# New Insights into Plant Responses to the Attack from Insect Herbivores

Jianqiang Wu and Ian T. Baldwin

Department of Molecular Ecology, Max Planck Institute for Chemical Ecology,
Hans-Knöll-Straße 8, 07745 Jena, Germany; email: jwu@ice.mpg.de, baldwin@ice.mpg.de

Annu. Rev. Genet. 2010. 44:1–24

The *Annual Review of Genetics* is online at genet.annualreviews.org

This article's doi:
10.1146/annurev-genet-102209-163500

0066-4197/10/1201-0001$20.00

## Key Words

plant-herbivore interaction, elicitor, defense, signaling, systemic response, natural variation

## Abstract

Plants have evolved sophisticated systems to cope with herbivore challenges. When plants perceive herbivore-derived physical and chemical cues, such as elicitors in insects' oral secretions and compounds in oviposition fluids, plants dramatically reshape their transcriptomes, proteomes, and metabolomes. All these herbivory-induced changes are mediated by elaborate signaling networks, which include receptors/sensors, $Ca^{2+}$ influxes, kinase cascades, reactive oxygen species, and phytohormone signaling pathways. Furthermore, herbivory induces defense responses not only in the wounded regions but also in undamaged regions in the attacked leaves and in distal intact (systemic) leaves. Here, we review recent progress in understanding plant perception of herbivory and oviposition, and the herbivory-induced early signaling events and their biological functions. We consider the intraspecific phenotypic diversity of plant responses to herbivory and discuss the underlying genetic variation. We also discuss new tools and technical challenges in studying plant-herbivore interactions.

**Secondary metabolites:** a very diverse group of compounds that are not directly required for plant development and reproduction and are distributed in specific plant lineages

**Direct defense:** toxins, repellants, antidigestive compounds, etc., that directly and negatively affect herbivore growth, reproduction, or fecundity

**Indirect defense:** production of volatile compounds that betray the location of feeding herbivores to their predators or substances that reward (e.g., extrafloral nectar) and thus nourish organisms that provide carnivore services for the plant and thereby reduce their herbivore loads

**MAPKs:** mitogen-activated protein kinases

# INTRODUCTION

There are almost a million insect species on this planet and nearly half of them feed on plants. This ongoing battle between plants and insects has lasted over 350 million years (24). During their coevolution with plants, insects have evolved to be able to locate their host plants for feeding and oviposition using physical or chemical cues from host plants. Generalist herbivorous insects feed on many plant species spanning different families, whereas specialists attack only one or a few plant species within the same family.

Accordingly, plants have evolved elaborate defense systems to resist insect herbivores. They equip themselves with physical barriers, such as thorns, trichomes, and cuticles. Moreover, many of the secondary metabolites in plants are powerful chemical weapons. There are estimated to be more than 500,000 secondary metabolites in plants (79). Overwhelming evidence has indicated the crucial importance of plant secondary metabolites in plant-herbivore interactions. Plants' direct defenses, which include glucosinolates, cyanogenic glucosides, alkaloids, phenolics, and proteinase inhibitors (PIs), function as toxins, repellents, or antidigestives. Plants' indirect defenses—green leaf volatiles, volatile organic compounds, and extrafloral nectars—attract the natural enemies (such as parasitoids) of herbivores. These two powerful defense systems, acquired by plants during the long arms race with herbivores, have enabled plants to survive (43). The timing of the deployment also defines defenses. Constitutive defenses are physical and chemical defensive traits that plants have regardless of the presence of herbivores; in contrast, inducible defenses are mounted only after plants are attacked by herbivores. In light of the energy and nutrient demands of the biosynthesis of defensive compounds (8, 148), it is not surprising that plants use highly sophisticated regulatory systems to balance growth against defense. Therefore, inducible defenses are particularly interesting, since they endow plants with flexible and economy-friendly defense systems.

Inducible defenses consist of three components: a perception component, which triggers a downstream regulatory network, which mediates the biosynthesis of metabolites that function as defenses. Highly complex pathogen-recognition mechanisms have been identified in many plant species. In contrast, little is known about how plants perceive herbivore-derived signals. However, exciting progress has been made in elucidating the regulatory elements in the networks that modulate herbivory-induced responses in plants; these include $Ca^{2+}$ ion fluxes, mitogen-activated protein kinases (MAPKs), jasmonic acid (JA), ethylene (ET), and reactive oxygen species (ROS).

In this review, we summarize the most recent understanding of how plants perceive herbivory, as well as the early herbivory-induced signaling events and short- and long-distance mobile signals that convey herbivory alerts to undamaged tissue and leaves. Furthermore, we consider the intraspecific diversity of plant responses to herbivory and discuss the underlying genetic basis and the ecological and evolutionary significance of this variability. Finally, we review recent advances in the development of genetic and molecular tools in plant biology that may benefit research on plant-herbivore interaction.

# PLANT PERCEPTION OF HERBIVORY

Defense is costly; thus discerning herbivory from casual mechanical wounding and promptly deploying increased levels of defensive compounds are critical skills in the battle between plants and herbivores. Many studies have demonstrated dramatic differences in plant responses to mechanical wounding and herbivory, although both result in tissue damage and loss (2, 7, 30, 101, 142). The rapid pace of advances into the molecular basis of plant perception of pathogens has been particularly inspiring for the study of how plants perceive herbivory. Various microbial (or pathogen)-associated molecular patterns (MAMPs or PAMPs) are recognized by specific receptors. The *R* gene–mediated defense system detects the presence of Avr proteins secreted from pathogens and initiates the hypersensitive response. Similarly, herbivore-derived elicitors or cues function as herbivore-associated molecular patterns (HAMPs) (83). These HAMPs may function in concert with herbivory-induced molecules originated from plants themselves to elicit the full patina of defense responses (31).

## Perception of Elicitors in Insects' Oral Secretions

Compared with the vast diversity of herbivores that attack plants, very few herbivore-derived elicitors are known, but the chemical and biological properties of those that have been identified are particularly intriguing. Fatty acid-amino acid conjugates (FACs) are a group of molecules that are the best studied among the known elicitors. The first fully characterized herbivore-derived elicitor, volicitin, a hydroxyl FAC [*N*-(17-hydroxylinolenoyl)-L-glutamine, **Figure 1*a***], was identified in *Spodoptera exigua* (beet armyworm) oral secretions (OS) (2). Since then, FACs have been isolated from several lepidopteran species (30, 97, 118, 119). Furthermore, FACs exist not only in caterpillars but also in crickets (*Teleogryllus taiwanemma*) and fruit flies (*Drosophila melanogaster*) (147). FACs are composed of two moieties: a fatty acid moiety [either linolenic acid (LA) or linoleic acid, as well as their derivatives, e.g., hydroxyl-modified] and an amino acid moiety (Glu or Gln). Interestingly, the fatty acid and the amino acid originate from plants and insects, respectively, and are synthesized in insects' midguts (91). FACs have recently been shown to play an essential role in insect nitrogen metabolism (146), and hence it may be difficult for insects not to produce FACs so as to feed stealthily on plants that use FACs to perceive insect attack.

The application of volicitin greatly enhances volatile emission in *Zea mays* seedlings, which attracts parasitoids to feeding larvae in laboratory assays (2). The biological functions of FACs have been intensively studied in *Nicotiana attenuata*, a wild tobacco plant that grows in western North America. Several forms of FACs were identified in *Manduca sexta* (a specialist herbivore feeding mainly on select solanaceous taxa) OS, the application of which to *N. attenuata*–wounded leaves induces the activation of MAPKs, JA, and ET biosynthesis and signaling, and the amplification and modification of wounding-induced transcriptomic, proteomic, and metabolomic responses, that have been shown to function as direct and indirect defenses in nature (25, 30, 78, 116, 142). Interestingly, FACs introduced into wounds during feeding are rapidly metabolized by lipoxygenases in the octadecanoid pathway to form additional active elicitors (A. van Doorn, I.T. Baldwin, G. Bonaventure, unpublished results).

The molecular mechanism of plant perception of FACs remains elusive. [$^3$H]-L-volicitin binds rapidly, reversibly, and saturably to maize cell membranes, and these binding capacities increase after methyl jasmonate (MeJA) treatment (132). This suggests the involvement of a FAC-specific receptor, whose abundance depends on JA signaling. Maischak et al. (72) proposed that the ion channel–forming properties of OS may contribute to the OS-induced ion fluxes and membrane depolarization, although FACs themselves aren't able to form stable channels. Nevertheless, because applying volicitin to several plant species showed no effect on JA and ET production, ion channel formation does not appear to be the function of FACs (110). Whether FACs and/or other components of OS form bona fide ion channels in plants, and whether and how ion fluxes and membrane depolarization are translated into cellular responses, deserve further study.

