Photosynthesis Research (2005) 85: 149–159 

*Review*

# Elicitors and priming agents initiate plant defense responses

Paul W. Paré[1,*], Mohamed A. Farag[2], Venkat Krishnamachari[1], Huiming Zhang[1], Choong-Min Ryu[3] & Joseph W. Kloepper[4]

[1]*Chemistry & Biochemistry Department, Texas Tech University, Lubbock, TX 79409, USA;* [2]*Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK 73402, USA;* [3]*Laboratory of Microbial Genomics, Korean Research Institute of Bioscience and Biotechnology, Yusong, Taejon, 306-600, S. Korea;* [4]*Department of Entomology & Plant Pathology, Auburn University, Auburn, AL 36849, USA; *Author for correspondence (e-mail: Paul.Pare@ttu.edu; fax: +1-806-742-1289)*

Received 15 October 2004; accepted in revised form 19 January 2005

*Key words:* chemical elicitors, plant defense responses, plant volatile emissions, priming agents, volicitin

**Abstract**

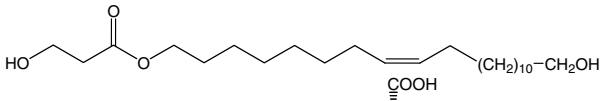
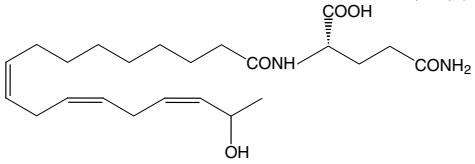
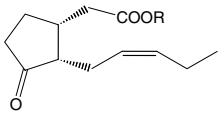
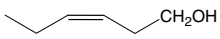
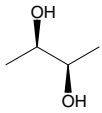
Biotic elicitors produced by plant pathogens or herbivore pests rapidly activate a range of plant chemical defenses when translocated to plant tissue. The fatty acid conjugate volicitin has proven to be a robust elicitor model for studying herbivore-induced plant defense responses. Here we review the role of insect-derived volicitin (*N*-[17-hydroxylinolenoyl]-L-glutamine) as an authentic elicitor of defense responses, specifically as an activator of signal volatiles that attract natural enemies of herbivore pests. Comparisons are drawn between volicitin as an elicitor of plant defenses and two other classes of signaling molecules, $C_6$ green-leaf volatiles and $C_4$ bacterial volatiles that appear to prime plant defenses thereby enhancing the capacity to mobilize cellular defense responses when a plant is faced with herbivore or pathogen attack.

*Abbreviations:* ADH – alcohol dehydrogenase; BAW – beet armyworm (*Spodoptera exigua*); FACs – fatty acid conjugates; HPL – hydroperoxide lyase; HPLC – high performance liquid chromatography; IF – isomerization factor; ISR – induced systemic resistance; JA – jasmonic acid; LOX – lipoxygenase; MeJA – methyl jasmonate; PAL – phenylalanine ammonia lyase; PGPR – plant growth promoting rhizobacteria; SAR – systemic acquired resistance; VOCs – volatile organic compounds

## Herbivore elicitors trigger plant defenses

Herbivores and plant pathogens alike can trigger responses in their host that cannot be mimicked by mechanical damage alone. Such plant defense responses have been ascribed to a wide array of chemical elicitors that activate specific down stream signal transduction pathways (Table 1). For herbivorous insects, two major classes of elicitors have been isolated from the oral secretions that alter wound responses in plants. The lytic enzyme group includes β-glucosidases (Mattiacci et al. 1995), glucose oxidases (Felton and Eichenseer 1999), and alkaline phosphatases (Funk 2001), while the fatty acid–amino acid conjugates (FAC) comprise a family of compounds consisting of 18-carbon polyunsaturated fatty acids coupled to L-glutamine or L-glutamic acid. Three structurally similar amides of linolenic acid, *N*-[17-hydroxylinolenoyl]-L-glutamine (volicitin), *N*-linolenoyl-L-glutamine, and *N*-linolenoyl-L-glutamic acid are thought to be responsible for a majority of elicitor activity associated with the oral secretions of the Lepidopteran larvae analyzed so far (Alborn et al. 1997; Pohnert et al. 1999; Halitschke et al. 2001). For example, FACs from *Manduca sexta* larvae

*Table 1.* Selected natural products that initiate plant defense responses.

