such eggs. 'Early herbivore alert' by insect egg deposition and resulting resistance induction has been described in several cases that comprise herbaceous as well as tree species.<sup>3-5</sup> Second, both pathogens and herbivores can move from one plant to another and several plant species can monitor the volatile organic compounds (VOCs) that are released from herbivore-infested neighbors, in order to mount their own anti-herbivore defense (reviewed in ref. 6). Finally, because herbivores and pathogens spread from the initial site of attack to other organs of the same

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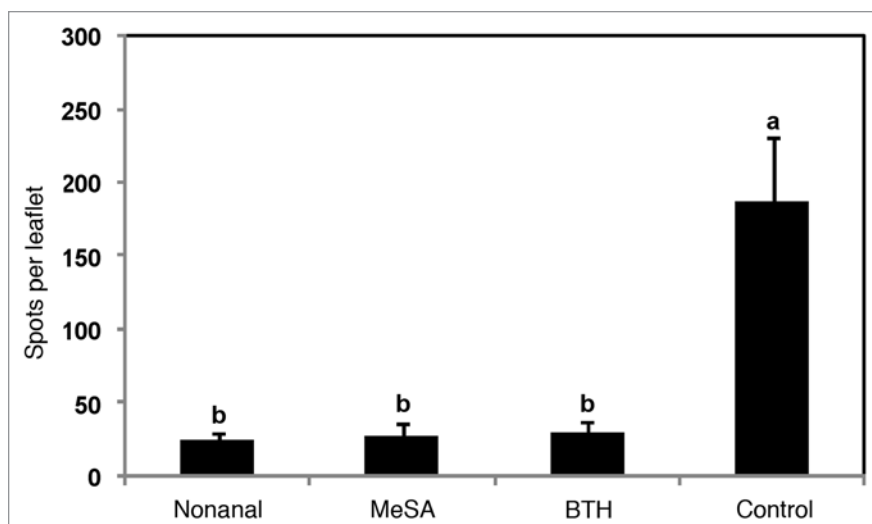
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**Figure 1.** Airborne induction of plant immunity against bacterial spot caused by *Pseudomonas syringae* pv. *syringae* in leaflets of lima bean plants treated before challenging with the resistance-inducing volatiles, nonanal and MeSA, or directly with BTH. Control was treated with water. Different letters indicate significant differences using Fisher's protected LSD test at  $p = 0.05$ .

plant, defensive plant responses are often not restricted to the damaged tissue but are expressed systemically, in yet undamaged organs.<sup>7-9</sup> However, local responses may suffice to kill the attacking insects or pathogens and plant enemies eventually will leave the plant for other reasons. Plants dispose, therefore, of another layer in their phenotypically plastic alarm response: priming, which prepares tissues to respond more rapidly and/or effectively to subsequent attack.<sup>10,11</sup> Although priming has been described mainly in the context of pathogen resistance, self-priming by herbivore-induced VOCs was found for poplar, lima bean and blueberry.<sup>12-14</sup>

In summary, plants possess three different strategies to prepare themselves for upcoming herbivore encounters before these have actually happened. Much less was known, however, on whether similar strategies are used also in the context of disease resistance. Most recently, we discovered that plants indeed perceive VOCs to prepare themselves for upcoming pathogen infection.<sup>15</sup> We used lima bean (*Phaseolus lunatus* L.) plants, which were exposed to the VOCs emitted from neighbors that expressed systemic acquired resistance (SAR) to biotrophic pathogens. The emitters were either treated with the chemical SAR elicitor benzothiadiazole (BTH = benzo(1,2,3)thiadiazole-7-