In addition to FACs, several other types of elicitors in insect OS have been discovered. Inceptins (**Figure 1*b***) are proteolytic products of the plant chloroplastic ATP synthase $\gamma$-subunit (cATPC). When fall armyworms (*Spodoptera frugiperda*) attack cowpea plants (*Vigna unguiculata*), the ingested cATPC is cleaved in insect

**R:** disease resistant
**Avr:** avirulent
**HAMPs:** herbivore-associated molecular patterns
**FACs:** fatty acid-amino acid conjugates

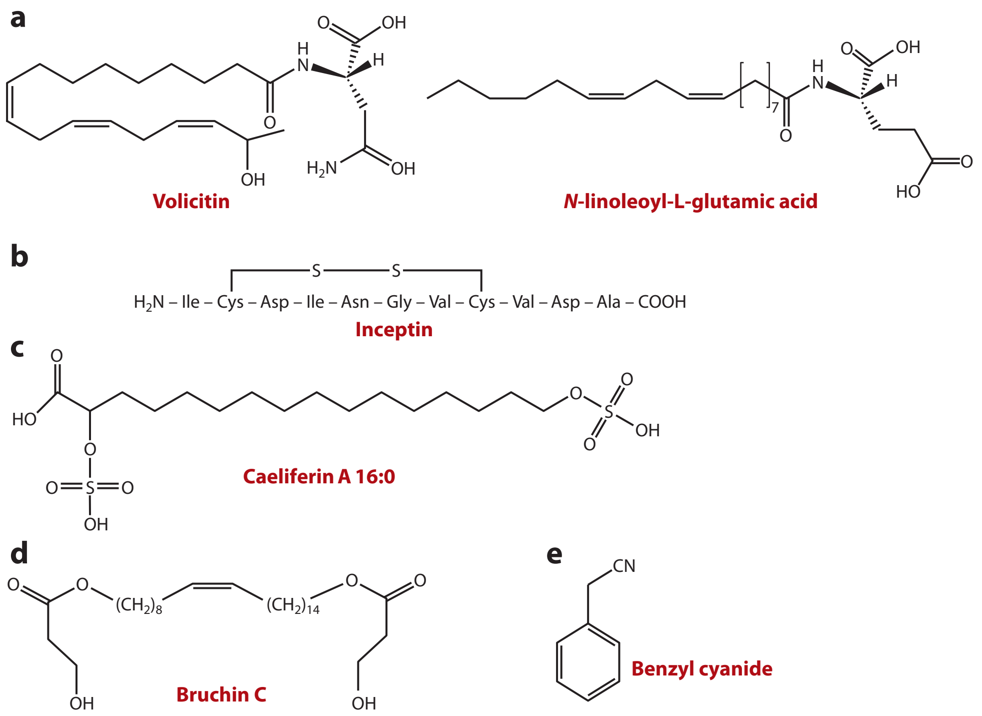

**Figure 1**

Structures of a sampling of the known herbivore-derived elicitors. (*a*) The fatty acid-amino acid conjugates, volicitin and *N*-linoleoyl-L-glutamic acid. (*b*) Inceptin is a proteolytic product of plant chloroplastic ATP synthase γ-subunit found in *Spodoptera frugiperda* oral secretions. (*c*) One of the caeliferins isolated from American bird grasshoppers (*Schistocerca americana*). (*d*) Bruchins are components of pea weevil oviposition fluids, which trigger the growth of neoplasma at the oviposition sites. (*e*) Benzyl cyanide in the oviposition fluids of the bufferfly *Pieris brassicae* induces the arrest of the egg parasitoid *Trichogramma brassicae* on Brussels sprouts.

midguts and forms inceptins; even a minute amount of inceptin introduced into mechanically damaged cowpea leaves dramatically augments the levels of JA, ET, and salicylic acid (SA) (109, 111). A new class of elicitors, caeliferins (**Figure 1*c***), was recently identified in American bird grasshoppers (*Schistocerca americana*). Like volicitin, caeliferins also elicit the release of volatile terpenes from maize seedlings (1). Given the great mobility of these grasshoppers, the ecological significance of eliciting volatile release in maize after grasshopper feeding (recognition of caeliferin) is unknown, as it seems unlikely that the volatile release could function as an indirect defense.

In addition to these elicitors with small molecular sizes, β-glucosidase in *Pieris brassicae* OS elicits parasitoid-attracting volatiles in cabbage (75). Glucose oxidase (GOX) in *Helicoverpa zea* saliva interestingly suppresses plant defense reactions (86), and GOX in *S. exigua* OS interferes with JA signaling by producing SA that antagonizes JA elicitation by the FACs that are also present in the OS (17). Testing the responses of various plant species from different families to FACs, inceptin, and a caeliferin highlighted the diversity of plant perception of elicitors in herbivore OS (110). There were no elicitors that induced responses (JA and ET accumulation) in all plant species; even closely related species may not have the same responsiveness to a given elicitor.

## Specific Patterns of Mechanical Wounding that Function as Herbivory Signals

The feeding behaviors of insect larvae are usually highly specific. The mode, speed, and frequency of tissue damage may be recognized by plants as herbivory-specific signatures. Using a programmable mechanical device that mimics the timing of herbivore feeding, but not its mechanical properties, Mithöfer et al. (84) revealed that in lima bean (*Phaseolus lunatus*), volatile bouquets elicited by continuous computer-controlled mechanical wounding qualitatively resembled those induced by attack from the caterpillar *Spodoptera littoralis* and the snail *Cepaea hortensis*. Therefore, the perception of certain temporal patterns of mechanical damage might also be an important part of the ability of plants to recognize herbivores.

## Sensing Oviposition

Many female adult herbivorous insects lay eggs directly into plants, and some species are known to perceive insects' oviposition activities and deploy direct and indirect defenses in response. After pea weevils (*Bruchus pisorum* L.) deposit eggs on pea plants (*Pisum sativum* L.), neoplasma forms at the sites of oviposition (19); as a result, the eggs are elevated above the surface of the leaves and may drop off. By expelling eggs, this response can be said to act as a direct defense. Rice plants produce an ovicidal substance that kills planthoppers' (*Sogatella furcifera*) eggs (113, 128). The oviposition by Colorado potato beetle (*Leptinotarsa decemlineata*) results in a hypersensitive response–like necrosis in a potato plant, and the necrotic regions to which eggs are attached disintegrate and detach the eggs (6). Oviposition also induces volatile signals in some plants that attract parasitoids to eggs (77).

Two substances in oviposition fluids are known to elicit defense responses in specific host plants. Bruchins were isolated from the oviposition fluid of pea weevils, which are long-chain $\alpha,\omega$-diols esterified at one or both oxygens with 3-hydroxypropanoic acid (**Figure 1*d***). Even 1 fmol (0.5 pg) of bruchins applied to the leaf surface results in neoplasma growth in a particular accession of pea pods (19). The other compound, benzyl cyanide (**Figure 1*e***), was found in the oviposition fluid of large cabbage white butterfly (*Pieris brassicae*) (22). One ng of benzyl cyanide induces the arrest of parasitoid *Trichogramma brassicae* on Brussels sprout plants (*Brassica oleracea* var. *gemmifera* cv. Cyrus), although whether the arrestment results in greater rates of parasitism and functions as a defense is unclear.

## *R* Gene–Mediated Herbivore Resistance

Aphids and whiteflies use their stylets to suck plant phloem sap to obtain nutrients. Although they don't produce substantial damage, aphids and whiteflies do elicit remarkable changes in plant signaling and secondary metabolism (135). Interestingly, an *R* gene, *Mi-1* (resistance to *Meloidogyne incognita*), was found to confer resistance to aphids, whiteflies, and nematodes (39). Recently, another *R* gene, *Bph14*, was found to confer resistance to brown planthoppers (*Nilaparvata lugens* Stål) in rice. Like the *Mi-1* gene in tomato, *Bph14* encodes a coiled-coil, nucleotide-binding site and leucine rich repeat (CC-NBS-LRR) protein (20). Interestingly, aphids and brown planthoppers are both phloem sap suckers, and both induce SA signaling. How these *R* genes are involved in herbivore resistance is unclear. Since these phloem sap suckers are known to introduce enzymes via their saliva into the plant during the feeding process, it will be interesting to discover whether R genes are involved in recognizing these potential elicitors.

**CC-NBS-LRR:** coiled-coil, nucleotide-binding site and leucine-rich repeat

Given the diversity of herbivore species and the very different fitness consequences of their attack for plants, it is reasonable to assume that like the rapidly evolving R proteins, plants have acquired multiple receptors and sensors that form a complex surveillance system for

**Membrane potential:** the voltage or electric potential differences between the inner and outer sides of a cell membrane

**CDPKs:** calcium-dependent protein kinases

**SIPK:** salicylic acid–induced protein kinase

**WIPK:** wound-induced protein kinase

herbivores. Depending on the HAMPs introduced by the herbivore into the plant, attack or oviposition may be perceived by a single receptor/sensor or a specific combination. Qualitatively and quantitatively different signaling events, though somewhat similar, may be triggered by these receptors/sensors.

## EARLY SIGNALING EVENTS INDUCED BY HERBIVORY

Herbivore attack induces a battery of molecular events in plant cells, which transduce the alarm signals and eventually result in the accumulation of defensive metabolites. Although little is known about how plants perceive herbivory, many small molecules, as well as proteins, have been identified to be the nodes of complex regulatory networks that enable plants to optimize energy and resource allocation and deploy appropriate defenses.