| Compound name | Structure | Plant response | Effective plant dose | Reference |
|---|---|---|---|---|
| Bruchin A |  | Neoplastic growth | 1 fmol | Doss et al. 2000 |
| Volicitin (*N*-17-hydroxy-linolenoyl)-L-glutamine) |  | Emissions of volatile organic compounds | 100 pmol | Alborn et al. 1997; Truitt et al. 2004 |
| 7-iso-jasmonic acid (R=H); 7-iso-methyl jasmonate (R=$CH_3$) |  | Defense regulation | 100 nmol–10 μmol | Farag et al. 2005; Rodrigues et al. 2001 |
| *cis*-3-hexenol |  | Defense regulation | 100 nmol | Farag and Paré 2002; Engelberth et al. 2004 |
| (*2R,3R*)-butanediol |  | Induced systemic resistance | 2 nmol | Ryu et al. 2004 |

elicit a suite of direct and indirect defense responses in its native host plant *Nicotiana attenuata*. The production of the broadly effective insect toxin nicotine as well as a bouquet of information-rich mono- and sesquiterpenes is increased with plant exposure to FACs. The emission of VOCs augments the numbers of predatory bugs that locate tobacco hornworm eggs and subsequent larval predation rates. In addition to plant volatiles released during the day that serve as a chemical beacon for host location by parasitoids, a second blend of nocturnal volatiles repel females and deter oviposition (DeMoraes et al. 2001; Kessler and Baldwin 2001). The ability of egg-laying moths to discriminate and appropriately select between insect-free plants and plants in which herbivore larvae have already taken up residency lowers competition for herbivore larvae during early development. At the same time, the emission of chemical cues by plants provides the distinct plant advantage that a visit by a second egg-laying moth is unlikely. The specificity of chemical signals transmitted from the feeding herbivore to a damaged plant, triggering a specific blend of VOCs to be released, was first established by having different species of caterpillar larvae which feed on separate plants (DeMoraes et al. 1988). Plant VOC analysis from plants damaged by different herbivore species exhibit a distinctly characteristic profile of plant volatiles that allow parasitic wasps to discriminate between host and non-host damaged plants based on odor. A comprehensive list of VOC emission signals and plant responses, broken down by plant species, has been compiled by van Poecke and Dicke (2004).

Although the series of specific defense responses that are activated, depend on the precise plant–herbivore interaction, several common global responses have emerged. Herbivore feeding usually triggers defense responses mediated by ethylene and jasmonic acid that act synergistically (Kahl et al. 2000; Schmelz et al. 2003) while pathogen attack typically elevates salicylic acid and corresponding defenses specific to the insipient infection (Vranova et al. 2002). Microarray studies confirm that many of the modifications of gene expression that occur in plants following attack by herbivores can be accounted for by the effects of chemical

elicitors released from chewing insects (Korth 2003). In *Arabidopsis*, insect wounding resulted in a down regulation of water stress-induced genes compared to wounding alone (Reymond et al. 2000) while in tobacco wound-induced transcripts were modified by insect regurgitant either by suppressing wound-induced transcripts systemically or by amplifying the wound response in the attacked leaves (Hermsmeier et al. 2001). To probe the role of FACs that make up a small fraction of the total oral secretions collected from *Manduca* larvae, transcriptional responses between wounded plants treated with the total mix of oral secretions and the two most abundant FACs in *Manduca* *N*-linolenoyl-L-Gln and *N*-linolenoyl-L-Glu were compared. Interestingly the two FACs tested accounted for greater than 50% of the oral secretion-specific transcript accumulations – a total of 37 gene sequences (Halitschke et al. 2003).

In addition to chemical "on" switches such as FACs that trigger altered levels of defense hormones (e.g. JA and ethylene) and large-scale transcriptional reorganization of defense responses, increased sensitivity in triggering inducible-plant defenses is achievable by some recently identified priming agents. Two examples of plant priming agents include green leaf volatiles generated from the $C_{18}$ oxylipin pathway, and bacterial alcohols generated by certain rhizobacteria that immunize plants against pathogen attack (Ryu et al. 2004b). The scope of this review does not allow for a rigorous distinction between chemical elicitors that directly activate plant defenses and priming agents that shorten a plant response time or heighten the magnitude of the defense response while requiring some other signal to trigger the full defense response. Factors such as plant history, elicitor and/or primer dose, and the sensitivity of response being monitored may play a significant role as to whether a particular signal chemical is perceived by the plant and/or the experimentalist as an elicitor or a priming agent. As a first approximation, fatty acid–amino acid conjugates are placed under the rubric of compounds that directly trigger defense responses (elicitors); whereas, $C_6$ green leaf volatiles and $C_4$ bacterial volatiles are grouped as components that trigger a state of enhanced ability to mobilize elicitor-induced cellular defense responses (priming agents). This review will highlight experimental data that elucidate the functioning of volicitin as an elicitor, as well as $C_4$ and $C_6$ alcohols as plant priming agents.

