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# PLANT RESPONSES TO INSECT HERBIVORY: The Emerging Molecular Analysis

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■ **Abstract** Plants respond to herbivore attack with a bewildering array of responses, broadly categorized as direct and indirect defenses, and tolerance. Plant-herbivore interactions are played out on spatial scales that include the cellular responses, well-studied in plant-pathogen interactions, as well as responses that function at whole-plant and community levels. The plant's wound response plays a central role but is frequently altered by insect-specific elicitors, giving plants the potential to optimize their defenses. In this review, we emphasize studies that advance the molecular understanding of elicited direct and indirect defenses and include verifications with insect bioassays. Large-scale transcriptional changes accompany insect-induced resistance, which is organized into specific temporal and spatial patterns and points to the existence of herbivore-specific *trans*-activating elements orchestrating the responses. Such organizational elements could help elucidate the molecular control over the diversity of responses elicited by herbivore attack.

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## WHY PLANT RESPONSES TO PATHOGENS AND HERBIVORES FREQUENTLY DIFFER



Autotrophs require sophisticated defenses if they are to survive in a world full of heterotrophs. Attacks from heterotrophs occur on spatial scales ranging from microbes to moose, and plants require defenses that are effective at all levels. Defenses against microbes can be highly effective on small spatial scales; the hypersensitive response (HR), in which cells immediately surrounding the infection site rapidly die and fill with antimicrobial compounds to prevent the spread of the pathogen, is the best-studied example (58). Although the HR is an extremely effective defense, this cellular suicide cannot be used without large costs and requires a sophisticated recognition system to avoid inappropriate deployment. Hence the HR, and the burst of reactive oxygen species (ROS) (114) that frequently precedes the HR and may contribute to apoptosis, are activated when a plant's surveillance system (*R*-genes, encoding receptor proteins) binds various elicitors from the attacking pathogen (proteins, peptides, lipids, polysaccharides) (58). Although the HR can be effective against sedentary herbivores that attack particular tissues [such as phloem-feeding aphids (73)], it is not effective against most free-living herbivores, which avoid an HR by simply moving to another feeding site. This autonomy and the resulting physiological independence of herbivores from their host plants profoundly expand the spatial scale of the plant-herbivore interaction to include not only whole-plant responses but also the community in which the plant lives (Figure 1).

Most insect herbivores arrive at a plant after their devoted mothers have carefully selected and, in some cases, manipulated [by microbial inoculations: e.g., bark beetles (88); or altering source-sink relationships: e.g., aphids (60)] the host plant and endowed the young herbivore nutritionally and developmentally, enabling it to launch its first attack. The young herbivore is fully equipped with mandibles and other feeding apparatus to force its way through the plant's protective covering, an efficient digestive tract in which plant parts can be digested and assimilated in a milieu controlled by the herbivore (112), and mobility and sensory systems that allow it to move in response to heterogeneity in plant suitability (90). This physiological and behavioral autonomy can account for overarching differences between how plants respond to herbivores and pathogens and in how an attack is perceived.

In contrast to pathogen attack, herbivore attack is frequently associated with wounding, and the "recognition" of herbivore attack frequently involves modifications of a plant's wound response (6, 56, 118). Moreover, the physiological and behavioral autonomy of herbivores also allows plants to use defenses that would

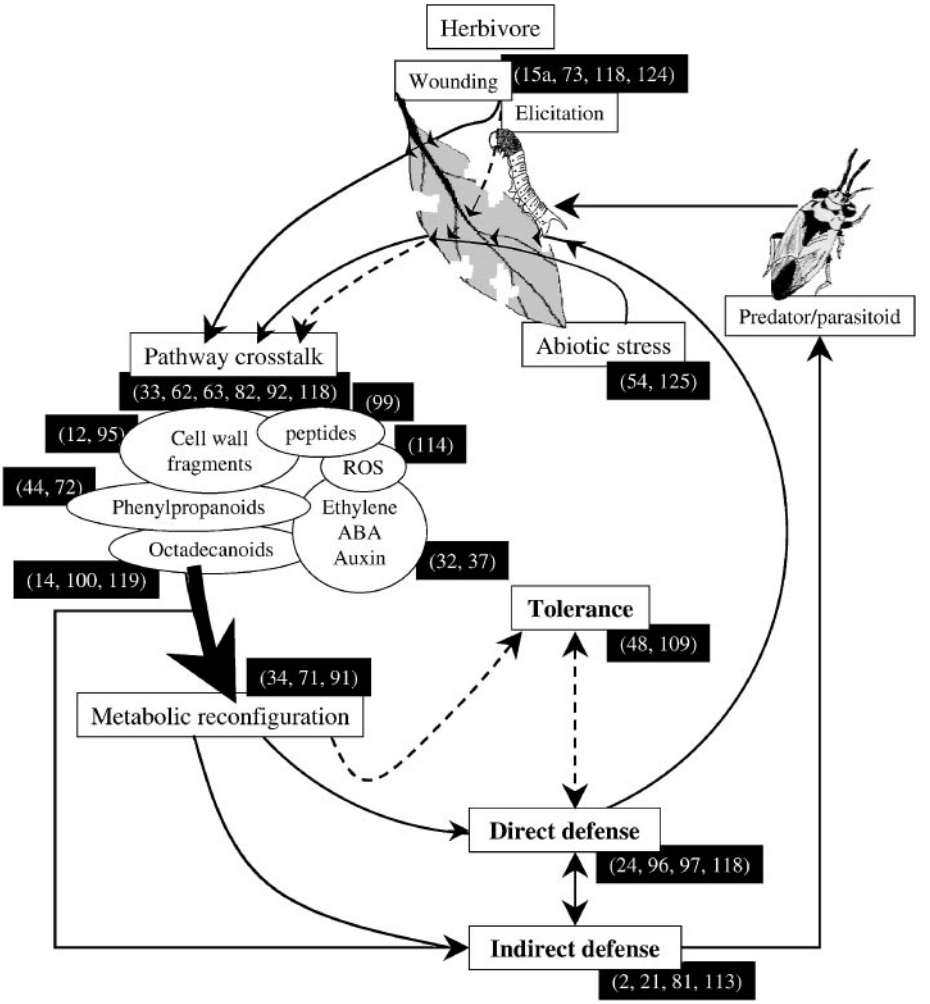



**Figure 1** The arena of plant-induced resistance to arthropods. An attacking herbivore wounds the plant and applies or injects elicitors. Wound- and herbivore-specific elicitors in combination with abiotic stresses differentially activate various signaling pathways. These signal cascades interact (pathway crosstalk) to either directly produce volatile signals that function as indirect defenses or effect a fine-tuned metabolic reconfiguration and the expression of defense-related genes. As a consequence of these changes, resources are allocated to regrowth (tolerance) or the production of compounds that directly affect the attacking herbivore (direct defenses: toxic, antinutritive, and antidigestive compounds) or indirectly (indirect defenses) by attracting natural enemies. Recent reviews summarizing the knowledge of particular parts of the arena are listed in black boxes. Evidence is emerging that arthropod-induced resistance results from a coordinated production of specific direct and indirect defenses that complement the existing constitutive defenses of the plant.

be ineffective against pathogens. For example, plants use secondary metabolites that are specifically targeted against organ systems unique to herbivores [nervous, digestive, endocrine, etc. (96)] and use higher trophic-level interactions defensively by providing information or nutritional encouragement to the predators of herbivores. The recruitment of the natural enemies of herbivores drastically increases the spatial scale of the interaction (21).

Plant-herbivore interactions are described in an enormous, largely ecological and entomological literature into which molecular techniques have only recently been injected. To assist molecularly oriented readers in coming to grips with this vast literature, we select recent reviews of different aspects of the interactions (Figure 1) and present a primer of the main concepts and terms used to describe the interaction. We follow this with a review of recent literature on advances in the molecular understanding of the "recognition" of herbivore attack by plants and the resistance mechanisms that have been verified with insect bioassays.