### Calcium Flux, Membrane Potential, and Calcium Sensors

$Ca^{2+}$ has been recognized as an important second messenger involved in numerous signaling actions in all eukaryotes. Under normal conditions, cytosolic $Ca^{2+}$ concentration is about 100 to 200 nM, which is $10^4$ times less than that in the apoplastic fluid and $10^4$ to $10^5$ times less than that in cellular organelles. $Ca^{2+}$ influx, which changes cytosolic $Ca^{2+}$ concentration, is often involved in stress responses and developmental regulation. Together with the fluxes of other ions, e.g., $Na^+$, $K^+$, and $Cl^-$, the fluxes of $Ca^{2+}$ ions usually result in temporary changes of cell membrane potentials. *S. littoralis* larvae feeding results in a large membrane depolarization in lima bean leaves; interestingly, the depolarization is located not only in the vicinity of the damage but throughout the leaf (70). Using a $Ca^{2+}$-specific dye, the authors also showed that herbivore damage induced a strong $Ca^{2+}$ influx in regions 30 to 200 μm away from the damage zones; the mechanical wounding-elicited $Ca^{2+}$ signal was weaker than that induced by herbivory, suggesting that an OS-recognition mechanism plays a role in activating $Ca^{2+}$ influxes (70). Pathogen-derived elicitors must be bound to their specific receptors to elicit changes of cytosolic $Ca^{2+}$ (10, 60). It is likely that herbivore-derived elicitors must also be bound to as-yet-unidentified receptors for $Ca^{2+}$ influxes to be triggered.

The changes in intracellular $Ca^{2+}$ are further translated into downstream actions through various $Ca^{2+}$ sensor proteins (61). These include calmodulins, calmodulin-binding proteins, calcium-dependent protein kinases (CDPKs), other EF-hand motif-containing $Ca^{2+}$-binding proteins, and $Ca^{2+}$-binding proteins without EF-hands. Among these, CDPKs are particularly interesting. They are plant-specific calcium sensors composed of many gene members (34 in *Arabidopsis*). A growing number of studies have revealed their roles in defense against biotic and abiotic stresses. Several CDPKs in *Arabidopsis* are involved in abscisic acid (ABA) signaling and thus plant resistance to drought or salt stress (85, 149). CDPKs are activated by pathogen elicitors and are important for disease resistance (11, 103). Furthermore, $Ca^{2+}$ is associated with ROS and nitric oxide production in plants. A potato NADPH oxidase is phosphorylated by two CDPKs in a $Ca^{2+}$-dependent manner, which in turn elevates its ability to produce ROS (51). To date, no CDPKs have been identified as part of a plant defense system against herbivores.

### MAPK Signaling

MAPK signaling is a well-conserved pathway that regulates various cellular processes in all eukaryotes. In plants, especially in *Nicotiana* spp. and in *Arabidopsis*, many studies have demonstrated the critical roles of MAPKs in plant stress signaling, especially pathogen stresses. Using a virus-induced gene silencing (VIGS) approach, Wu et al. (142) revealed that both salicylic acid–induced protein kinase (SIPK) and wound-induced protein kinase (WIPK) play a central role in plant responses to herbivory. Herbivory highly elicits MAPK activity, and OS-elicited JA, ET, and the transcriptional regulation of many defense-related genes are dependent on the MAPK pathway. Similar results were obtained in tomato plants overexpressing the prosystemin gene: silencing the tomato orthologues of *SIPK* and *WIPK* impairs plant JA accumulation after herbivory and thus defenses against *M. sexta* (40). Moreover, the homologues of *SIPK* and *WIPK* in potato are also involved in *Mi-1*-mediated resistance to aphids (68). Given the large gene family of MAPKs (20 in *Arabidopsis* genome), we expect the number of MAPKs that regulate plant resistance to herbivores will increase.

In mammals, the major targets of MAPKs are transcription factors, the phosphorylation of which changes these proteins' stability, localization, or activity. Emerging evidence supports the same scenario in plants. Although little is yet known about which transcription factors are MAPKs' direct phosphorylation targets in plants (18, 80), a few studies have demonstrated the central roles of MAPKs in regulating plant transcriptomes (48, 99, 142). In addition to transcription factors, other proteins may also be substrates for MAPKs. An elegant study showed that MPK6 (an *Arabidopsis* homolog of SIPK) directly phosphorylates two 1-aminocyclopropane-1-carboxylic acid synthases (ACSs), ACS2 and ACS6, resulting in the enhanced stability of these proteins and, in turn, higher levels of pathogen elicitor-induced ET (69). SIPK also regulates herbivory-induced ET biosynthesis in *N. attenuata*, probably through a similar pathway (142). Studies on how kinases modulate herbivory-induced JA accumulation and transcriptional responses are still lacking.

$Ca^{2+}$ influxes appear to be associated with protein phosphorylation events. Overexpressing a voltage-gated $Ca^{2+}$ channel in rice enhances elicitor-induced MAPK activity (56). In tobacco suspension cultures, the application of lanthanum chloride and calmodulin antagonist W7 inhibits the pathogen elicitor-elicited MAPK activation (104). Nevertheless, evidence supporting the location of $Ca^{2+}$ influxes downstream of phosphorylation events also exists. For example, staurosporine, a general inhibitor of serine/threonine protein kinases, fully inhibits the cryptogein-induced elevation of cytosolic $Ca^{2+}$ in *N. plumbaginifolia* cell cultures (60). Yet nothing is known about the interaction between herbivory-induced kinase signaling, especially MAPK signaling, and $Ca^{2+}$ influxes. The application of $Ca^{2+}$-specific fluorescence dyes and the transformation of plants with $Ca^{2+}$-sensing aequorin and GFP proteins are all potentially powerful tools with which to explore the regulation of $Ca^{2+}$ influxes.

In *Arabidopsis*, a complete MAPK pathway composed of MEKK1, MKK4/MKK5, MPK3/MPK6, and WRKY22/WRKY29 transcription factors has been identified to function downstream of FLS2 (the flagellin receptor) (5). A growing number of studies have revealed more and more elements of MAPK pathways, especially those involved in plant-pathogen interactions. Many questions about MAPK signaling in plant-herbivore interactions remain to be answered: in addition to SIPK and WIPK, which MAPKs are also involved in plant defenses against herbivores? Which MAPKKKs and MAPKKs are their upstream kinases? Which proteins, presumably transcription factors, are MAPKs' direct substrates, and how do the phosphorylation events change their activity or localization, and thereby modulate the transcriptome?

## Reactive Oxygen Species

Superoxide anion ($O_2{}^-$), hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), and hydroxyl radical ($^{\cdot}OH$) are collectively called ROS; they are produced in mitochondria, chloroplasts, and peroxisomes, as well as on the external surfaces of plasma membranes. ROS production is an essential part of plant stress responses; the biological function of ROS in particular has been very well recognized in plant-pathogen interactions (58). Several studies have revealed that ROS are also implicated in herbivory-induced responses in plants. Feeding of *Helicoverpa zea* on soybean plants leads to considerably elevated ROS levels (9). Wounding alone doesn't induce a detectable amount of ROS in *Medicago truncatula*, but herbivory does (63). An $H_2O_2$-sensitive dye also revealed an increase in ROS levels in lima bean after *S. littoralis* attacks (71).

The production of pathogen-induced ROS is mainly dependent on plasma membrane-bound NADPH oxidases. In *Arabidopsis*, two *RBOH* genes (respiratory burst oxidase homologues, encoding an important subunit of NADPH oxidase), *AtrbohD* and *AtrbohF*, are required for pathogen-induced ROS production (131); these two genes also contribute greatly to the ROS burst after ABA treatment in stomata (57). Pharmacological evidence has suggested that NADPH oxidase is involved in wounding-induced ROS in tomato plants (89, 90). Similar results were obtained in tomato plants expressing a partial *RBOH* sequence in the antisense orientation (105). Although it has yet to be examined, the herbivory-induced elevation of ROS levels also likely results from the activation of NADPH oxidases.

The existence of $Ca^{2+}$-binding EF hands in all plant RBOH proteins suggests that stress-induced $Ca^{2+}$ influxes modulate NADPH oxidase activity (42). In line with this, an in vitro study showed that plant NADPH oxidase can be directly activated by $Ca^{2+}$ (106). However, the relationship between ROS and $Ca^{2+}$ influxes is complex. $Ca^{2+}$ appears to be both upstream and downstream of ROS, and the relationships are likely specific to cell type and signaling pathway (23, 65, 95, 96). Whether herbivory-induced $Ca^{2+}$ influxes are required for the ROS production is still unknown. Apparently, other layers of regulation also contribute to ROS production. In potato plants, StCDPK4 and StCDPK5 phosphorylate StRBOHB directly at the N terminal, which enhances the activity of NADPH oxidase and in turn results in increased ROS (51). In *N. benthamiana*, SIPK transcriptionally regulates *RBOHB* (4).