## Fatty acid–amino acid conjugates trigger plant volatile emissions

Since the first report that FACs can be potent activators triggering plant VOC emissions (Alborn et al. 1997), chemical studies of caterpillar larvae have provided detailed information about the biosynthesis (Paré et al. 1998), as well as subcellular localization of enzymes responsible for the assembly of FACs (Lait et al. 2003). For beet armyworm (BAW) (*Spodoptera exigua*), biosynthesis of volicitin and linolenoyl-L-glutamine, the linolenic acid portion has been shown to be derived from the larva's diet while the glutamine fatty acid coupling occurs within the caterpillar (Paré et al. 1998). The enzyme(s) that catalyze biosynthesis of *N*-linolenoyl-L-glutamine have been localized to the integral membrane protein fraction extracted from microsomes of tobacco hornworm larvae (*Manduca sexta*) (Lait et al. 2003). Enzymatic decomposition of FAC elicitors from regurgitant has also been observed in larvae from *Heliothis virescens* and *Helicoverpa zea* (Mori et al. 2001).

Besides the fact that exogenous applications of synthetic or insect-derived volicitin triggers VOC emissions in whole and cut corn seedlings (Alborn et al. 1997), at least two lines of evidence support an essential role for the FAC elicitor volicitin in the regulation of plant volatile emissions in maize. First is that the beet armyworm elicitor volicitin is in the right place at the right time. By allowing radiolabeled larvae to feed on unlabeled maize seedlings, radioactive components were observed to be translocated from the herbivore larvae to the insect-damaged plant tissue. By extracting the lipids from damaged leaf tissue 9 h after feeding, collection and scintillation counting of HPLC fractions indicated that radioactivity was associated with volicitin as well as components with the same retention time as the other insect oral secretion components (Figure 1). Radioactivity associated with volicitin after HPLC analysis was 5 nCi. Estimating that ca. 1% of the total radioactivity in the regurgitant is associated with volicitin, approximately 4 μl of oral secretions is transferred to corn leaf wound site with 9 h of caterpillar feeding.

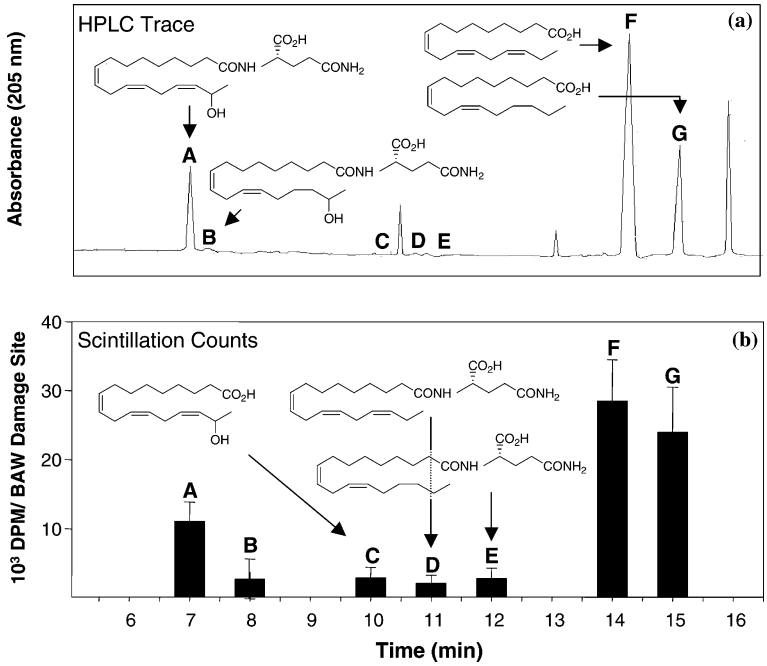



*Figure 1.* (a) HPLC profile of radiolabeled regurgitant components extracted from corn tissue after beet armyworm feeding including (A) *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin); (B) *N*-(17-hydroxylinoleoyl)-L-glutamine; (C) 17-hydroxylinolenic acid; (D) *N*-linolenoyl-L-glutamine; (E) *N*-linoleoyl-L-glutamine (F) linolenic acid; (G) linoleic acid. BAW larvae were fed on radiolabeled corn tissue for 6 h prior to feeding on unlabeled seedlings. Plant extracts were spiked with unlabeled volicitin (100 pmol) to facilitate HPLC peak recognition. (b) Radioactivity associated with individual HPLC fractions after background subtraction ($n = 3$).