## A PRIMER IN PLANT-HERBIVORE INTERACTION TERMINOLOGY

### Fitness-Based Evaluations of Resistance Traits

Much of the interest in plant-herbivore interactions among ecologists stems from a seminal paper by Ehrlich & Raven (25), which coined the term "coevolution" and stimulated entomological studies of how plants and insects influence each other's evolutionary trajectories. As a result of this evolutionary focus, the functional analysis of plant traits is frequently evaluated at higher-level integrations of plant performance, namely the correlates of Darwinian fitness (production of seeds, pollen, tubers, etc.). The adjective "defensive" is usually reserved for traits that increase plant fitness correlates when plants are under attack (50). This evolutionary emphasis and fitness-based analysis of plant traits have focused interest on the factors that contribute to maintaining the variability in resistance so frequently observed in nature. A central thrust has been to understand why plants are not always resistant, when it has such clear fitness benefits. This emphasis on understanding variability has focused attention on the fitness costs of different types of resistance: their modes of expression (constitutive or induced) and the environmental, evolutionary, and developmental constraints on them (42, 96, 97). Resistance traits can be broadly categorized into three defense strategies: direct and indirect defenses and tolerance (50). Plants are either constitutively resistant as a result of preformed resistance traits, or they become resistant after an attack as a result of herbivore-induced changes that are either localized to the tissues adjacent to an attack site or systemically expressed throughout the plant.

### Costs of Defense

Just as the indiscriminate deployment of the HR would likely severely compromise a plant's performance, the production of resistance traits when they are not

needed is likely to be costly for a number of reasons. First, resistance traits can be costly to produce if fitness-limiting resources (such as nitrogen) are invested (5) or if the traits are also toxic to the plant. However, resistance costs can also arise from higher-level ecological processes. For example, specialized herbivores may sequester a plant's defenses and use them for their own defense against predators, or compounds that provide defense against generalist herbivores may attract specialist herbivores, which use them as host-location signals (113). Moreover, the defenses may disrupt important mutualistic interactions, such as pollination, which are also mediated by insects (1). These fitness costs probably provide the selection pressure behind the evolution of inducible resistance, if inducible expression allows plants to forego these fitness costs when the defense is not needed.



## Direct Defenses

Direct defenses are any plant traits (e.g., thorns, silica, trichomes, primary and secondary metabolites) that by themselves affect the susceptibility to and/or the performance of attacking arthropods and thus increase plant fitness in environments with herbivores. Defensive secondary metabolites are categorized by their mode of action (24). Proteinase inhibitors (PI) (antidigestive proteins) are inducible by wounding and herbivory and influence herbivore performance by inhibiting insect digestive enzymes (55, 110). Polyphenol oxidases are antinutritive enzymes that decrease the nutritive value of the wounded plant by cross-linking proteins or catalyzing the oxidation of phenolic secondary metabolites to reactive and polymerizing quinones. Toxic compounds (e.g., alkaloids, terpenoids, phenolics) poison generalist herbivores, forcing specialists to invest resources in detoxification mechanisms that in turn incur growth and development costs.

## Indirect Defenses

Indirect defenses are plant traits that attract predators and parasitoids of herbivores and increase the carnivore's foraging success and thereby facilitate top-down control of herbivore populations (50). Volatile organic compounds (VOCs) released by herbivore-attacked plants are known to be attractive to arthropod predators and parasitoids in laboratory experiments on agricultural plants (21) and have recently been shown to function defensively under natural conditions (52). The VOC response can be highly specific; parasitic wasps often use this specificity to locate particular hosts or even the particular instars of their hosts (113). However, generalist predators are also attracted by single components of the VOC bouquet, which are commonly emitted after attack from a diverse set of herbivore species (52). In addition to attracting natural enemies of the herbivores, the VOC release can function as a direct defense by repelling the ovipositing herbivores (19, 52). Finally, it may be involved in plant-plant interactions (27). In addition to supplying information to natural enemies about the location and activity of foraging herbivores, plants also provide food and shelter to the enemies of herbivores (3). Extrafloral nectaries increase their rate of nectar secretion after herbivore attack, and these carbohydrates and proteins (from various food bodies or even pollen)

provide nutritional encouragement for predators to increase their foraging rate in certain areas of a plant (40). Shelter is provided as specialized structures (leaf domatia) or modifications of existing structures (hollow thorns, stems).

### Tolerance

Tolerance decreases the fitness consequences of herbivore attack for a plant. A plant genotype is termed tolerant if it can sustain tissue loss with little or no decrease in fitness relative to that in the undamaged state (109). Although genotypes clearly vary in their tolerance, the mechanisms underlying such variation are not understood. Certain morphological traits, such as meristem sequestration and reactivation, as well as photoassimilate storage in below-ground and stem structures, in addition to physiological responses, such as herbivore-induced increases in photosynthetic capacities, and nutrient uptake, are correlated with compensatory growth following herbivore attack (109). However, the functionally mysterious transcriptional reconfiguration that follows herbivore attack is most likely to hold the key to a more detailed mechanistic understanding of tolerance responses.



## ELICITORS FROM HERBIVORES

Any compound that comes from herbivores and interacts with the plant on a cellular level is a potential elicitor. So far, herbivore-specific elicitors have been isolated from oral secretions of lepidopteran species and the oviposition fluid of weevil beetles—the two insect fluids that regularly come in contact with plant wounds (Figure 2). Additionally, some evidence suggests that microbes present in the digestive organs of herbivores are involved in the production of elicitors found in oral secretions (107).

Two classes of elicitors have been isolated from the oral secretions of lepidopteran larvae; both elicit indirect defense responses. The first class includes lytic enzymes, such as $\beta$-glucosidase that was isolated from *Pieris brassicae* and elicits the release of terpenoid volatiles from cabbage leaves (66). Other lytic enzymes have been found in the saliva of other lepidopteran species, such as glucose oxidases in *Helicoverpa zea* saliva (29); in piercing insects, such as alkaline phosphatase in whitefly (*Bemisia tabaci*) saliva (31); and in a wide array of watery digestive enzymes from aphid saliva (73); but their roles as elicitors of defense responses have not yet been established.

The second class of elicitors comprises fatty-acid–amino-acid conjugates (FACs), which have been found in the regurgitant of larval Sphingidae (36), Noctuidae, and Geometridae (4, 86). The FAC volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine], from *Spodoptera exigua*, induces excised maize seedlings to release the same odor blend of volatile terpenoids and indole that is released when they are damaged by caterpillar feeding (4). Unfortunately a recent study with intact maize seedlings found volicitin to only elicit VOC release when applied to plants at midnight and that the release from excised seedlings was much greater than that from