Although the function of ROS in plant-pathogen interaction is well understood, the biological significance of ROS in plant resistance to herbivores remains elusive. In tomato plants, inhibiting NADPH oxidase activity pharmacologically results in diminished transcript levels of defense-related genes, such as *PI*s and a *polyphenol oxidase* (90). The antisense expression of the *RBOH* gene in tomato also compromises the wounding-induced transcript abundance of *PI*s (105). Identifying genes involved in the regulation and production of ROS, studying the function of ROS in mediating downstream responses, and carrying out bioassays of herbivore performance on genetically modified plants with altered levels of ROS will provide important insight into how these small, reactive molecules are involved in the battles between plants and herbivores.

## Jasmonic Acid, Ethylene, and Salicylic Acid

JA's pivotal role in plant development and resistance to biotic stresses has been well documented (12, 32, 138). JA appears unimportant for plant vegetative growth, but plants with impaired JA biosynthesis or perception are male sterile due to insufficient stamen maturation and pollen function. As a part of the plant immune system, JA confers resistance to necrotrophic pathogens (55, 133). Many studies have demonstrated that JA is the most important hormone that controls plant defense against herbivores. Drastically decreased resistance was observed in plants with impaired biosynthesis or perception of JA; the compromised resistance is usually associated with the highly attenuated accumulation of defensive compounds in these plants (29, 33, 44, 94, 135, 137). Moreover, transcriptome analyses using microarrays indicated that a large portion of wounding- and herbivory-induced responses are mediated through the JA pathway (101, 102).

JA is synthesized consecutively in chloroplasts and peroxisomes through an octadecanoid pathway. Almost all the enzymes involved in JA biosynthesis have been identified in *Arabidopsis*. Phospholipase A catalyzes the hydrolysis of chloroplast membrane lipids and thus releases free LA (34, 38). After a series of reactions catalyzed by enzymes localized in chloroplasts [lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC)], LA is further converted to 12-oxo-phytodienoic acid (OPDA). After being transported to peroxisomes, OPDA is catalyzed by an OPDA reductase (OPR); after three steps of β-oxidation, JA is formed (138). Moreover, although 13-LOXs convert LA to 13-hydroperoxy LA, different LOXs channel these fatty acid hydroperoxides to either green leaf volatile or the JA biosynthesis pathway (S. Allmann, R. Halitschke, R.C. Schuurink, I.T. Baldwin, unpublished results). Importantly, genetic work identified JA-isoleucine conjugate (JA-Ile; catalyzed by JAR enzymes, JA-Ile is formed from JA and Ile) as the molecule that activates the majority of JA-induced molecular responses (122). An F-box protein, COI1, has long been recognized to play a central role in JA signaling. Recently, the identification of JASMONATE ZIM-DOMAIN (JAZ) proteins as the repressors of JA-induced responses and COI1 as the receptor of JA-Ile has greatly advanced our understanding of JA signaling (14, 130, 144). JA-Ile specifically binds to the COI1 protein and thus promotes the physical interaction between COI1 and JAZs. This binding event facilitates the ubiquitination of JAZs by the $\mathrm{SCF}^{\mathrm{COI1}}$ E3 ubiquitin ligase, which leads to the subsequent degradation of JAZs in the 26S proteasome. JAZs bind to JA-responsive transcription factors, such as MYC2, and the decreased levels of JAZs triggered by wounding, herbivory, or pathogen attack release the JA-responsive transcription factors and finally activate JA (more accurately JA-Ile)-induced transcriptional responses. The mode of JA perception is strikingly similar to that of auxin and gibberellic acids (GAs): binding of auxin and GAs to their receptors, F-box proteins TIR1 and GID1, activates the ubiquitination and subsequent degradation of the transcriptional repressors Aux/IAA and DELLA.

JA also forms conjugates with other amino acids (Leu, Val, and Phe), and with ACC, the precursor of ethylene (122), although their biological functions remain elusive. Interestingly, some evidence indicates that JA-Ile executes only a portion of JA-induced responses; for example, in *Arabidopsis*, wounding *jar1* mutants highly induces jasmonate-dependent wounding-induced genes (127), and applying JA-Ile to *N. attenuata* plants impaired in JA biosynthesis (antisense *LOX3* plants) doesn't fully restore JA-mediated defense traits (137). Similarly, in addition to being the precursor of JA, OPDA is also believed to be a signaling molecule that activates certain wounding- or herbivory-induced responses (124).

The regulation of JA accumulation and signaling after wounding or herbivory is particularly important for plants' ability to initiate defense reactions in a timely fashion. JA biosynthesis is generally believed to be substrate limited. It is not yet known what signal triggers the biosynthetic responses in chloroplasts and peroxisomes after wounding and herbivory. A mutation in a cellulose synthase gene, *AtCeSA3*, leads to highly increased levels of both JA and ET, suggesting that the biosynthesis of plant cell walls is implicated in the regulation of JA homeostasis, at least in *Arabidopsis* (21). In both *Nicotiana* and tomato plants that overexpress the prosystemin gene, silencing *SIPK* and *WIPK* impairs the accumulation of wounding- or herbivory-induced JA (40, 114, 142). However, activating SIPK and WIPK in *Nicotiana tabacum* by transiently overexpressing their upstream MAPK kinase *MEK2* in a constitutively active form doesn't elicit JA accumulation (47). Therefore, certain molecules induced by wounding or herbivory are probably required for JA induction in addition to MAPK activation.

The mechanism of MAPKs' involvement in JA accumulation is unclear. Chemical analyses of some precursors of JA in plants deficient in *SIPK* and *WIPK* have indicated that both kinases mediate wounding- and FAC-elicited JA accumulation in different fashions (38). The suppression effect of SA on JA accumulation and signaling is well known. In *Arabidopsis*, NPR1 (nonexpressor of pathogenesis-related genes 1), which is a key element in SA signal transduction, is required for the antagonistic function of SA (120). Recent biochemical and genetic work has revealed that high ET levels induced by biotic or abiotic stresses abolish the

NPR1-dependency of SA's antagonism on JA (17, 64). In *N. attenuata*, NPR1 also modulates the herbivory-induced accumulation of JA, although the mechanism is unclear (38, 100). Interestingly, JA signaling also has a feedback effect on the regulation of the accumulation of JA, given that greatly decreased JA levels were detected in *COI1*-silenced plants (93). We expect to see an increasing number of the regulators of JA biosynthesis and JA signaling identified in the near future.

Although it has a very simple structure, ET regulates a wide range of physiological processes in plant development and in abiotic and biotic stress resistance. The biosynthesis pathway of ET has been intensively studied. Catalyzed by *S*-adenosylmethionine (*S*-AdoMet) synthetase (SAM), *S*-AdoMet is formed from methionine, which is further converted to ACC by ACC synthases (ACSs); after being oxidized by ACC oxidases (ACOs), ACC forms ET, $CO_2$, and cyanide. Among these steps, the formation of ACC is thought to be rate limiting. After herbivore attack, plants rapidly activate the biosynthesis of ET. The application of *M. sexta* OS or FACs to wounded *N. attenuata* plants induces a burst of ET in quantities much greater than those induced by mechanical wounding (134). Several other plant species also recognize elicitors in certain herbivores' OS and respond with highly elevated ET levels (54, 110, 123). An elegant study revealed that in *Arabidopsis*, the phosphorylation of ACS2 and ACS6 by MPK6 stabilizes these proteins and hence greatly enhances the rate of ET production (69). The MPK6-ACS2/6 pathway accounts for roughly half of the pathogen elicitor, flagellin-induced ET. Using a reverse genetic approach, Wu et al. (142) also demonstrated that silencing *SIPK* but not *WIPK* abolishes around 50% of herbivory-elicited ET. Some evidence suggests that the phosphorylation of ACSs by an unidentified CDPK might be responsible for the other half of ET production (129). Whether ET induced by other types of herbivore-derived elicitors (e.g., caeliferins and inceptin) is also partially dependent on MAPK signaling will be interesting to explore.

ET signaling has been intensively studied in *Arabidopsis*. Five proteins comprise the ET receptors—ETR1, ETR2, ERS1, ERS2, and EIN4—all of which are homologous to bacterial two-component histidine kinases involved in sensing environmental changes (136). Genetic work identified CTR1 as being directly downstream of ET receptors, where it functions as a negative regulator for downstream signaling responses (46). CTR1 encodes a Raf-like MAPKKK, but the exact mode of CTR1's action in ET signaling remains unclear. EIN2 and nuclear-localized EIN3 and other EIN3-like transcription factors are sequentially further downstream of CTR1 (3, 13). Interestingly, SCF E3 ubiquitin ligase complex-mediated protein degradation is also part of the ET signaling network: two F-box proteins, AtEBF1 and AtEBF2, interact with EIN3 to facilitate its removal by the 26S proteasome (28, 98). Thus, EIN3 is likely constantly degraded, and enhanced ET levels inhibit this process. Recently, a MAPKK MKK9 in *Arabidopsis* was shown to be implicated in modulating EIN3 stability (145). EIN3 and other EIN3-like transcription factors target promoters of transcription factors such as ERF1 (117). These transcription factors act as further transcription activators or repressors of ET-responsive genes.