A second line of evidence that volicitin specifically initiates maize volatile emissions is the close link between chemical structure and biological activity: BAW regurgitant and synthetic volicitin were equally active in triggering VOC emissions in maize, while the synthetic analog of volicitin, linolenoyl-L-glutamine triggered only a 40% relative release rate of VOC emissions. Other volicitin analogs tested, including 17-hydroxylinolenoyl-D-glutamine (D-volicitin), 17-hydroxylinolenic acid, and L-glutamine, did not trigger maize volatile emissions at a level greater than emissions observed for the buffer control treatment. A corollary of the structure–activity relationship of FACs is the binding activity of ligands to a plasma membrane-localized binding protein of FACs. This recently reported volicitin-binding protein has been identified from maize based on filter binding assays using radiolabeled volicitin as a tag (Truitt et al. 2004). From plasma membrane enriched fractions, binding was observed as early as 1 min after the introduction of radiolabeled volicitin into the assay and the extent of binding increased up to 7 min. Non-specific binding remained constant throughout the experiment and represented approximately 10% of the total binding. The competition of or between volicitin and volicitin analogues was assessed by adding non-radioactive volicitin and volicitin analogues to the enriched plasma membrane binding assay mixture. The competition assays were performed by the addition of a 100-fold molar excess of analog to the reaction mixture prior to the addition of 10 nM [$^3$H]-L-volicitin. The binding of [$^3$H]-L-volicitin to the enriched plasma membrane preparation decreased to background levels in the presence of unlabeled L-volicitin. D-volicitin containing the same charge and hydrophobicity produced only a 15% decrease for [$^3$H]-L-volicitin bound by the enriched plasma membrane preparation. Unlabeled L-volicitin and linolenoyl-L-glutamine competed for the binding sites with half-maximal inhibitory concentrations ($IC_{50}$) of 9 and 22 nM, respectively (Figure 2a). D-volicitin and the fatty acid and amino acids of volicitin did not produce greater than a 15% decrease in [$^3$H]-L-volicitin binding at concentrations up to 1 mM. To relate

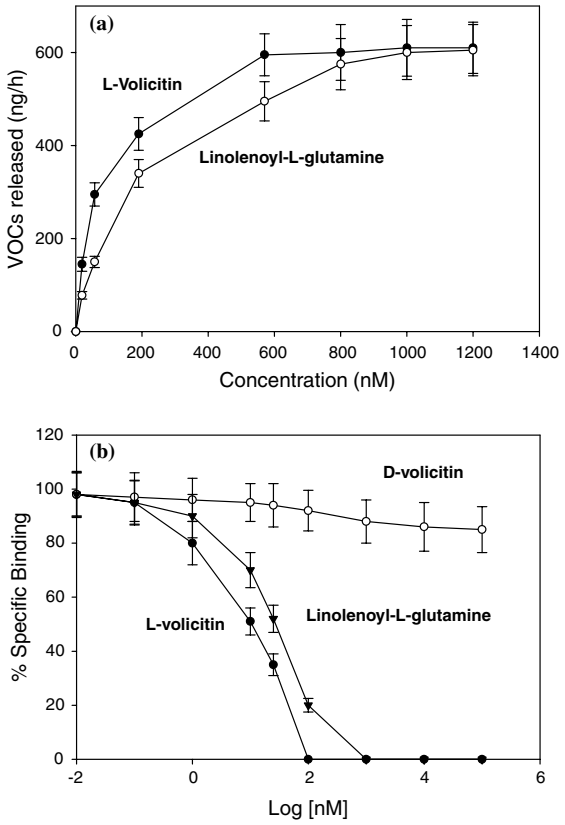



*Figure 2.* (a) Saturation analysis of L-volicitin and linolenoyl-L-glutamine VOC induction with maize seedlings. Maximum release of volatiles collected for 2 h from six maize seedlings that had been treated with the indicated concentration of either L-volicitin (closed circles) or linolenoyl-L-glutamine (open circles). The combined amount in nanograms of caryophyllene, α-transbergamontene, (*E*)-β-farnesene, (*E*)-nerolidol, and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11,-tridecatetraene was used to calculate the release of seedlings treated with 15 µl of test solution. (b) Competition analysis of [$^3$H]-L-volicitin binding by L-volicitin, D-volicitin, and linolenoyl-L-glutamine determined by treating the enriched plasma membrane preparations with the indicated concentration of unlabeled analog and by calculating the percentage of specific binding as a ratio of specific binding at the indicated concentration to maximal specific binding found in the presence of 100-fold excess of unlabelled volicitin. L-volicitin (closed circles), D-volicitin (open circles), and linolenoyl-L-glutamine (triangle). Error bars indicate standard error ($n$ = 6).

the binding data with the volatile assays, half-maximal effective concentration ($EC_{50}$) of L-volicitin and linolenoyl-L-glutamine was determined by measuring volatile release from maize seedlings with increasing concentrations of elicitor (Figure 2b). $EC_{50}$ values for L-volicitin and linolenoyl-L-glutamine were measured to be 58 and 165 nM, respectively.