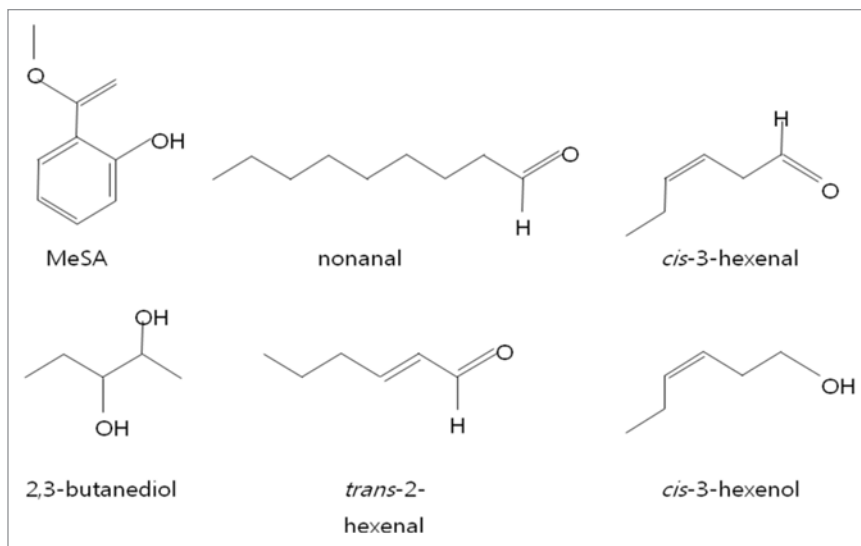
carbothioic acid S-methyl ester) or had been induced biologically. Resulting changes in the resistance of the receivers were monitored at the phenotypic (Fig. 1) and the gene expression level. Experiments conducted both under field conditions and in the growth chamber confirmed that exposition to the air coming from SAR-expressing plants rendered receiver plants phenotypically more resistant to the bacterial pathogen, *Pseudomonas syringae*. This effect became obvious both from the visual inspection of the challenged plants and when quantifying the pathogen population per leaflet. Thus, airborne signals indeed can enhance resistance of lima bean to bacterial pathogens.<sup>15</sup>

Interestingly, we found no significant reduction of growth in the exposed plants, although delayed growth is a common cost usually associated with direct resistance induction.<sup>16</sup> When we assessed for the occurrence of such costs, the exposed plants showed normal growth rates and leaf shape, although they exhibited an enhanced resistance when being challenged. This pattern is redolent of primed plants, in which the defense arsenal had been sensitized rather than fully induced.<sup>17</sup> Priming allows plants to prepare for a more rapid and/or effective response to subsequent attack<sup>11,17</sup> but comes at much lower costs than direct resistance induction.<sup>18</sup>

Indeed, two VOCs that were released from the induced plants (nonanal and methyl salicylate, MeSA) primed the expression of the resistance marker gene, *Phaseolus lunatus* pathogenesis-related protein-2.<sup>15</sup>

Our data indicate that plants pre-empt future pathogen infection and prepare themselves for this important threat in a similar way as they cope with herbivore attack: VOCs released from neighboring plants that are already infected are used as alarm-cues. As yet uninfected neighbors respond to the perception of these VOCs with an induction, or priming, of their resistance. How common might this phenomenon be? One of the two VOCs that we identified in our recent study, MeSA, represents the volatile derivative of the central SAR-eliciting hormone, salicylic acid<sup>19-21</sup> (Fig. 2) and has been reported to function in the systemic signaling of tobacco and in airborne resistance induction against viruses.<sup>22,23</sup> Every plant that expresses an active methyl salicylate esterase and is, thus, able to convert MeSA into the active hormone, salicylic acid,<sup>24</sup> should therefore have the capacity to respond to volatile MeSA. Moreover, even volatiles that are released from plant growth-promoting rhizobacteria can interfere positively with plant growth rates and induce plant resistance against pathogen infection.<sup>25,26</sup> It appears, therefore, likely that an induction of resistance to pathogens by airborne cues represents a common phenomenon, but future studies will have to investigate how common this phenomenon is.

"Plant-plant communication" via airborne cues could cause evolutionary conflicts when benefiting genetically independent receiver individuals at the cost of the emitter. As a solution, a role of VOCs in within-plant signaling has been predicted<sup>27</sup> and was indeed found for three plant species by now: poplar, lima bean and blueberry.<sup>12-14,28</sup> As stated by Gershenzon,<sup>29</sup> plant volatiles "carry both private and public messages" and their distribution cannot be controlled once they have been released into the air. VOCs can, however, serve as reliable within-plant signals only when mainly reaching other leaves of the same plant<sup>7</sup> and when they carry specific information on the type of enemy that is attacking the emitter tissue.