The function of ET in plant resistance to herbivores is considered to be primarily fine-tuning JA-induced responses. In tomato plants, ET potentiates the JA-induced transcript accumulation of *PI*s (87). Treating *Arabidopsis* plants with ethephon, a synthetic ET donor, transiently elevates the levels of JA and *AOS* transcript (59). Blocking ET perception with 1-MCP diminishes herbivory-induced volatile emission, which is mainly regulated by JA (108). Using plants that ectopically express an ETR gene with mutations that cause loss-of-function, von Dahl et al. (134) showed that compromising ET signaling results in decreased basal levels of nicotine but enhanced inducibility after herbivory. An antagonistic effect of ET on JA-induced responses has also been demonstrated. In both *N. attenuata* and *N. sylvestris*, ET suppresses *putrescine N-methyltransferase* transcript levels and thus negatively regulates the accumulation of JA-induced nicotine (115, 141). ET signaling in *Arabidopsis* also plays a negative role in plant resistance to a generalist herbivore, *S. littoralis* (126).

Phenylalanine ammonia lyase (PAL) and isochorismate synthase (ICS) are two key enzymes in the biosynthesis of SA. Phloem-feeding insects, such as aphids and silverleaf whiteflies, induce transcriptional responses in plants somewhat similar to those induced by SA treatment or attack from biotrophic pathogens (135). However, the function of SA in influencing the performance of phloem-feeding insects is species-specific. Tomato plants' defense against potato aphids is dependent on SA, given that aphids survive longer on tomato plants ectopically expressing *NahG* (salicylate hydroxylase) than on wild-type plants (68). In contrast, aphids (*Myzus persicae* and *Brevicoryne brassicae*) perform better on wild-type *Arabidopsis* plants than on *npr1* mutants, which have impaired SA-induced responses (81). Whether attack from different chewing insects alters plants' SA levels depends on the plant and herbivore species (15, 17, 63) and the magnitude of the ET burst elicited by the attack (17). The function of SA in plant defense against chewing insects remains largely unknown.

Many of the early signaling events are very similar in plants challenged by distinct herbivores; these events include $Ca^{2+}$ influxes, ROS production, MAPK activation, and JA and ET accumulation (summarized in **Figure 2**). How plants accurately and promptly transmit herbivore species–specific alert signals down the signaling network and thereby tailor the biosynthesis of defensive compounds is particularly interesting. Different HAMPs are perceived by their specific receptors/sensors. Thereafter, certain molecular events, which differ qualitatively and quantitatively but are largely similar, modify the output of different HAMP inductions. Certain signal transmission pathways may be on or off for different HAMPs, resulting in qualitatively different regulatory effects. Perhaps most important, the intensity and the temporal and spatial distribution of the signaling events greatly influence the specific regulatory output. After perceiving a specific HAMP, the unique signature in each step of a molecular reaction is gradually amplified and translated into a specific combination of defensive secondary metabolites.

## SYSTEMIC SIGNALING

Plants accumulate defensive compounds not only in herbivore-damaged leaves but also in distal intact (systemic) leaves. Clearly, a signal travels to other parts of the plants and transmits an herbivory alert. Although this systemic response was found almost forty years ago, the identity of the mobile signal remains unknown (27, 41). Solanaceous plants have long been used as model organisms for studying systemic signaling. Recently, research in *Arabidopsis* has begun to shed light on systemic responses in other plant species (26, 52).

Although the nature of the systemic signal has long been debated, several studies have indicated that the vascular system is involved in the transportation of this mobile signal (35, 88, 107). Electric and hydraulic signals were proposed to play a role (73, 121). A recent study on broad bean (*Vicia faba*) and barley (*Hordeum vulgare*) supports the involvement of electric signals in systemic responses (150). In tomato, systemin was thought to travel out of wounded leaves to convey wounding or herbivory alert to systemic leaves (76). In an excellent study using grafting techniques and plants deficient in JA biosynthesis and responses, Li et al. (67) demonstrated that systemin is not the mobile signal in tomato and is not required for the induction of systemic responses. Instead, the production of JA in damaged local leaves and the perception of JA in distal leaves are necessary for inducing systemic responses; therefore, JA or a JA-induced compound travels to systemic leaves and elicits defenses (**Figure 3*a***). The critical role of JA production in local leaves in eliciting systemic responses was further confirmed in tomato *acx1* mutants (66). In *Nicotiana*, a mobile signal travels from attacked leaves to roots and initiates the biosynthesis

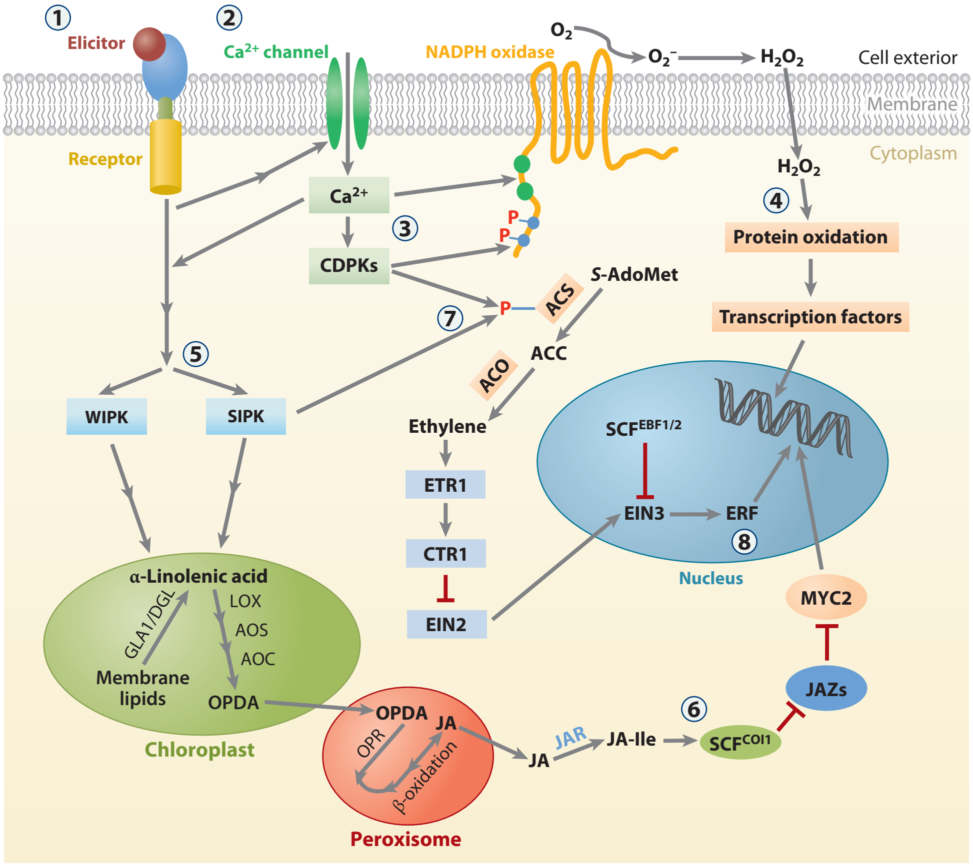

Figure 2

A model summarizing early signaling events in a cell in an herbivore-attacked leaf. Step 1: Herbivore-derived elicitors are perceived by unidentified receptors on the plasma membranes. Step 2: The perception events trigger the activation of $Ca^{2+}$ channels and result in $Ca^{2+}$ influxes. Step 3: Binding of $Ca^{2+}$ to NADPH oxidase and its phosphorylation by CDPKs enhance the activity of NADPH oxidase and lead to the production of ROS. Step 4: Oxidation of proteins by reactive oxygen species (ROS) further modifies the activity of ROS-responsive transcription factors. Step 5: MAPKs are also rapidly activated; among those activated, SIPK and WIPK trigger the biosynthesis of JA and thus JA-Ile. Step 6: Binding of JA-Ile to the COI1 receptor leads to the rapid degradation of JAZ proteins, resulting in the release of their inhibitory effect on MYC2 and MYC2-like transcription factors. Step 7: SIPK and likely a CDPK phosphorylate ACS proteins and enhance ACS stability, and thereby increase ethylene production. Step 8: After several steps of signaling events, elevated ethylene production leads to the increased activity of ethylene-responsive transcription factors, such as ERF. These early signaling events are gradually translated into the accumulation of metabolites that function as defensives.

of nicotine; whether the same signal travels to both roots and systemic leaves is unclear. Interestingly, *Arabidopsis* seems to have a distinct systemic signaling mechanism. Koo et al. found that in *Arabidopsis*, the de novo biosynthesis of both JA and JA-Ile in systemic leaves is required for activating systemic responses, and this process is independent of the JA and JA-Ile production in local leaves (52). This suggests that a mobile signal generated in the wounded leaves, whose production is independent of JA, conveys an alert to systemic leaves, which in turn initiates JA and JA-Ile biosynthesis (**Figure 3*b***). Investigating systemic signaling in other plant