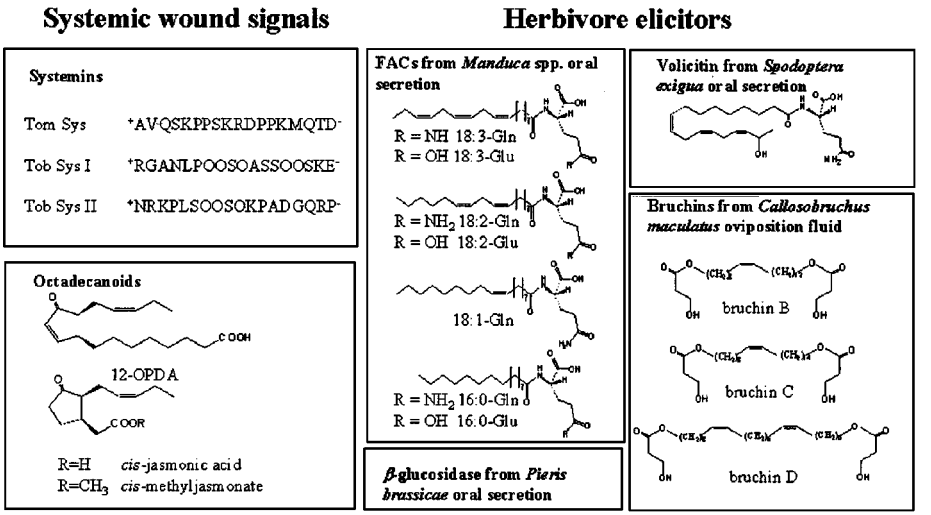



**Figure 2** Examples of systemic wound- and herbivore-specific signals demonstrated to elicit either direct or indirect defenses in plants. Systemins are polypetide systemic wound signals from solanaceous plants, such as tomato (Tom Sys) and tobacco (Tob Sys I and II), which activate the octadecanoid pathway but are also inducible by various oxylipins (83, 99). Oxylipins, as illustrated by the octadecanoids, 12-oxophytodienoic acid (12-OPDA), jasmonic acid, and methyl jasmonate, elicit defense gene expression, numerous secondary metabolites, and insect resistance (14). Herbivore-specific elicitors have been identified in insect oral secretions and oviposition fluids, the two fluids that commonly come into contact with the wounded plant tissue. *Manduca* and *Spodoptera exigua* oral secretions contain the class of fatty-acid-amino-acid conjugates (FACs) found to actively elicit volatile organic compounds (VOCs), which function as indirect defenses (36, 113). The FACs from *Manduca* also elicit other herbivore-specific changes (Figure 3). *Pieris brassicae* oral secretion contains the enzyme $\beta$-glucosidase that also elicits VOC emission (66). The novel class of elicitors from cowpea weevil oviposition fluid (bruchins) elicits neoplasmic tissue formation in peas, which expels the oviposited egg from the pea leaf tissue (23).



intact plants. Moreover, volicitin was less effective than JA in eliciting VOC release (103a). Finally it is interesting to note that none of the separated enatiomers of volicitin were active in lima bean, which is also a host plant of the beet armyworm (108), suggesting that volicitin as an elicitor deserves additional research.

Recently, another class of herbivore-specific elicitors has been determined to induce a novel type of defense response in peas. These elicitors, long chain diols that are mono- and diesterified with 3-hydroxypropanoic acid, are called bruchins because they are excreted with the oviposition fluid of pea and cowpea weevils (Bruchidae) (Figure 2). In certain genotypes of peas, bruchins elicit neoplastic growth on pods, which lifts the egg out of the oviposition site and impedes larval entry into the pod (23). Because the neoplastic growth exposes the larvae to

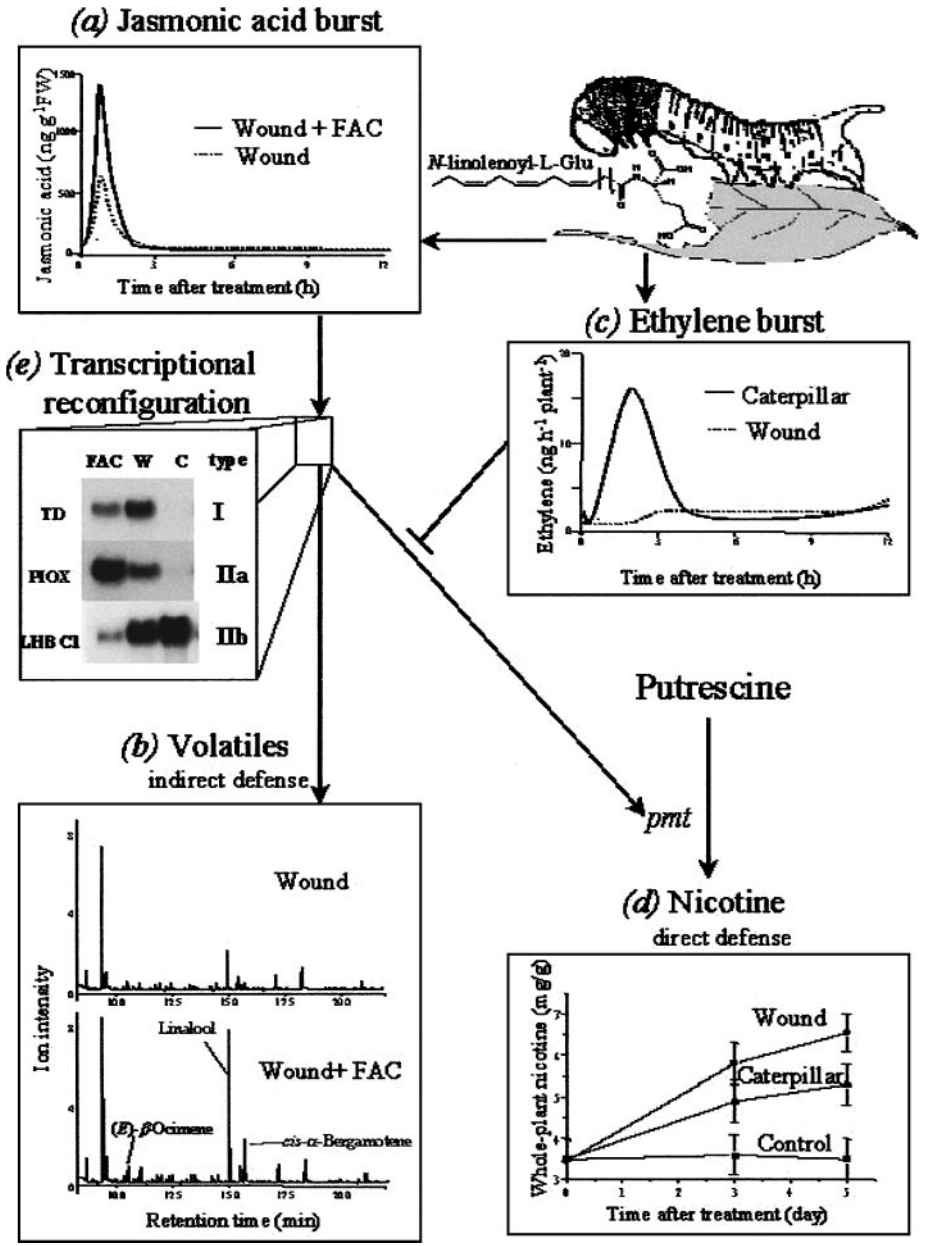
predators, parasites, and desiccation, it may function as an indirect defense. Similarly, the oviposition of elm leaf beetles, *Xanthogaleruca luteola*, elicits the emission of specific VOCs from the field elm host trees. The oviposition-induced VOC release attracts parasitic wasps that attack eggs (70), but the chemical basis of this elicitation remains unknown.

The early steps in the herbivore elicitation process remain to be elucidated. No receptors comparable to the *R*-gene products for pathogen recognition have been found for herbivore elicitors. Glucose oxidases may increase $H_2O_2$ production at the site of attack and may potentiate elicitation by forming ROS (29). Another mechanism has been suggested by Engelberth et al. (26), who found that the channel-forming peptide alamethicin, produced by the parasitic fungus *Trichoderma viridae*, elicited the release of volatiles in lima bean that are comparable to those elicited when the jasmonic acid (JA) cascade is antagonized by the salicylic acid (SA) cascade. However, such channel-forming peptides have not yet been found in the saliva of insect herbivores.