**Figure 2.** Chemical structures of compounds with resistance-enhancing effects. MeSA was released from resistance-expressing tobacco or lima bean plants and induced disease resistance in conspecific neighbors,<sup>15,23</sup> nonanal fulfilled the same function in lima bean,<sup>15</sup> trans-2-hexenal, cis-3-hexenal and cis-3-hexenol rendered *Arabidopsis thaliana* more resistant to *Botrytis cinerea*<sup>33</sup> and the bacterial volatile 2,3-butanediol induced systemic disease resistance in the same species.<sup>26</sup>

## Future Perspectives

Several questions are open and require future scientific scrutiny. (i) First, the question over which distances these signals remain active represents a central one in order to understand the evolutionary stability of airborne plant-plant signaling and its relevance in natural and agronomic ecosystems. By now, the ranges for which signaling among different plants has been reported ranged from few centimeters up to less than a meter.<sup>13,30,31</sup> It appears, thus, to be likely that VOCs emitted from a certain organ will mainly reach other parts of the same plant,<sup>7,32</sup> but systematic studies on the distances over which VOCs remain active are lacking. (ii) Second, VOCs for which an enhancing effect on plant resistance to diseases has been reported so far comprise MeSA,<sup>15,22,23</sup> nonanal,<sup>15</sup> trans-2-hexenal, cis-3-hexenal, cis-3-hexenol<sup>33</sup> and the bacterial acetoin and 2,3-butanediol.<sup>26</sup> What do these compounds have in common (Fig. 2) and can their perception lead to specific responses? Specific motifs characterizing volatiles that induce gene expression in plants have been discussed<sup>34</sup> but could not be confirmed in later studies.<sup>35</sup>

Lima bean responds to herbivore attack and pathogen infection with the emission of clearly different bouquets of volatiles<sup>15</sup> and nonanal, the compound that primed resistance to pathogens (Fig. 1), proved inactive in the context of extrafloral nectar secretion,<sup>35</sup> an indirect defense against herbivores. The potential for specific responses by the receivers when different types of enemies threaten the emitter is clearly there. Future studies will have to figure out which VOCs are mainly emitted under which conditions and which ones of these carry the active and specific message that plants use when they preempt future enemy attack by monitoring VOCs emitted from plant organs in their immediate vicinity.

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## References

1. Heil M. Plastic defence expression in plants. *Evol Ecol* 2010; in press.
2. Karban R, Baldwin IT. *Induced Responses to Herbivory*. Chicago and London: University of Chicago Press 1997.
3. Hilker M, Kobs C, Varama M, Schrank K. Insect egg deposition induces *Pinus sylvestris* to attract egg parasitoids. *J Exp Bot* 2002; 205:455-61.
4. Hilker M, Meiners T. Early herbivore alert: insect eggs induce plant defense. *J Chem Ecol* 2006; 32:1379-97.
5. Doss RP, Oliver JE, Proebsting WM, Potter SW, Kuy SR, Clement SL, et al. Bruchins: Insect-derived plant regulators that stimulate neoplasm formation. *Proc Natl Acad Sci USA* 2000; 97:6218-23.
6. Heil M, Karban R. Explaining evolution of plant communication by airborne signals. *Trends Ecol Evol* 2010; publ. online.
7. Heil M, Ton J. Long-distance signalling in plant defence. *Trends Plant Sci* 2008; 13:264-72.
8. Truman W, Bennett MH, Kubigsteltig I, Turnbull C, Grant M. *Arabidopsis* systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. *Proc Natl Acad Sci USA* 2007; 104:1075-80.
9. Wasternack C. Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann Bot* 2007; 100:681-97.
10. Conrath U, Beckers GJ, Flors V, Garcia-Agustin P, Jakab G, Mauch F, et al. Priming: getting ready for battle. *Mol Plant Microbe Interact* 2006; 19:1062-71.
11. Bruce TJ, Matthes MC, Napier JA, Pickett JA. Stressful "memories" of plants: Evidence and possible mechanisms. *Plant Science* 2007; 173:603-8.
12. Frost C, Appel H, Carlson J, De Moraes C, Mescher M, Schultz J. Within-plant signalling by volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecol Lett* 2007; 10:490-8.
13. Heil M, Silva Bueno JC. Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proc Natl Acad Sci USA* 2007; 104:5467-72.
14. Rodríguez-Saona CR, Rodríguez-Saona LE, Frost CJ. Herbivore-induced volatiles in the perennial shrub, *Vaccinium corymbosum*, and their role in inter-branch signaling. *J Chem Ecol* 2009; 35:163-75.
15. Yi H-S, Heil M, Adame-Álvarez R-M, Ballhorn D, Ryu C-M. Airborne induction and priming of plant resistance to a bacterial pathogen. *Plant Physiol* 2009; 151:2152-61.
16. Heil M, Baldwin IT. Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends Plant Sci* 2002; 7:61-7.
17. Goellner K, Conrath U. Priming: it's all the world to induced disease resistance. *Eur J Plant Pathol* 2008; 121:233-42.
18. Heil M, Walters D. Ecological consequences of plant defence signalling. In: Van Loon LC, ed. *Plant Innate Immunity*. London: Elsevier 2009; 667-716.
19. Durrant WE, Dong X. Systemic acquired resistance. *Annu Rev Phytopathol* 2004; 42:185-209.
20. Hammerschmidt R, Smith-Becker JA. The role of salicylic acid in disease resistance. In: Agrawal AA, Tuzun S, Bent E, eds. *Induced plant defenses against pathogens and herbivores: Biochemistry, Ecology and Agriculture*. St. Paul, Minnesota, USA: The American Phytopathological Society Press 1999; 37-53.
21. Kumar D, Klessig DF. High-affinity salicylic acid-binding protein 2 is required for plant innate immunity and has salicylic acid-stimulated lipase activity. *Proc Natl Acad Sci USA* 2003; 100:16101-6.

22. Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science* 2007; 318:113-6.
23. Shulaev V, Silverman P, Raskin I. Airborne signaling by methyl salicylate in plant pathogen resistance. *Nature* 1997; 385:718-21.
24. Forouhar F, Yang Y, Kumar D, Chen Y, Fridman E, Park SW, et al. Structural and biochemical studies identify tobacco SABP2 as a methyl salicylate esterase and implicate it in plant innate immunity. *Proc Natl Acad Sci USA* 2005; 102:1773-8.
25. Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare PW, et al. Bacterial volatiles promote growth in *Arabidopsis*. *Proc Natl Acad Sci USA* 2003; 100:4927-32.
26. Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW. Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol* 2004; 134:1017-26.
27. Farmer EE. Surface-to-air signals. *Nature* 2001; 411:854-6.
28. Karban R, Shiojiri K, Huntzinger M, McCall AC. Damage-induced resistance in sagebrush: volatiles are key to intra- and interplant communication. *Ecology* 2006; 87:922-30.
29. Gershenzon J. Plant volatiles carry both public and private messages. *Proc Natl Acad Sci USA* 2007; 104:5257-8.
30. Dolch R, Tschardt T. Defoliation of alders (*Alnus glutinosa*) affects herbivory by leaf beetles on undamaged neighbours. *Oecologia* 2000; 125:504-11.
31. Karban R, Maron J, Felton GW, Ervin G, Eichenseer H. Herbivore damage to sagebrush induces resistance in wild tobacco: evidence for eavesdropping between plants. *Oikos* 2003; 100:325-32.
32. Heil M, Silva Bueno JC. Herbivore-induced volatiles as rapid signals in systemic plant responses. *Plant Signaling and Behavior* 2007; 2:191-3.
33. Kishimoto K, Matsui K, Ozawa R, Takabayashi J. Volatile C6-aldehydes and allo-ocimene activate defense genes and induce resistance against *Botrytis cinerea* in *Arabidopsis thaliana*. *Plant Cell Physiol* 2005; 46:1093-102.
34. Almeras E, Stolz S, Vollenweider S, Reymond P, Mene-Saffrane L, Farmer EE. Reactive electrophile species activate defense gene expression in *Arabidopsis*. *Plant J* 2003; 34:202-16.
35. Heil M, Lion U, Boland W. Defence-inducing volatiles: in search for the active motif. *J Chem Ecol* 2008; 34:601-4.

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