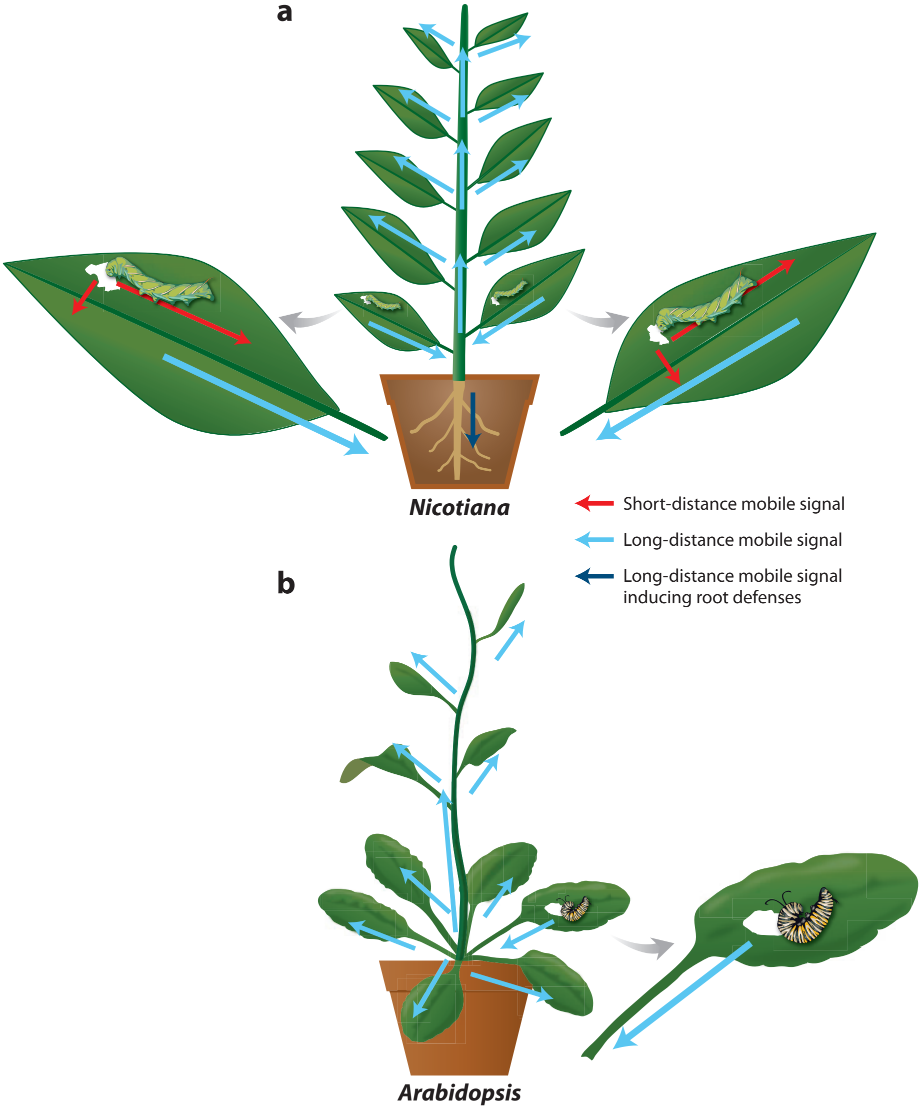

**Figure 3**

Systemic signaling in plants. (*a*) Systemic signaling in solanaceous plants. Herbivory rapidly induces a short-distance mobile signal (*red arrows*) that travels in particular directions that are both facilitated and constrained by leaf vasculature to the undamaged parts of the attacked leaves and thus activates MAPKs and triggers JA accumulation. Thereafter, a long-distance mobile signal (*light blue arrows*), which is either JA or a JA-induced compound, is produced. It moves to systemic leaves, where it is converted to JA-Ile or it elicits the accumulation of JA-Ile. Defense responses, such as increased activity of proteinase inhibitors, are induced after the perception of JA-Ile in both local and systemic leaves. In *Nicotiana* plants, another long-distance mobile signal (*dark blue arrow*) of unknown identity travels to roots and induces defenses, e.g., nicotine biosynthesis. (*b*) Systemic signaling in *Arabidopsis* plants. After herbivores attack, a mobile signal produced independently of JA signaling is generated in wounded leaves and travels to undamaged leaves and induces the biosynthesis of JA and JA-Ile, and thereby activates defense responses in these leaves.

systems will shed further light on how this important defense mechanism evolved in different plants. Although different mechanisms may exist, the JA pathway is likely to play a pivotal role in all plant systemic responses. Interestingly, *N. tabacum* and *Arabidopsis* were also shown to have distinct mechanisms of systemic acquired resistance (37, 92).

Little is known about how after wounding or herbivory the cells that perceive herbivore-derived elicitors transmit alerts to the cells in other parts of the damaged leaf. After tomato was wounded at the tip of its leaves, elevated transcript levels of a *PI* gene were detected; interestingly, even higher levels of *PI* transcript accumulated in undamaged basal parts of the leaves (33, 62). Thus, after a leaf is wounded, a mobile signal moves from the wounded region to the other parts of the damaged leaf and activates defense responses. Using a MAPK activity assay and mRNA blotting as imaging techniques, Wu et al. (142) demonstrated that after herbivores attack certain regions of *N. attenuata* leaves, a short-distance mobile signal rapidly disperses to specific undamaged areas in the attacked leaves; these areas activate MAPKs and elicit JA accumulation and transcriptional responses. The identity of the signal is unclear, although it appears to be distinct from the one that travels to systemic leaves (the long-distance mobile signal), given that this short-distance signal is capable of activating MAPKs and no MAPKs are activated in systemic leaves in *N. attenuata* (142). One possibility is that FACs in OSs applied to wounds are transported to other parts of the attacked leaves and thus act as the short-distance mobile signal that activates downstream responses, or it may be a compound elicited by wounding and/or FACs. Given that the long-distance signal is either JA or a JA derivative, its production is likely dependent on the short-distance signal. The defined movement of the short-distance mobile signal probably results from the constraints of the leaf's vasculature (142). Consistent with this hypothesis, Stork et al. (125) applied OS to *N. attenuata* leaves wounded with a needle and measured JA content in different regions; the data suggest that plant vasculature is likely to be involved in transporting this short-distance signal as well as constraining the spread of the JA response to the region of the leaf lamina that was elicited.

## DIVERSITY OF PLANT RESPONSES TO HERBIVORES

Within a species, heritable genetic and phenotypic variations among populations and even among individuals are the driving force for evolutionary changes. Substantial genetic variation has been demonstrated in humans, *Saccharomyces cerevisiae*, *Drosophila*, *Caenorhabditis elegans*, and *Arabidopsis*.

Owing to its abundant genetic and genomic resources, *Arabidopsis* has been intensively studied for its diversity in many phenotypic traits (53). The high genetic variation leads to great diversity of plant metabolites. Using an untargeted metabolic profiling approach, Keurentjes et al. (45) showed that only 13.4% of the mass peaks from the liquid chromatography–mass spectrometry (LC-MS) analyses were common among 14 accessions of *Arabidopsis*, indicating the remarkable intraspecific diversity of plant metabolites. Great variation among the glucosinolates, antiherbivore compounds in Brassicaceae, was also detected among 39 *Arabidopsis* accessions (50), as well as among various populations of close relatives of *Arabidopsis* (140). Intraspecific variation of herbivory-induced plant volatiles was found in maize, lima bean, tomato, cabbage, etc., as well as among wild plants *Datura wrightii* and *N. attenuata* (16). In addition to the intraspecific diversity of antiherbivore secondary metabolites, variation among the regulatory signaling events that dictate downstream herbivory-induced reactions have been described. Large differences in herbivory-induced early signaling events (MAPK activity, JA and ET production, and transcript accumulation of transcription factors) were observed in two accessions of *N. attenuata* (143). In line with this, within a *N. attenuata* population, plants grown in close proximity also display extremely heterogeneous levels of herbivory-induced JA and volatile compounds (112).

Heritable genetic variation in plant secondary metabolites and their underlying regulatory elements are essential for a plant's ability to adapt to the abiotic and biotic stresses of their natural environments. By exploiting the natural phenotypic variations in plant resistance to insects and studying the genetic diversity that determines these phenotypic variations, we increase our understanding of the coevolutionary process that has shaped the molecular machinery of plant defense systems against insects.