## $C_6$ green leaf volatiles that prime plant defense responses

Unlike many plant volatiles such as terpenes and shikimic acid derivatives that vary among plant species, $C_6$-volatiles produced from the catalytic activity of hydroperoxide lyase (HPL) can be generated in all green tissues and are among the earliest components to be released from damaged leaves. The biosynthesis of $C_6$-volatiles from an 18-carbon fatty acid precursor involves two enzymatic steps catalyzed by lipoxygenase (LOX) and HPL (Figure 3). Depending on the degree of saturation of the substrate, HPL produces either (*Z*)-3-hexenal or hexanal. Alcohol dehydrogenase (ADH), an isomerization factor (IF), and/or acetylation leads to the production of other $C_6$-volatiles including (*E*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-3-hexenol, hexenol and (*Z*)-3-hexenyl acetate (Hatanaka 1993). The $C_{12}$-component is processed to the plant wounding signal traumatin (12-oxo-*E*-10-dodecenoic acid) via a 12-oxo-*Z*-9-dodecenoid acid intermediate (Figure 3).

Leaf-tissue emitted $C_6$-compounds, often referred to as green leaf volatiles, can trigger responses in neighboring plants, including phytoalexin accumulation in cotton (Zeringue 1992), lower insect feeding rates in tomato (Hildebrand et al. 1993) and reduced germination frequency in soybean (Gardener et al. 1990). Several of these green leaf compounds can also act as anti-microbial agents on their own (Croft et al. 1990). As a signal molecule, exogenous application of (*E*)-2-hexenal to *Arabidopsis* seedlings induces a group of genes that closely mimic methyl jasmonate (MeJA) induction as well as trigger the upregulation of LOX pathway and PAL genes (Arimura et al. 2001). LOX and PAL gene induction also occurs in maize on plant exposure to (Z)-3-hexenol (Farag et al. 2005). Interestingly (*E*)-2-hexenal treatment of *Arabidopsis* plants does not induce HMGR-1, the gene that encodes a key regulatory step enzyme involved in isoprenoid biosynthesis (Nicholas and Steven 1988). The moderate level of VOC emissions and gene induction on plant exposure to green leaf volatiles relative to plant exposure to herbivore damage presented the possibility of an indirect role for $C_6$ components in triggering plant defenses. The signaling role and metabolic turnover of (*E*)-2-hexenal as well as other $C_6$-volatile components in triggering plant

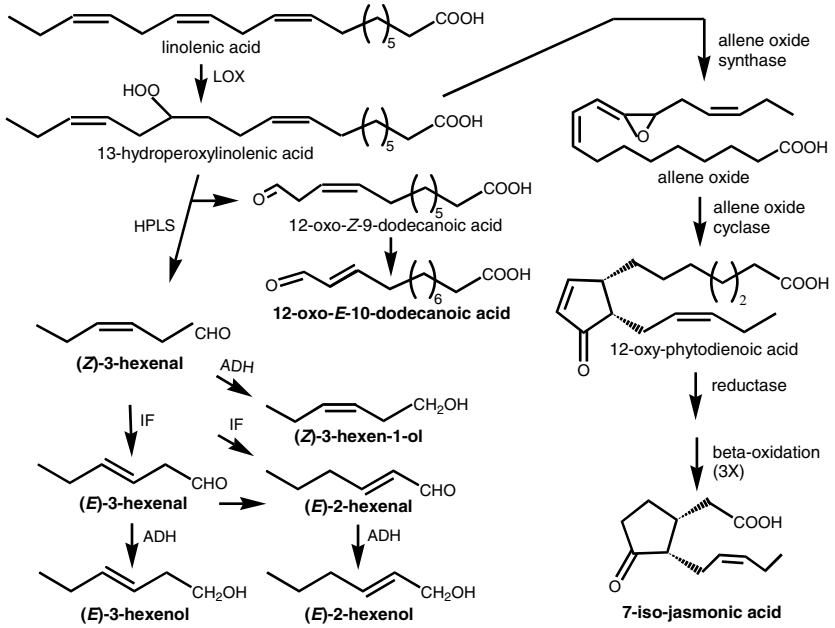



*Figure 3*. Three linolenic-acid derived signaling pathways that prime and/or elicit plant defense responses. Enzyme abbreviates include lipoxygenase (LOX), hydroperoxide lyase (HPLS), aldehyde dehydrogenase (ADH), isomerization factor (IF).

VOC emissions has begun to come into focus primarily based on two recent studies using maize as a model system: (i) the ability of $C_6$ volatile components to prime neighboring plants against impending herbivory (Engelberth et al. 2004), and (ii) the deactivation and exportation of $C_6$ components from leaf tissue by acetylation of $C_6$ components *in planta* (Farag et al. 2005).