The only system so far to demonstrate a link from the elicitor to subsequent signaling steps is the *Nicotiana attenuata-Manduca* system (Figure 3) (see Crosstalk below). FACs in the oral secretions of two *Manduca* species elicit JA and ethylene



**Figure 3** Alteration of the wound response of wild tobacco plants (*Nicotiana attenuata*) by *Manduca* caterpillar feeding. (*a*) Wounding of the leaf tissue results in a JA burst, which is amplified by caterpillar feeding and the application of FACs from larval oral secretions to the wound (36, 103). (*b*) Caterpillar attack, or the application of FACs to wounds, but not wounding alone induces the production of volatiles that function as predator attractants in the plant's indirect defense (35, 36). (*c*) Caterpillar feeding and the application of their oral secretions to wounds cause an ethylene burst (49), which (*d*) attenuates the wound- and JA-induced accumulation of nicotine by suppressing the accumulation of transcripts for a key regulatory step in nicotine biosynthesis (pmt: putresine N-methyl transferase) (121). The attenuation of the direct defense, nicotine, may be an adaptation to the feeding of a specialized herbivore, which is able to tolerate high alkaloid concentrations and can potentially use them for its own defense (49). (*e*) Caterpillar attack and the addition of FACs to plant wounds also result in a transcriptional reconfiguration of the plant's wound response (43). This reconfiguration of the wound response consists of three temporal and spatial alterations. Addition of FACs antagonizes the wound-induced increase (W) of transcripts encoding threonine deaminase (TD), representing a type-I expression pattern, which spreads systemically throughout the plant from the wound site. The wound-induced increase in transcripts in a type-IIa expression pattern [exemplified by PIOX (pathogen-induced oxygenase)] is further amplified after application of FACs to wounds. In contrast, the genes with a type-IIb expression pattern are suppressed after wounding and further suppressed with the addition of FACs, as exemplified by the gene encoding the light-harvesting complex subunit LHB C1. Both type-IIa and -IIb patterns are found only in the leaves directly suffering the herbivore attack (102).



bursts, which are involved in the alterations of the *N. attenuata* wound responses (36, 103, 121).

## WOUND-ELICITED RESPONSES

Wound-induced resistance is to a large extent mediated by products of the "octadecanoid" ($C_{18}$-fatty acids) pathway. The production of various defense-related compounds, e.g., toxins, antinutritive and antidigestive enzymes, requires signaling by octadecaniods, such as 12-oxophytodienoic acid (OPDA), JA, and methyl jasmonate (MJ), all derived from linolenic acid (14). Our understanding of the local and systemic signaling pathways that transduce the signals produced at the wound site into changes in defense-related gene expression throughout the plant and of how herbivore-specific elicitors modify these signaling pathways is still sketchy. An encouraging trend has been the increase in studies using herbivore bioassays to evaluate the consequences of manipulations, in particular signal transduction chains, as well as those using insect attack and transcriptional profiling to identify new signaling pathways.



### Wound Signals

Both herbivore feeding and mechanical damage induce systemic responses that are rapidly propagated throughout the plant as well as responses that are restricted to the wound site. Systemic responses require mobile signals; these could be electrical (41), hydraulic (65), and chemical (99). In tomato, PIs are induced by signals transported in both xylem and phloem tissues. Steam girdling the petiole, which kills the phloem but leaves the xylem intact, does not prevent systemic induction of PIs after severe wounding, suggesting a xylem-transported signal. Small crushing wounds do not, however, induce a xylem-mediated signal; rather, they elicit PI production in organs distal to the wound and with intact phloem connections (94). Moreover, *Pin2* (PI II) gene expression can be induced by electrical signals that are associated with wounding. The response and propagation of wound-induced electrical signals apparently requires intact abscisic acid (ABA) signaling pathways because they both are lacking in ABA-deficient tomato mutants (41).

Immediately after wounding, plants transiently produce ROS, such as the superoxide anion, locally in the damaged tissue and $H_2O_2$ both locally and systemically throughout the plant (114). Because wound-induced oligogalacturonides transiently elicit ROS production, they are thought to be a primary signal of tissue damage (62). Moreover, both oligogalacturonides and fungal-derived chitosan can elicit ROS and the expression of wound-inducible PI genes. However, oligogalacturonides have limited mobility and are also induced by systemins (Figure 2), and they probably represent a local, intermediate step in signaling following systemin production, rather than a mobile primary signal (62).

Systemin, the first described oligopeptide with phytohormonal function, is thought to represent the primary wound signal in some solanaceaous plants (tomato, pepper, black nightshade, tobacco). Cleaved from a 200–amino acid precursor called prosystemin, and probably transported in the phloem, it is active at femtomolar levels and is the best-verified mobile signal (99). Transformed tomato plants expressing prosystemin cDNA in an antisense orientation do not express PI I and II after wounding and are more susceptible to attacking *Manduca sexta* larvae (80). Plants transformed to overexpress the prosystemin gene, in turn, exhibit constitutively activated wound responses and PI transcript expression (69). Systemin-binding proteins in the plasma membrane of tomatoes and on the surface of *Lycopersicon peruvianum* suspension cultured cells initiate a complex wound cascade after binding systemin. The wound cascade concludes with the activation of a phospholipase $A_2$, which releases linolenic acid from the plasma membrane, supplying the substrate for the initial step in the octadecanoid pathway (99). The systemin-mediated wound cascade is likely to be the main cascade that is subsequently directly modified by herbivore-specific elicitors or indirectly through the recruitment of other signaling cascades. With the recognition that $C_{16}$-fatty acids and other lipids can be used in the production of JA and other potential signal molecules, the term "octadecanoids" ($C_{18}$-fatty acids) should be broadened to "oxylipins" (28). However, because the major advances in the endogenous manipulation of wound- and herbivore-induced oxylipins are derived from $C_{18}$-fatty acid substrates, we retain the term octadecanoids in the following discussion.

## Octadecanoids

The importance of the signaling function of octadecanoids for plant-insect interactions has recently been demonstrated by manipulating three enzymes in the pathway: lipoxygenase (LOX), hydroperoxide lyase (HPL), and JA carboxyl methyltransferase (JMT).

LOX, the nonheme iron-containing dioxygenase, catalyzes the oxygenation of linolenic acid to the 9- and 13-hydroperoxides, which are in turn converted to aldehydes and oxoacids. Products from 13-hydroperoxy linolenic acid can be further elaborated by enzymatic cyclization, reduction, and $\beta$-oxidation to produce JA. The LOX proteins play roles in plant growth and development, in maturation and senescence, and in the metabolic responses to pathogen attack and wounding that are thought to be mediated by their role in the biosynthesis of oxylipin signals such as JA and OPDA (14, 100).

A number of studies suggest that different LOX isoforms have different functions. In *A. thaliana*, cosuppression-mediated depletion of a LOX isoform led to a decrease in the wound-induced JA levels but did not affect basal JA levels (9). Different LOX isoforms are encoded by multigene families, of which at least three have been found in potato. Transgenic potato plants devoid of one 13-LOX isoform (LOX-H3) through antisense-mediated depletion of the LOX mRNA do not

accumulate PIs in response to wounding. Moreover, when Colorado potato beetle larvae and beet armyworm larvae fed on antisense plants, they grew significantly larger than those fed on wild-type plants. More puzzling, however, was the observation that the effect of LOX-H3 on resistance was apparently not through its involvement in the wound-induced increase of JA. JA levels after wounding were similar in both antisense and wild-type plants, and the exogenous application of JA was not able to recover wild-type PI levels in LOX-H3 antisense plants (98). These results suggest that LOX-H3 is producing a JA-independent signal by one of several possible mechanisms: LOX-H3 may directly affect a *pin* gene response; it may be involved in a regulatory network that influences the expression of other LOX genes; or it may affect the wound-responsive production of ethylene that is required, together with JA, for maximal *pin2* expression in tomato (79). It is interesting to note that LOX-H3 antisense potato plants produced more flowers and on average 20% more tuber mass than did wild-type plants, suggesting that the resistance mechanisms mediated by this isoform of LOX incur fitness costs when they are not needed (98).

One of the LOX-catalyzed products, 13(*S*)-HPOT, is a substrate for several other enzymes that are thought to be important in plant-insect interactions, including HPL, which cleaves the 13-hydroperoxide into $C_6$-aldehydes and $C_{12}$-oxoacids (100). The $C_6$-aldehydes are thought to protect the wound site from microbial infection and to function as a direct defense against some herbivores, whereas the $C_{12}$-products, which include traumatin and traumatic acid, may be involved in wound healing (78). More recently, the $C_6$-aldehydes have been suggested to play a role in directly eliciting defense-related gene expression (8), systemin-based signaling (106), and signaling between plants (27).