## PLANT-HERBIVORE INTERACTIONS: TOOLS AND CHALLENGES

Widely used in the model plants *Arabidopsis* and rice, forward genetics, especially the analyses of artificially generated mutants that have desired phenotypic changes, has played an enormous role in advancing our understanding of the genetic basis of plant development, hormone signaling, and stress responses. Unfortunately, the systems that have proven most useful for our understanding of the ecological significance of plant-herbivore interaction lack the characteristics of good genetic model systems: genetic markers, small sizes with short generation times, facile transformation systems, and small compact genomes, all the traits that facilitate forward genetic analyses. Despite these drawbacks, the great genetic diversity of herbivore-resistance traits in many plants is a valuable resource for identifying new herbivore-resistance genes. The *Mi-1* gene, which confers resistance to aphids and nematodes in tomato, and the brown planthopper–resistance gene *Bph14* in rice were both identified by map-based cloning (20, 82). When the traits are controlled by many loci, quantitative trait loci (QTL) mapping and association (or linkage disequilibrium) mapping are powerful tools with which to identify the genetic elements underlying diverse phenotypes, including genes implicated in herbivore resistance. Notably, although its application is still limited mostly in human disease research, genome-wide association mapping has recently been used in *Arabidopsis* research (139). Given the rapid development in sequencing technologies, we expect this powerful linkage disequilibrium mapping approach will be used for studying the genetic basis of plants' resistance to herbivores. Other new tools have also emerged in recent years. The rapid development in microarray and second generation sequencing technology has greatly facilitated the identification of genes using expression QTL (eQTL) mapping (36, 49). In this analysis, different expression levels of genes are regarded as QTLs to map genes that account for these differences. Instead of analyzing just a few traits, eQTL mapping deals with thousands of traits simultaneously. These forward genetic approaches are not only widely used in model plants, such as *Arabidopsis*, rice, and crop plants, but they probably also offer the best ways to study genes involved in herbivore resistance in nonmodel organisms, especially in those that can't be genetically transformed.

**QTL:** quantitative trait loci

Reverse genetics has become increasingly important. In reverse genetic studies, plants with altered transcript levels of specific genes are studied and the functions of these genes are explored. Plants carrying mutations at genes-of-interest can be obtained from public mutant seed banks, which are created by physical or chemical mutagenesis or *Agrobacterium*-mediated T-DNA insertion. Plants transformed with genes in sense, antisense or inverted-repeat orientations are also very valuable for reverse genetics. In species that are not transformable or are difficult to transform, TILLING (targeting induced local lesions in genomes) is emerging as an important tool in reverse genetics. Using reverse genetic approaches, researchers can investigate the functions of candidate genes efficiently and rapidly.

The advance in the secondary generation sequencing technology will surely become an essential driving force in studying plant-herbivore interactions. Together with genetic tools, genomic tools such as microarrays, deep sequencing, and other transcriptional profiling tools (subtractive hybridization, differential display, cDNA-AFLP, SAGE, etc.) and rapidly developing mass spectrometry–based proteomic and metabolic platforms all will greatly facilitate our understanding of the plant defense network on a molecular level.

## FUTURE PERSPECTIVES

Substantial progress has been made in recent years in understanding the signaling pathways involved in plant herbivore resistance, especially the recent development in MAPK and JA signaling. Nevertheless, many questions remain to be answered. How do plants recognize specific predators and deploy herbivore-specific responses? What are the receptors? How do these receptors and other yet-to-be-identified effectors activate MAPK signaling? What is the molecular mechanism for how MAPKs modulate phytohormone accumulation and reshape the transcriptome? How is JA biosynthesis rapidly activated? Finding R proteins in pathogen resistance has greatly enriched our understanding of the defenses plants use against pathogens. Similarly, searching for *R* genes responsible for phloem-sucking insect resistance and deciphering how plants perceive elicitors such as FACs in chewing insects' OS will bring us exciting knowledge about the plant first line of defense against herbivores. Using mapping technology, some progress has been made in identifying *R* genes for aphids and brown planthoppers. However, due to a lack of appropriate reporter/screening systems, forward genetics using direct artificial mutagenesis in plants has so far seemed impractical. An overwhelming number of receptor-like genes makes the success of reverse genetics unlikely. Given the advancement in rapidly producing genetic markers for nonmodel plant species and other techniques facilitating mapping (36), we believe that identifying plant populations that have significant differences in early chewing herbivore-elicited responses (e.g., MAPK activity and JA accumulation) and finding the genetic basis for these differences using mapping will shed light on the identity of genes involved in plants' very early responses, including the receptors for herbivore elicitors. Many studies have been carried out to elucidate the functions of pathogen effectors in suppressing plant defense reactions. Whether components of herbivore OS, especially those of aphids and other phloem-sucking insects that activate defense responses in plants similarly to those induced by pathogens, suppress plant defenses deserves attention. Our understanding of plant-pathogen interaction has benefited remarkably from our ability to genetically manipulate pathogens (e.g., *Pseudomonas syringae*); in contrast, genetically modifying insects remains an underdeveloped challenge. Although the range of applicable herbivore species is unclear, expressing herbivore-targeting RNAi constructs in plants to silence the expression of particular genes in herbivores is an exciting new approach that would revolutionize the study of the coevolutionary dynamics of both partners in the plant-herbivore interactions (74).

### SUMMARY POINTS

1. Herbivory and oviposition lead to increased levels of defenses in plants, which are composed of direct and indirect defenses.
2. Plants sense the existence of herbivores and initiate changes in a battery of signaling pathways, including $Ca^{2+}$ influxes, membrane depolarization, kinase activation, and jasmonate accumulation. These pathways form sophisticated intertwined regulatory networks that orchestrate specific defense responses according to the species of the herbivore.
3. The jasmonate pathway, which is well conserved in all plant species, plays a central role in modulating plant resistance to herbivores.

4. A short-distance mobile signal travels from damaged regions in an herbivore-attacked leaf to certain regions of the undamaged portion and initiates defense reactions; moreover, a long-distance mobile signal conveys an herbivory alert to distal intact leaves and subsequently triggers defenses in these systemic leaves. Although different plant species may have different mechanisms with which to activate systemic responses, the jasmonate pathway is required for systemic responses.
5. Large intraspecific variations in herbivory-induced signaling events and secondary metabolites exist among different plant populations and even individuals within a population.

## FUTURE ISSUES

1. What are the mechanisms with which plants perceive herbivory and oviposition?
2. Which proteins constitute complete MAPK cascades, including MAPKKK, MAPKK, MAPK, and downstream transcription factors that are activated by herbivory?
3. What are the additional regulators of JA accumulation and signaling, especially the direct regulator that activates OPDA biosynthesis in chloroplasts?
4. What are the components of herbivores' oral secretions that likely suppress plants' herbivore-resistance responses?

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

We thank the Max Planck Society for funding, our colleagues for sharing unpublished data, and Emily Wheeler for editorial assistance. Because of space limitation, we could not fully cite all the publications in this field; we apologize to all the researchers whose relevant work has not been mentioned here.

## LITERATURE CITED

1. Alborn HT, Hansen TV, Jones TH, Bennett DC, Tumlinson JH, et al. 2007. Disulfooxy fatty acids from the American bird grasshopper *Schistocerca americana*, elicitors of plant volatiles. *Proc. Natl. Acad. Sci. USA* 104:12976–81
2. Alborn T, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, Tumlinson JH. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276:945–49
3. Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR. 1999. EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. *Science* 284:2148–52
4. Asai S, Ohta K, Yoshioka H. 2008. MAPK signaling regulates nitric oxide and NADPH oxidase-dependent oxidative bursts in *Nicotiana benthamiana*. *Plant Cell* 20:1390–406
5. Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, et al. 2002. MAP kinase signaling cascade in *Arabidopsis* innate immunity. *Nature* 415:977–83