To test what role do green leaf volatiles play in priming a plant's chemical defenses, jasmonic acid, a pivotal endogenous signaling molecule that orchestrates direct and indirect defense responses in plants, as well total sesquiterpene emissions, was monitored in insect-treated corn plants with or without pretreatment with $C_6$ VOCs (Engelberth et al. 2004). After overnight exposure to green leaf volatiles or pure $C_6$ compounds, caterpillar induced sesquiterpenes and the resting level of jasmonic acid in these plants were the same as control plants. However 30 min after treatment with caterpillar regurgitant, the level of endogenous jasmonic acid rose in green leaf volatiles-pretreated plants to twice the level of untreated control plants on a gram fresh weight tissue basis. This elevatation of jasmonic acid in green leaf volatiles-pretreated plants remained higher over a period of 3 h. Interestingly, only elicitor-induced jasmonic acid was affected, whereas wound-induced production of jasmonic acid remained unchanged. The same pattern of green leaf volatile pretreatment augmenting plant response to caterpillar regurgitant was also observed in the emissions of caterpillar-induced VOCs. The green leaf volatiles pretreated plants released ca. 4 μg of total VOCs 4–6 h post-induction compared to 2.4 μg from non-primed control plants.

A burst in (*Z*)-3-hexenyl acetate emissions with (*Z*)-3-hexenol treatment in maize plants raised the

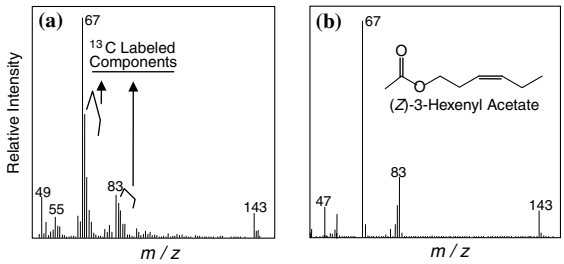



*Figure 4*. Abundance of $^{13}C$ label in plant metabolized (*Z*)-3-hexenyl acetate with mechanical damage (a) or $^{12}C$ (*Z*)-3-hexenol exposure (b) as analyzed by electron ionization mass spectroscopy. The ion species at *m*/*z* 68–73 were used to calculate $^{13}C$ enrichment levels in untreated (a) and (*Z*)-3-hexenol-treated plants (b).

possibility that (*Z*)-3-hexenyl acetate is derived at least in part from (*Z*)-3-hexenol. To determine the source of (*Z*)-3-hexenyl acetate released in response to exogenous (*Z*)-3-hexenol treatment, endogenous and exogenous $C_6$-volatile components were separately labeled with $^{13}C$ and $^{12}C$, respectively (Farag et al. 2005). Plants were grown under $^{13}CO_2$ conditions and chemically labeled plants were exposed to unlabeled [$^{12}C_6$]-(*Z*)-3-hexenol. Dilution of $^{13}C$ label in $C_6$-volatiles was used as a measure of exogenous [$^{12}C_6$]-(*Z*)-3-hexenol incorporation into hexenyl acetate emissions (Figure 4). The 88% dilution of the $^{13}C$ label with the application of unlabeled (*Z*)-3-hexenol indicated a biochemical conversion of the $C_6$ alcohol to the acetylated form in maize plants. Although (*Z*)-3-hexenyl acetate-treated plants showed no significant difference than untreated plants in the level of VOC emissions or PAL gene induction, Engelerth et al. (2004) did report that (*Z*)-3-hexenyl acetate was effective in priming defense responses in maize. In tomato, both $C_6$-aldehydes and alcohols reduce aphid fecundity while (*Z*)-3-hexenyl acetate was biologically inactive; the biological activity is hypothesized to be due to induced changes in leaves initiate by plant interactions with $C_6$ volatiles (Hildebrand et al. 1993). At least in the induction of some genes and VOC emissions, the acetylated form of (*Z*)-3-hexenol is less active than the free alcohol form and may serve a biological role in the inactivation and rapid turnover of $C_6$ components. This deactivation by chemical modifications is not unprecedented; the biochemical conversion of JA to cis-jasmone is another example of a biochemical conversion of a signal molecule to a less active form (Koch et al. 1997).

### $C_4$ bacterial volatiles trigger plant defenses

In response to an avirulent pathogen attack, plants often mount a series of direct defenses at the site of infection characterized by a hypersensitive response (localized cell death), elevated peroxide production and increased synthesis of defense compounds (phytoalexins). Systemic biochemical changes referred to as systemic acquired resistance (SAR) are usually mediated by a salicylic acid signal transduction pathway. Enhanced chemical defenses against a broad spectrum of plant pathogens can also be activated by the colonization of the roots by selected strains of non-pathogenic bacteria. In many cases rhizobacteria-induced systemic resistance (ISR) functions independently of salicylic acid, although chemical signaling is required by the plant hormones jasmonic acid and/or ethylene. In contrast to pathogen-induced SAR, rhizobacteria-mediated ISR is usually not associated with changes in the expression of genes encoding pathogenesis-related proteins. Plant growth-promoting rhizobacteria (PGPR)-elicited ISR was initially observed in carnation with reduced susceptibility to *Fusarium* wilt (van Peer et al. 1991), in common bean with reduced susceptibility to halo blight (Alström 1991), and in cucumber with reduced susceptibility to *Colletotrichum orbiculare* (Wei et al. 1991). PGPR-mediated ISR has since been reported for several other plant–pathogen systems (Maurhofer et al. 1994; Zhou and Paulitz 1994; Liu et al. 1995; Leeman et al. 1996; Benhamou et al. 1998). PGPR that colonize root systems with seed applications and protect plants against foliar diseases include *Pseudomonas fluorescens*, *P. putida, Bacillus pumilus*, and *Serratia marcescens* (Liu et al. 1995; Raupach et al. 1996; Kloepper et al. 1999; Pieterse et al. 2002; Ryu et al. 2003b, 2004b).