Recent experiments with HPL-depleted potato plants showed that HPL gene expression is developmentally controlled and the activity levels are posttranscriptionally regulated (115), complementing results from tomato studies (45). Moreover, normal wound-induced gene (*AOS*, *LOX-H1*, *LOX-H3*, *prosystemin*, *Pin2*, *LAP*) expression was found in these plants, despite diminished hexenal/3-hexenal production, suggesting that these metabolites do not play a signaling role. It is interesting to note that *Myzus persicae* aphids performed better on HPL-depleted plants than they did on wild-type plants, suggesting that the $C_6$-aldehydes produced by HPL may play an important role in direct defenses (115).

If the 13(*S*)-HPOT produced by LOX is not metabolized by HPL, it can become a substrate for allene oxide synthase (AOS), which produces the unstable allene oxide; this, in turn, is cyclized by allene oxide cyclase (AOC) to form enatiomerically pure 9*S*,13*S*-OPDA (100). A subsequent reduction and $\beta$-oxidation of OPDA produces JA. Both OPDA and JA actively regulate defense gene expression and elicit resistance (14). Most of the genes that encode enzymes in the JA biosynthetic pathway are activated by wounding, and some (e.g., *LOX*, *AOS*, *12-OPDA reductase*) are also upregulated by exogenous JA application, suggesting positive feedback control (62). Overexpression of flax AOS in potato increased constitutive JA concentrations but did not influence the expression of *pin2* genes (38), which

suggests a JA-independent signal for *pin*-gene expression. However, the resistance of the AOS overexpressing potato lines against insect attack was not reported.

Although most of the octadecanoids are involved in systemic responses, their function as a mobile signal has yet to be conclusively demonstrated. Applications of $^{14}C$ labeled JA to leaves of *Nicotiana sylvestris* plants provided evidence for shoot-to-root transport with a kinetic that was identical to the appearance of a transient increase in endogenous JA concentrations in the roots of plants after leaf wounding, suggesting that JA was transported from wounded leaves to roots in these plants (123). Glucuronidase (GUS) reporter gene fusion experiments with the AOS promotor from *A. thaliana* and *N. tabacum* found AOS activation locally and systemically upon wounding. In contrast to the findings from *N. sylvestris*, this induction remained restricted to the application site of JA or OPDA, suggesting that these two octadecanoids were not systemically transported in the transformed plants (57). However, recently the gene for *S*-adenosyl-L-methionine–JMT from *A. thaliana*, which catalyzes the formation of MJ from JA, was cloned (104). JMT transcripts were induced both locally and systemically after wounding and MJ treatments. Transgenic plants overexpressing *JMT* had elevated MJ levels and constitutively expressed JA responsive genes, such as *VSP* and *PDF1.2*, but they exhibited wild-type-JA contents. The expression of defense genes may have contributed to enhanced levels of resistance against the pathogen *Botrytis cinerea* (104). These results suggest that MJ may regulate defensive gene expression and have the potential for mobile intra-and interplant signal function. Earlier work had proposed that MJ was an airborne signal that functioned in interplant communication for defense responses (27, 51).



## Crosstalk

Although it is becoming increasingly clear that single signal cascades, as illustrated by the oxylipins, can produce a bewildering array of potential secondary signal molecules with potentially different functions (14, 28, 119), it has also become apparent that herbivore attack frequently involves the recruitment of several signal cascades, the interaction ("crosstalk") among which may explain the specificity of the responses. Reymond & Farmer (92) proposed a tunable dial as a model for the regulation of defensive gene expression, based on the crosstalk of the three signal pathways for JA, ethylene, and SA. According to this model, a plant tailors its defensive responses to a specific attacker by eliciting signal molecules from the three pathways to different degrees. The elicitation of multiple pathways after attack is likely common and may be necessary if plants are to tailor their responses adaptively to the diverse herbivore species that attack them (118). Herbivores frequently attack in guilds, specifically or opportunistically vectoring microbes into the resulting wounds. These interactions of herbivore and pathogen attack and the resulting signal crosstalk may compromise a plant's responses. Some attackers may induce a pathway (e.g., pathogens activating primary SA responsive genes) that influences the activity of another (e.g., chewing herbivores activating JA responsive

genes), thereby compromising the plant's defensive reaction against one or both of the enemies (63, 82).

How these responses are fine-tuned to optimize the defense against a particular herbivore species or guild is the subject of recent investigations and reviews (11, 118). One of the clearest differences in defense responses to herbivores exists between chewing caterpillars and phloem-sap-sucking whiteflies or aphids. That attack from *Manduca sexta* caterpillars is "recognized" by the plant as evidenced by a JA burst far greater than that produced by the wounding that herbivores' feeding behavior causes (68, 103) (Figure 3). This JA burst is associated with expression of both wound-responsive genes and a set of novel JA-independent genes. The introduction of oral secretions from the feeding caterpillar into the wound site can account for the differences (36, 56, 102). Aphid feeding, in contrast, induces the expression of pathogen-responsive, SA-, and wound-responsive, JA-regulated genes (118). Green peach aphids, *Myzus persicae*, induced SA-dependent transcription of *PR-1* and *BGL2* in wild-type plants but not in *npr1* mutant plants, which are deficient in SA signaling (76). In addition, plants attacked by aphids had higher mRNA levels of *PDF1.2* (encoding defensin) and *LOX2* (encoding lipoxygenase), both of which are wound inducible and involved in the JA signaling cascade. SA- and JA-dependent genes have also been induced in plants attacked by phloem-feeding whiteflies. These whiteflies induce the expression of genes involved in lignin production, SA biosynthesis, oxidative burst, as well as in pathogenesis-related and JA-responsive PR proteins (118). Moreover, silverleaf whitefly feeding on squash induces accumulation of transcripts encoding *SLW3*, a gene that is not responsive to any known wound or defense signal. This suggests that there are other defense signal cascades waiting to be discovered (116).

The observation that whitefly and aphid feeding elicit both JA- and SA-induced genes runs counter to well-described observations that SA and other cyclooxidase inhibitors can effectively inhibit wound-induced JA production and JA-elicited gene expression (7, 84). Moreover, pathogen-inducible SA, such as is elicited by TMV infection, inhibits wound-inducible JA accumulation and secondary metabolite accumulation (87). The stimulation of SAR (systemic acquired resistance) with the SA mimic, BTH (benzothiadiazole), attenuated the JA-induced expression of polyphenol oxidase in tomato plants and increased the performance of *Spodoptera exigua* caterpillars, suggesting compromised defense responses. Other examples further undermine the belief that SA exclusively mediates pathogen responses and JA exclusively mediates herbivore responses. *Pseudomonas syringae*, a tomato pathogen, induces responses typically associated with SAR and SA signaling, such as PR-protein expression, but also expression of PI genes,which are normally considered to be JA induced. This activation of different pathways may underlie the observed crossresistance of *P. syringae*–attacked tomato plants against both the pathogen and subsequently feeding noctuid larvae (11).

Crosstalk between ethylene and octadecanoid pathways can be either synergistic or antagonistic. Synergistic effects of JA and ethylene have been reported from *A. thaliana*, in the expression of defensive genes [e.g., *PDF1.2* (85)]; from tomato, for maximal induction of *PI* gene expression (79); and from cultivated

tobacco, *Nicotiana tobacum*, for the expresssion of two *PR* genes encoding PR1b and osmotin (122). In contrast, in wild tobacco plants (*N. attenuata*), ethylene antagonizes JA-induced transcript accumulation after herbivore damage (Figure 3). In this species, wounding and mammalian herbivore attack increase the production of a potent defense metabolite, nicotine, which in turn is activated by proportional changes in endogenous JA production (7) as well as exogeneous JA applications (5). Attack by the tobacco hornworm, *M. sexta*, a solanaceous specialist, or application of its regurgitant to wounds results in a JA burst (36, 103) and reduces induced nicotine production (68). The attenuation of nicotine accumulation results from an ethylene burst after hornworm feeding (49), which antagonizes the wound-induced transcriptional increase in the nicotine biosynthetic genes *NaPMT1* and *NaPMT2* (121).