The development of enhanced defensive capacity against a broad spectrum of plant pathogens by the colonization of plant roots with select non-pathogenic bacteria has been attributed to strengthening of root epidermal and cortical cell walls. Such additional callose deposits in the roots with PGPR treatments are often observed to be infiltrated with phenolic compounds (Benhamou et al. 1996, 1998). Mutations in plant signaling pathways point to an active role by salicylic acid or jasmonic acid and/or ethylene in activating ISR (Kloepper et al. 2004). Cross-talk between salicylate- and jasmonate-dependent defenses have been identified (Spoel et al. 2003). Mediation of ethylene levels by microbial 1-aminocyclopropane-1-carboxylates (ACC) may also play a key role in signal transduction. For example, the ISR-inducing *Pseudomonas fluorescens* bacteria enhance bacterial ACC-converting capacity and leads to potentiated levels of ethylene emissions in *Arabidopsis* infected by a plant pathogen (Hase et al. 2003). Volatile signals generated by certain non-pathogenic bacteria have also been shown to trigger defense responses in *Arabidopsis* (Ryu et al.

2003a, 2004). Unlike airborne VOCs such as $C_6$ green leaf volatiles that can be easily sampled by head-space collections of the living plant, rhizosphere emissions by PGPR present the complication of de-adsorbing low molecular wei-ght compounds from the soil matrix. By growing PGPR and *Arabidopsis* seedlings on separate sides of divided petri dishes, Ryu et al. (2004a) were able to examine the role of airborne bacterial metabolites in triggering ISR.

Exposure of *Arabidopsis* seedlings to volatiles from the *Bacillus* sp. for as little as 4 days was sufficient to activate ISR as demonstrated by a significant reduction in symptomatic leaves, 24 h after inoculation with the soft rot-causing pathogen *Erwinia carotovora* (Ryu et al. 2004). Gas chromatographic analysis of volatiles collected from the growth-promoting bacteria *Bacillus subtilis* (strain GB03) and *B. amyloliquefaciens* (strain IN937a) revealed consistent differences in the composition of volatile blends released by the non-growth promoting bacterial strain DH5α. The two most abundant compounds 2,3-butanediol and 3-hydroxy-2-butanone (also referred to as acetoin) were consistently released from the GB03 and IN937a strains while these metabolites were not released from the DH5α or water-treated MS media. Other components of the complex bacterial bouquet that did not exhibit ISR priming activity included dodecane, 2-undecanone, 2-tridecanone, 2-tridecanol and tetramethyl pyrazine.

In *Bacillus* sp., 2,3-butanediol and acetoin are produced under low atmospheric $O_2$ partial pressure to provide an alternative electron sink for the regeneration of $NAD^+$ when usual respiration is not possible (Figure 5); this additional metabolic pathway functions analogously to alcohol fermentation activated in yeast under anaerobic conditions. The biological activity of 2,3 butanediol in triggering ISR was surmised in *Arabidopsis* when pre-exposure of plants to low doses (pg to ng range) of 2,3-butanediol activated ISR. The priming activity of 2,3-butanediol to reduce a plant's susceptibility to disease was confirmed when Bacilli strains genetically blocked in the production of 2,3-butanediol exhibited no disease protection (Figure 6).

The involvement of known signaling pathways in *Arabidopsis* were screened by exposing defined mutants and transgenic plant lines to bacterial emissions containing 2,3-butanediol. ISR triggered by GB03 emissions was independent of salicylic acid, NPR1, and jasmonic acid signaling pathways, but did appear to be mediated by ethylene. Interestingly ISR activation by strain IN937a was independent of all the signaling pathways that were tested, opening up the possibility that additional VOCs may be utilizing alternative pathways to trigger ISR.

In Ryu et al.'s report (2004a), petri dish assays expose the whole plant to the plume of bacterial VOCs; this leaves open as to whether the site of plant VOC perception is above or below ground for soil-grown plants. The sphere of microbial emissions for rhizosphere bacteria may be within the soil and/or above ground; the possibility exists that VOCs are produced at a sufficient level for aerial tissues to perceive and respond to bacterial volatiles. An alternative scheme is that an endog-

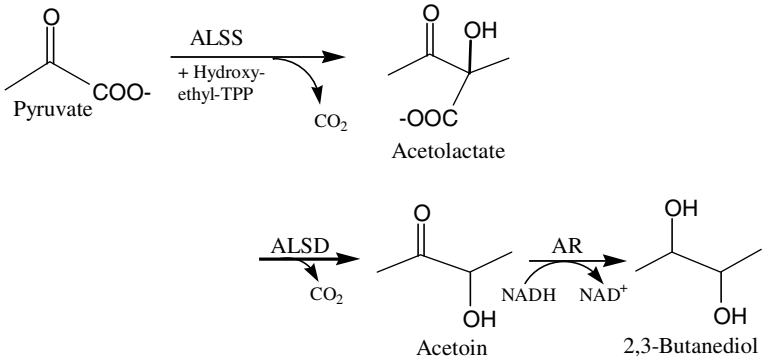



*Figure 5*. Proposed pathways for anaerobic fermentation in *Bacillus subtilis*. Enzymes with known coding genes include pyruvate dehydrogenase (PDH), lactate dehydrogenase (LDH), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), acetolactate synthase (ALSS), and acetolactate decarboxylase (ALSD) and acetoin reductase (AR).

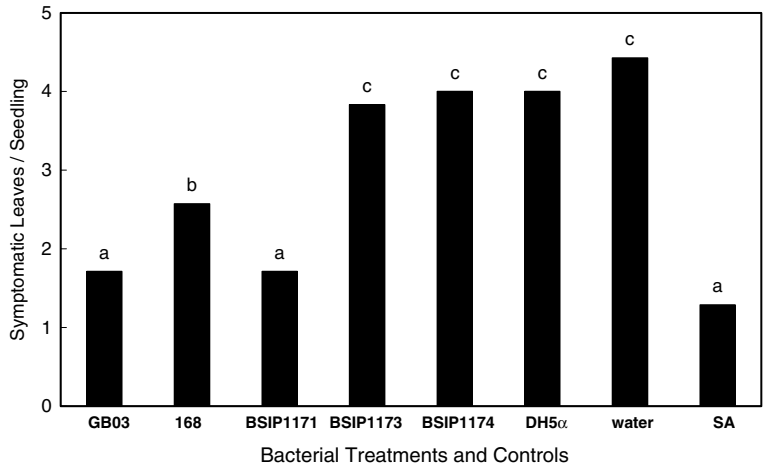



*Figure 6*. Infection of *Arabidopsis thaliana* by *Erwinia carotovora* subsp. carotovora strain SCC1 after exposure to wild (GB03, and 168) overproducing 2,3-butanediol (BSIP1171) and mutants with attenuated 2,3-butanediol synthesis (BSIP1173 and BSIP1174). *Escherichia coli* strain DH5α, and water treatment were used as negative controls. Different letters indicate significant differences between treatments according to LSD at $P = 0.05$.

enous signal or signals transport information from the root zone to the aerial portion of the plant. The observation that induced resistance is systemic, necessitates the presence of some mobile messenger within the plant.

To study the mechanism of systemic defense responses triggered by PGPR, the transcriptional response of over 8000 genes have been surveyed during rhizobacteria-mediated ISR (Verhagen et al. 2004). Although a substantial change in the expression of almost 100 genes was observed locally in the roots, none of the genes tested showed a consistent change in expression in response to effective colonization of the roots. This invariant pattern in transcript profiles suggests that the onset of ISR in the leaves is not associated with detectable changes in gene expression. However after PGPR-treated plants were challenged with a bacterial leaf pathogen, over 80 genes showed an augmented expression pattern in ISR-expressing leaves, suggestive of a priming mechanism triggered by plant exposure to PGPR that allowed the plant to respond faster and more strongly upon pathogen attack. The monitoring of gene expression in plants exposed to bacterial VOCs will provide insight into how such chemical agents may serve to prime pathogen-induced genes allowing plants to react more effectively to a particular pathogen or a broad spectrum of possible invaders.

## Conclusions

Priming of defense pathways by external signals allows for a potentiated induction of defense responses without an immediate activation of defense signal cascades and the accompanied expenditure of energy for defense mobilization (Conrath et al. 2002). Airborne green-leaf volatiles are one venue by which information-rich chemicals can be relayed from a damaged individual to neighboring plants that may be at an elevated risk of herbivore damage (Schmelz et al. 2004). In the case of PGPR priming of plant defenses, it is hypothesized that induction of the primed state results in an increase in the amount or activity of cellular components with important roles in defense signaling and not associated with direct changes in gene expressions in the leaves (Verhagen et al. 2004). We expect that comparisons of signal transduction cascades activated by elicitors in the presence or absence of external priming agents will provide insight into the impact of elicitors, and priming agents in triggering plant defense responses as well as the long-term fitness of a plant.

## Acknowledgements

Funding is provided in part by grants from the Robert A. Welch Foundation (D1478) and the Herman Frasch Foundation for chemical research.

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