Genoud & Metraux (33) summarized examples of the crosstalk between different signal cascades and modeled them as Boolean networks with logical gates and circuits. This model complements that of Reymond & Farmer (92) and makes concrete predictions regarding the outcome of the crosstalk between pathways. The utility of this approach is limited by our incomplete understanding of all the cascades that are involved. Also lacking from the model is how crosstalk translates to ecological interactions among players on the second and third trophic levels and how compromised plant defense responses translate into plant fitness. An understanding of the functional consequences of crosstalk requires a sophisticated understanding of whole-plant function, which the *Manduca-Nicotiana* interaction illustrates (Figure 3).

In this natural interaction, JA and ethylene bursts are both induced when larval-specific elicitors (FACs) (Figure 2) are introduced to the feeding sites and result in reduced nicotine induction. As such, the crosstalk appears to benefit the herbivore and suggests that *Manduca* is feeding in a "stealthy" fashion, reducing its dietary intake of nicotine by suppressing the nicotine (68). However, parallel to the nicotine attenuation, *Manduca* feeding and application of oral secretions or FACs to the wound induce the emission of a suite of VOCs (35) that function as an indirect defense by attracting predators to the feeding herbivore (52). With the downregulation of a direct defense and the parallel upregulation of an indirect defense, *N. attenuata* may be optimizing the defensive function of its volatile release by suppressing nicotine production, which could be sequestered by the herbivore and used against predators attracted by the volatile release. Plant defense compounds are commonly sequestered by adapted herbivores for their own defense, and thus induced nicotine production may wreak havoc with the plant's ability to use "top-down" processes as a defense (113).

## MOLECULAR ADVANCES IN DIRECT DEFENSES

Almost any plant trait can be manipulated to function as a direct defense; however, most of the research has focused on the veritable arsenal of secondary metabolites that function as poisons, digestibility reducers, and repellants. Although many

potential direct defenses have been identified, definitive proof of the defensive function of a particular metabolite is in large part limited to the few examples in which the molecular basis of production is sufficiently understood to allow its expression to be manipulated. We limit our review to those studies that demonstrably influence a plant-insect interaction or contribute to an understanding of how variability in direct-defense profiles is generated. In the evolutionary arms race between plants and insects, plants are expected to be under strong selection to evolve new defenses as insects evolve resistance to the initial suite of defenses (25). The mechanisms responsible for the generation of variability in direct defenses are therefore of particular interest. Because a majority of the molecular efforts have focused on model solanaceous and brassicaceous plant systems, these are the systems with the most advances.

The defensive function of PIs was first described in solanaceous plants and is now one of the best-verified groups of direct defenses. PIs are expressed in seeds and tubers and also in vegetative tissue after wounding. Wound-induced PIs have been shown to enhance plants' resistance to insects by inhibiting the proteolytic enzymes of the attacking insect. A majority of the described plant PIs are inhibitors of trypsin and chymotrypsin (55), but recent work with maize (110) describes an herbivore-induced PI that inhibits both elastase and chymotrypsin in the midgut of *Spodoptera littoralis* larvae. The defensive effectiveness of PIs depends on their affinity and specificity for the midgut proteinases of the attacking insect and the ability of the insect to alter its proteinase profile and overexpress proteinases, which are PI insensitive after ingestion of PI-laced food (55). Hence a PI that is able to inhibit two types of insect proteinases may be particularly difficult for the herbivore to counter and may be useful in engineering durable resistance in crops.

Because PIs and other defensive proteins are direct gene products, their defensive effects have been tested by genetic transformation in a number of plant species. A recent example is the transformation of white poplar with an *A. thaliana* cystein proteinase inhibitor gene (*Atcys*), which conferred resistance to a major insect pest, *Chrysomela populi*, by inhibiting most of the digestive proteinase activity of this chrysomelid beetle (18). However, when novel PIs are expressed in a host plant, resistance to all of a plant's herbivores is rarely achieved, and the degree of resistance is in part determined by the insect's counter responses. A study that compared the performance of three generalist lepidopteran herbivores on each of three different host plants (tobacco, *Arabidopsis*, and oilseed rape), each transformed to express the mustard trypsin inhibitor MTI-2, demonstrated that the chemical milieu in which the PI is expressed influenced its defensive function (17). To keep one step ahead of rapidly adapting herbivores, plants have evolved mechanisms to produce many new active PIs. Some of these may be able to retain their defensive function in the constantly changing chemical environments of a plant as it matures and senesces. The multidomain structure of some PIs may allow a plant to produce inhibitors against a broad spectrum of proteases that retain their defensive function in different chemical environments.

The serine proteinase inhibitors of the potato type-II inhibitor family consist mostly of two repeated domains (124), whereas in the stigmas of *Nicotiana alata* flowers, the PIs are produced from a precursor protein with six repeat domains (61) that after proteolytic processing eventually produces six single-domain PIs (four trypsin and two chymotrypsin PIs) and a novel two-domain PI. A four-domain PI from the stigmas of the same plant species has recently been discovered (74). These multidomain structures probably allow plants to target a large number of different proteases within a relatively short period of time (74). Over evolutionary time, the reactive sites in the PI genes have accumulated a larger number of mutations, which we would expect to be the signature of an evolutionary arms race to diversify PI properties (39).

*Arabidopsis* produces many different glucosinolates that can protect plants against generalist herbivores and pathogens but also function as feeding and/or oviposition attractants for Brassicaceae specialists (13). Heterogeneous selection pressures may therefore maintain the variation in metabolite profiles within populations. A model for how the qualitative and quantitative diversity of glucosinolate profiles is maintained was recently proposed by Kliebenstein et al. (53). They examined 39 *Arabidopsis* ecotypes and found that polymorphisms at only five loci, each coding for different branch points in the glucosinolate biosynthetic pathway, were sufficient to generate 14 qualitatively different leaf glucosinolate profiles, including 34 different structures, most of which are derived from methionine by chain elongation. Moreover, a single locus appeared to control a majority (nearly 75%) of the observed quantitative variation.

Transformation has been used to examine the importance of both qualitative and quantitative variation in glucosinolate profiles of *A. thaliana*. For example, the *CYP79A1* gene from *Sorghum bicolor*, which encodes an enzyme that normally converts L-tyrosine to *p*-hydroxyphenylacetaldoxime in the biosynthesis of cyanogenic glycosides, produces *p*-hydroxybenzylgucosinolate, sinalbin (a glucosinolate not normally found in *A. thaliana* plants) when transferred to *A. thaliana* plants (77). The expression of this gene caused a fourfold increase in total glucosinolate levels (largely owing to increased sinalbin production) but did not alter the acceptance of the plants by two brassicaceous specialist flea beetles (*Phyllotreta nemorum* and *P. cruciferae*) in choice tests. This demonstrates that the plant is unlikely to realize lower herbivore loads from these specialists even with an enormous increase in investment in glucosinolate production.

For a plant to rid itself of its specialist herbivore community might require the evolution of an entirely new defense system. Advances in the ability to transform plants with all of the enzymes required for an entire secondary metabolite pathway have allowed researchers to recreate exactly such an evolutionary event. Cyanogenic glucosides, such as dhurrin, are not normally found in any brassicaceous plants, but recently the complete biosynthetic pathway for dhurrin was transferred to *A. thaliana* by expressing two multifunctional microsomal P450 enzymes (CYP79A1 and CYP71E1) and a soluble UDPG-glucosyltransferase (sbHMNGT) (111). It is remarkable that these three enzymes self-organized into a functional

complex that efficiently transferred reaction products so that transformed plants were able to produce and store large amounts of dhurrin. Transformed plants were completely resistant to the Brassicaceae-specialist *P. nemorum*. The ability to transfer entire secondary metabolite pathways between species will allow researchers to test a fundamental tenet of the theory by Ehrlich & Raven (25) of plant-herbivore coevolution: that the diversity of secondary metabolites represents constraints on the evolution of herbivore-host selection.

Resistance to herbivores in *Arabidopsis* is correlated with glucosinolate production and breakdown (myrosinase) and the presence of trichomes (67). However, a recent QTL analysis revealed a locus that did not map to any locus of the previously known resistance traits. Jander et al. (47) crossed two commonly studied *Arabidopsis* ecotypes that differed in their susceptibility to the larvae of *Trichoplysia ni* (a generalist noctuid), the Landsberg *erecta* ecotype, and the Columbia ecotype (which is considerably more resistant). Susceptibility mapped to the *TASTY* locus on chromosome 1, which was distinct from genes that affect trichome density, disease resistance, glucosinolate content, and flowering time (47), but close to a recently discovered locus, *esp*, which causes the formation of epithionitriles during the hydrolysis of glucosinolates instead of isothiocyanates (59). Glucosinolates are hydrolyzed during wounding and herbivore attack, and this finding underscores the importance of studying direct defenses not only in planta, but also in the insect digestive system.

Because herbivores take plant material into an environment that they chemically control (112) and adapt their digestive and detoxification systems to neutralize the effects of direct defenses (90), studying these mechanisms and the chemical dynamics that occur in insect guts might provide important insights into the plant traits that provide direct defense. However, it is also clear that the spatial scale needs to be broadened beyond the insect gut to include insect behavior and the natural enemies of insects. For example, when *Brassica napus* plants were transformed with the gene coding for a potato PI, diamondback moth larvae compensated for their decreased digestive efficiency by eating more leaf material (120). Such compensatory responses demonstrate that many direct defenses, particularly those that slow herbivore growth but do not kill them, may not function as defenses if they are not expressed in concert with indirect defenses, namely plant traits that increase the foraging efficiency of the natural enemies of herbivores (75). A combination of defenses that slow the growth of herbivores and increase the probability of their mortality before they become reproductively mature is likely to strongly suppress the growth of herbivore populations and represent a particularly effective defense.



## MOLECULAR ADVANCES IN INDIRECT DEFENSES

Analysis of the costs of resistance suggests that certain direct defenses can incur substantial metabolic loads and decrease plant fitness when other demands are made on a plant, such as when they are grown with competitors (5). Perhaps as a

consequence of these costs and other constraints on the use and effectiveness of direct defenses, plants have evolved defensive mutualisms in which, for a small investment in information-containing VOC releases or nutritional rewards, insects from higher trophic levels are recruited for a plant's defensive needs. Natural selection should favor both plant genotypes that use traits that enhance effectiveness of natural enemies on one hand and predator genotypes that are able to use such plant traits on the other hand (21). Many plant species express these indirect defense traits when they are attacked by herbivores, and many predators clearly use these traits to increase their foraging efficiency (Figure 1). Although evidence for the effectiveness of these defenses in nature is mounting, much remains to be discovered about the mechanisms responsible for expression of these traits. In the following section, we review the molecular advances in two indirect defenses: One is widespread among plants, herbivore-induced VOC emissions, and one is limited to a few taxa, the induction of extrafloral nectar production.



## Volatile Organic Compounds

The VOC emission of more than 15 plant species involved in plant–spider mite–predatory mite, plant-caterpillar-parasitoid (21), plant–leaf beetle–egg parasitoid (70), and plant–caterpillar–predatory bug interactions (52) has been examined. In all systems, the host plant releases wound- and herbivory-inducible volatiles that function as signals in tritrophic interactions. Detailed analysis of the released volatile bouquets has identified many signals that are common to many different plant species, but there are also many compounds that are species specific and are elicited by herbivore-specific cues (21, 36).

The volatiles originate from at least three biosynthetic pathways. First, the so-called green-leaf volatiles, $C_6$-alcohols and -aldehydes, are produced from $\alpha$-linolenic acid and linoleic acid via their respective hydroperoxides (78). Some of the green-leaf volatiles may function as direct defenses, as was elegantly demonstrated in a study of transgenic potato plants with depleted HPL (see Octadecanoids), which exhibit lower resistance to aphids (115). Green-leaf volatiles also play a role as kairomones (signals with information content). *Cis*-3-hexen-1-ol is commonly found in the headspace of plants after herbivore attack (35, 113), and enhancing its release from plants in a field study attracted a generalist predator (52). *Trans*-2-hexenal, another green-leaf volatile with biocide effects (15) and commonly emitted after herbivore wounding (19), elicits the accumulation of sesquiterpenoid phytoalexins in wounded cotton and *Arabidopsis*, suggesting a potential role in intra- and interplant signaling. The electrophile $\alpha,\beta$-unsaturated carbonyl group of many green-leaf volatiles may confer the ability to induce stress and defense responses in plants (8, 27), but these potential functions need to be verified.

Second are the terpenes derived from the two (mevalonate and nonmevalonate) isoprenoid pathways. Both mono- and sesquiterpenes play a major role

as kairomones in attracting predators and parasitoids to attacked plants (113) as well as functioning as phytoalexins (46). Elicitors present in the insect regurgitant induce the release of mono- and sesquiterpenes after herbivore attack (4, 21, 36). Some of the terpenoids induced in lima beans after herbivore damage [homoterpenes 3*E*-4,8-dimethyl-1,3,7-nonatriene (DMNT) and 3*E*,7*E*-4,8,12-trimethyl-1,3,7,11-tridecatetraene] are synthesized de novo in response to herbivore attack (22), and DMNT emission has recently been shown to depend on the herbivore-specific expression of (*E*)-neridol synthase, which catalyzes the synthesis of the sesquiterpene precursor (3*S*)-(*E*)-neridol (16). Many terpenoids are emitted transiently and systemically after arthropod damage (21, 35, 113), and initial evidence suggests that the release of some compounds may be under transcriptional regulation (105).

Two regulatory enzymes in terpenoid biosynthesis are hydroxymethyl glutaryl-CoA reductase (HMGR), which catalyzes the first committed step to the mevalonate terpenoid pathway, and the family of terpenoid synthases, which catalyzes isomerizations and cyclizations of prenyl diphosphates into mono- and sesquiterpenes. The plastidial monoterpene synthases and the cytosolic sesquiterpene synthases share a high degree of sequence similarity and reaction mechanisms (10), which has made functional knockouts difficult to generate. Although the accumulation of HMGR transcripts is induced rapidly by wounding and amplified by herbivore regurgitants (56) and $C_6$ aldehydes (8), no mutants are currently available for this gene. A first cyclase mutant identified from maize (105) carried an *Ac* (transposition mutation) insertion in the sesquiterpene cyclase, *stc1*, which is normally induced 15- to 30-fold by insect damage, insect oral secretion, and purified volicitin (Figure 2) in wild-type plants. In contrast, *stc1* was not induced in mutant plants, and an analysis of volatiles revealed that *stc1* encodes a synthase for a naphthalene-based sesquiterpene (105). Naphthalene is unfortunately not a major component of the VOC mixture of maize plants, and its function in indirect defense is still unknown.

The third pathway involves a group of volatile compounds emitted by herbivore-damaged plants that are derived from shikimate. This pathway links metabolism of carbohydrates to the biosynthesis of aromatic compounds in microorganisms and plants (44). Methyl salicylate, derived from this pathway, is emitted after herbivore damage but not after mechanical wounding by lima beans and wild tobacco. When applied to lima beans, methyl salicylate was attractive to foraging predatory mites (20) but was not attractive to predatory bugs foraging on wild tobacco in a field experiment (52). Another shikimate-derived metabolite is indole, which is released from maize seedlings after damage by beet armyworm caterpillars but not after mechanical damage. The blend of terpenoids and indole released from maize is attractive to the endoparasitic wasp *Cotesia marginiventris*, which attacks larvae of several Lepidoptera species (113). The enzyme indole-3-glycerol phosphate lyase catalyzes the formation of free indole and is, like naphthalene-containing terpenoids selectively activated by volicitin (Figure 2), an elicitor derived from caterpillar regurgitant (30).

The ecological function of VOCs—namely attracting predators and parasitoids and reducing the herbivore load of the plant—has been demonstrated in nature (52), but direct proof that plants that rely on this indirect defense experience fitness benefits is still lacking. Understanding the mechanisms responsible for the herbivore-induced VOC release and manipulating these mechanisms under field conditions will provide such proof and, additionally, evaluate the agricultural utility of this defense mechanism. Crops that release volatile signals in response to herbivore attack could provide the basis for a new era in sustainable biological control of agricultural pests.



## Extrafloral Nectar

In addition to VOC releases, plants use bait to attract the natural enemies of herbivores. These have been particularly elaborated in ant plants, which produce extrafloral nectar as well as Pearl and Mullerian food bodies to attract ants (3). These ant mutualists provide defense, which is so effective that, through evolutionary time, ant-housing acacias have apparently lost their chemical defenses [e.g., cyanogenic glucosides (89)]. Extrafloral nectaries are found in at least 66 families, and several studies have documented the defensive role they play for the plants by attracting wasps and ants that attack herbivorous insects and hence reduce damage from herbivores (3). Several studies have shown that the density of extrafloral nectaries and amino acid concentration of the nectar increases after herbivore attack, but until recently, nothing was known about the underlying mechanisms of elicitation. In *Macaranga tanarius* trees, extrafloral nectar secretion increased after herbivory, mechanical leaf damage, and exogenous JA application (40). In addition, phenidone, an antagonist of endogenous JA biosynthesis, inhibited wound-induced extrafloral nectar secretion, and both the transient increase in JA as well as the wound-induced nectar flow were strongly correlated with the amount of damage. Higher nectar secretion resulted in higher numbers of visitors and defenders, which significantly reduced herbivory. The JA cascade is involved in this indirect defense, as it is in herbivore-induced VOC release.

Ecological research into indirect defenses that are mediated by plant traits has demonstrated that the defensive value of the VOC release and nectar production lies principally in the spatial information these traits provide to the predators. Specifically, plants are helping small predators locate actively foraging herbivores in the vastly larger spatial dimensions of host plants. As a result, if indirect defenses are to be successfully applied in agricultural crops, the spatial information content of the signal must be preserved. If crops were engineered to constitutively release VOCs by, for example, a terpene synthase under control of a constitutive promotor, predators and parasites would rapidly learn to ignore these signals or, worse, to associate them with hunger and thus avoid emitting plants. Therefore the identification of herbivory-responsive regulatory elements will likely be important for the use of these defenses in biotechnology applications.

## COMPLEXITY AND COORDINATION OF INSECT-INDUCED RESPONSES

The changes in plant metabolism in response to herbivore or pathogen attack are probably orchestrated by complex transcriptional changes that include genes coding for both primary and secondary metabolism. Procedures for the analysis of differential expression (microarrays, subtractive libraries, AFLP-cDNA display, and DDRT-PCR) allow researchers to study changes in the "transcriptome," which are elicited in response to herbivore attack or to identify differences in expression between genotypes that differ in resistance. The first results of these techniques, which are just beginning to be applied to plant-insect interactions, suggest that the changes elicited by herbivore attack are comparable in scope and magnitude to those elicited by pathogen attack (64, 101).

The first microarray study of plant-insect interactions analyzed the timing, dynamics, and regulation of the expression of 150 wound-induced genes in *A. thaliana* (93). A time-course analysis of responses elicited by wounding identified groups of genes with similar behaviors, one of which was correlated with the appearance of signals from the jasmonate cascade: OPDA, dnOPDA (dinoroxophytodienoic acid), and JA. But not all genes in this group depended on the JA signaling, as revealed by the comparison of responses from the coronatine-insensitive *coi1-1* mutant (which is JA insensitive) with wild-type plants. Moreover, a comparison of expression patterns in mechanically wounded and *Pieris*-caterpillar-wounded plants revealed very different transcript profiles, particularly in the expression of the water-stress-induced genes, which were reduced in insect-attacked plants. However, the timing and the magnitude of damage caused by insect feeding were not mimicked in the mechanical wound treatment, so it is difficult to know whether the lack of drought-associated expression was a specific response to *Pieris* feeding (93). This microarray study examined a plant-insect interaction with a "boutique" chip consisting of a preselected group of genes. With the availability of microarrays that cover the complete genome of *A. thaliana*, we look forward to the first truly unbiased analyses of the transcriptional changes induced by herbivore attack in this model plant.

For studies with other plant systems that are not supported by genome sequencing projects, other less-expensive approaches provide unbiased analyses of insect-induced transcriptional changes. In one such analysis, differential display (DDRT-PCR) was used to analyze the transcriptional changes in *N. attenuata* after damage by the specialist herbivore *Manduca sexta*. The putative functions of induced and repressed transcripts could be crudely categorized as being involved in photosynthesis, electron transport, cytoskeleton, carbon and nitrogen metabolism, and pathogen response (43). Transcripts involved in photosynthesis were strongly downregulated, whereas transcripts responding to stress, wounding, and invasion of pathogens or involved in shifting carbon and nitrogen were upregulated. From this study, it was estimated that more than 500 genes responded to the attack of this specialized herbivore. To separate the wound-induced changes from the changes

elicited by the *M. sexta* oral secretion and regurgitant, a subset of the differentially expressed transcripts was analyzed, and three discrete patterns of expression were identified (102). Regurgitant modified the wound-induced responses by suppressing wound-induced transcripts systemically in the plant (type I) or amplifying the wound response in the attacked leaves (type II). This amplification was either a downregulation of wound-suppressed transcripts (type IIb) or an upregulation of wound-increased transcripts (type IIa) (Figure 3). It is interesting to note that all three patterns of *Manduca*-induced transcriptional changes of the wound response of *N. attenuata* could be fully mimicked by adding minute amounts of FACs to wounds (36) (Figure 3). The amounts of FACs required are so small that they may be transferred to the plant during normal feeding (102).

These two studies demonstrate that herbivore attack causes a coordinated transcriptional reorganization of the plant, which, in turn, points to the existence of herbivore- and wound-specific *trans*-acting factors that mediate the coordinated changes. Although nothing is known about the identity of such transcription factors, the recently discovered ORCA3, a JA-responsive APETALA2 (AP2)-domain transcription factor from *Catharanthus roseus* that regulates the expression of genes from both primary and secondary metabolism required for the production of terpenoid indole alkaloids (117), provides support for the concept. Identification of such regulatory factors will represent a major advance in understanding the bewilderingly complex transcriptional and phenotypic changes that are elicited after herbivore attack.



## CONCLUSION

Plant-herbivore interactions, in contrast to plant-pathogen interactions, are characterized by greater physiological independence of the actors, which has two important consequences for future work. First, the physiological independence of insect herbivores means that for many plant-insect interactions, the wound response will play a prominent role. The seminal work of Ryan and colleagues (99) in understanding the wound response provides an important foundation from which to understand how elicitors from herbivores modify these responses. Second, the arena in which the interaction is played out is clearly very large and includes not only the whole plant but its surrounding biotic community. These larger-scale interactions have been extensively studied by ecologists, and whole-organism entomologists and molecular biologists interested in understanding the function of the transcriptional changes observed after insect attack will benefit from establishing collaborations with these research communities.

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