6. Balbyshev NF, Lorenzen JH. 1997. Hypersensitivity and egg drop: a novel mechanism of host plant resistance to Colorado potato beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 90:652–57
7. Baldwin IT. 1990. Herbivory simulations in ecological research. *Trends Ecol. Evol.* 5:91–93
8. Baldwin IT. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc. Natl. Acad. Sci. USA* 95:8113–18
9. Bi JL, Felton GW. 1995. Foliar oxidative stress and insect herbivory—primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. *J. Chem. Ecol.* 21:1511–30
10. Blume B, Nurnberger T, Nass N, Scheel D. 2000. Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. *Plant Cell* 12:1425–40
11. Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, et al. 2010. Differential innate immune signaling via $Ca^{2+}$ sensor protein kinases. *Nature* doi:10.1038/nature08794 Letter
12. Browse J. 2009. Jasmonate passes muster: a receptor and targets for the defense hormone. *Annu. Rev. Plant Biol.* 60:183–205
13. Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR. 1997. Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell* 89:1133–44
14. Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, et al. 2007. The JAZ family of repressors is the missing link in jasmonate signaling. *Nature* 448:666–73
15. De Vos M, Van Oosten VR, Van Poecke RM, Van Pelt JA, Pozo MJ, et al. 2005. Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Mol. Plant-Microbe Interact.* 18:923–37
16. Dicke M, Baldwin IT. 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends Plant Sci.* 15:167–75
17. Diezel C, von Dahl CC, Gaquerel E, Baldwin IT. 2009. Different lepidopteran elicitors account for cross-talk in herbivory-induced phytohormone signaling. *Plant Physiol.* 150:1576–86
18. Djamei A, Pitzschke A, Nakagami H, Rajh I, Hirt H. 2007. Trojan horse strategy in Agrobacterium transformation: abusing MAPK defense signaling. *Science* 318:453–56
19. Doss RP, Oliver JE, Proebsting WM, Potter SW, Kuy S, et al. 2000. Bruchins: insect-derived plant regulators that stimulate neoplasm formation. *Proc. Natl. Acad. Sci. USA* 97:6218–23
20. Du B, Zhang W, Liu B, Hu J, Wei Z, et al. 2009. Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. *Proc. Natl. Acad. Sci. USA* 106:22163–68
21. Ellis C, Karafyllidis I, Wasternack C, Turner JG. 2002. The *Arabidopsis* mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. *Plant Cell* 14:1557–66
22. Fatouros NE, Broekgaarden C, Bukovinszkine'Kiss G, van Loon JJ, Mumm R, et al. 2008. Male-derived butterfly antiaphrodisiac mediates induced indirect plant defense. *Proc. Natl. Acad. Sci. USA* 105:10033–38
23. Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, et al. 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 422:442–46
24. Gatehouse JA. 2002. Plant resistance towards insect herbivores: a dynamic interaction. *New Phytol.* 156:145–69
25. Giri AP, Wunsche H, Mitra S, Zavala JA, Muck A, et al. 2006. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VII. Changes in the plant's proteome. *Plant Physiol.* 142:1621–41
26. Glauser G, Grata E, Dubugnon L, Rudaz S, Farmer EE, Wolfender JL. 2008. Spatial and temporal dynamics of jasmonate synthesis and accumulation in *Arabidopsis* in response to wounding. *J. Biol. Chem.* 283:16400–7
27. Green TR, Ryan CA. 1972. Wound-induced proteinase inhibitor in plant leaves—possible defense mechanism against insects. *Science* 175:776–77
28. Guo H, Ecker JR. 2003. Plant responses to ethylene gas are mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. *Cell* 115:667–77
29. Halitschke R, Baldwin IT. 2003. Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. *Plant J.* 36:794–807

30. Halitschke R, Schittko U, Pohnert G, Boland W, Baldwin IT. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. *Plant Physiol.* 125:711–17
31. Heil M. 2009. Damaged-self recognition in plant herbivore defense. *Trends Plant Sci.* 14:356–63
32. Howe GA, Jander G. 2008. Plant immunity to insect herbivores. *Annu. Rev. Plant Biol.* 59:41–66
33. Howe GA, Lightner J, Browse J, Ryan CA. 1996. An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *Plant Cell* 8:2067–77
34. Hyun Y, Choi S, Hwang HJ, Yu J, Nam SJ, et al. 2008. Cooperation and functional diversification of two closely related galactolipase genes for jasmonate biosynthesis. *Dev. Cell* 14:183–92
35. Jones CG, Hopper RF, Coleman JS, Krischik VA. 1993. Control of systemically induced herbivore resistance by plant vascular architecture. *Oecologia* 93:452–56
36. Jones N, Ougham H, Thomas H, Pasakinskiene I. 2009. Markers and mapping revisited: finding your gene. *New Phytol.* 183:935–66
37. Jung HW, Tschaplinski TJ, Wang L, Glazebrook J, Greenberg JT. 2009. Priming in systemic plant immunity. *Science* 324:89–91
38. Kallenbach M, Alagna F, Baldwin IT, Bonaventure G. 2010. *Nicotiana attenuata* SIPK, WIPK, NPR1, and fatty acid-amino acid conjugates participate in the induction of jasmonic acid biosynthesis by affecting early enzymatic steps in the pathway. *Plant Physiol.* 152:96–106
39. Kaloshian I. 2004. Gene-for-gene disease resistance: bridging insect pest and pathogen defense. *J. Chem. Ecol.* 30:2419–38
40. Kandoth PK, Ranf S, Pancholi SS, Jayanty S, Walla MD, et al. 2007. Tomato MAPKs LeMPK1, LeMPK2, and LeMPK3 function in the systemin-mediated defense response against herbivorous insects. *Proc. Natl. Acad. Sci. USA* 104:12205–10
41. Karban R, Baldwin IT. 1997. *Induced Responses to Herbivory*. Chicago: Univ. Chicago Press
42. Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C. 1998. A plant homolog of the neutrophil NADPH oxidase gp91phox subunit gene encodes a plasma membrane protein with $Ca^{2+}$ binding motifs. *Plant Cell* 10:255–66
43. Kessler A, Baldwin IT. 2002. Plant responses to insect herbivory: the emerging molecular analysis. *Annu. Rev. Plant Biol.* 53:299–328
44. Kessler A, Halitschke R, Baldwin IT. 2004. Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science* 305:665–68
45. Keurentjes JJB, Fu JY, de Vos CHR, Lommen A, Hall RD, et al. 2006. The genetics of plant metabolism. *Nat. Genet.* 38:842–49
46. Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. 1993. CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. *Cell* 72:427–41
47. Kim CY, Liu Y, Thorne ET, Yang H, Fukushige H, et al. 2003. Activation of a stress-responsive mitogen-activated protein kinase cascade induces the biosynthesis of ethylene in plants. *Plant Cell* 15:2707–18
48. Kim CY, Zhang S. 2004. Activation of a mitogen-activated protein kinase cascade induces WRKY family of transcription factors and defense genes in tobacco. *Plant J.* 38:142–51
49. Kliebenstein D. 2009. Quantitative genomics: analyzing intraspecific variation using global gene expression polymorphisms or eQTLs. *Annu. Rev. Plant Biol.* 60:93–114
50. Kliebenstein DJ, Kroymann J, Brown P, Figuth A, Pedersen D, et al. 2001. Genetic control of natural variation in *Arabidopsis* glucosinolate accumulation. *Plant Physiol.* 126:811–25
51. Kobayashi M, Ohura I, Kawakita K, Yokota N, Fujiwara M, et al. 2007. Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. *Plant Cell* 19:1065–80
52. Koo AJ, Gao X, Jones AD, Howe GA. 2009. A rapid wound signal activates the systemic synthesis of bioactive jasmonates in *Arabidopsis*. *Plant J.* 59:974–86
53. Koornneef M, Alonso-Blanco C, Vreugdenhil D. 2004. Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annu. Rev. Plant Biol.* 55:141–72

54. Kruzmane D, Jankevica L, Ievinsh G. 2002. Effect of regurgitant from *Leptinotarsa decemlineata* on wound responses in *Solanum tuberosum* and *Phaseolus vulgaris*. *Physiol. Plantarum.* 115:577–84
55. Kunkel BN, Brooks DM. 2002. Cross talk between signaling pathways in pathogen defense. *Curr. Opin. Plant Biol.* 5:325–31
56. Kurusu T, Yagala T, Miyao A, Hirochika H, Kuchitsu K. 2005. Identification of a putative voltage-gated $Ca^{2+}$ channel as a key regulator of elicitor-induced hypersensitive cell death and mitogen-activated protein kinase activation in rice. *Plant J.* 42:798–809
57. Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, et al. 2003. NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. *EMBO J.* 22:2623–33
58. Lamb C, Dixon RA. 1997. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:251–75
59. Laudert D, Weiler EW. 1998. Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signaling. *Plant J.* 15:675–84
60. Lecourieux D, Mazars C, Pauly N, Ranjeva R, Pugin A. 2002. Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells. *Plant Cell* 14:2627–41
61. Lecourieux D, Ranjeva R, Pugin A. 2006. Calcium in plant defense-signaling pathways. *New Phytol.* 171:249–69
62. Lee GI, Howe GA. 2003. The tomato mutant *spr1* is defective in systemin perception and the production of a systemic wound signal for defense gene expression. *Plant J.* 33:567–76
63. Leitner M, Boland W, Mithofer A. 2005. Direct and indirect defences induced by piercing-sucking and chewing herbivores in *Medicago truncatula*. *New Phytol.* 167:597–606
64. Leon-Reyes A, Spoel SH, De Lange ES, Abe H, Kobayashi M, et al. 2009. Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. *Plant Physiol.* 149:1797–809
65. Levine A, Pennell RI, Alvarez ME, Palmer R, Lamb C. 1996. Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. *Curr. Biol.* 6:427–37
66. Li C, Schilmiller AL, Liu G, Lee GI, Jayanty S, et al. 2005. Role of beta-oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. *Plant Cell* 17:971–86
67. Li L, Li C, Lee GI, Howe GA. 2002. Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. *Proc. Natl. Acad. Sci. USA* 99:6416–21
68. Li Q, Xie QG, Smith-Becker J, Navarre DA, Kaloshian I. 2006. Mi-1-mediated aphid resistance involves salicylic acid and mitogen-activated protein kinase signaling cascades. *Mol. Plant-Microbe Interact.* 19:655–64
69. Liu Y, Zhang S. 2004. Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in *Arabidopsis*. *Plant Cell* 16:3386–99
70. Maffei M, Bossi S, Spiteller D, Mithofer A, Boland W. 2004. Effects of feeding *Spodoptera littoralis* on lima bean leaves. I. Membrane potentials, intracellular calcium variations, oral secretions, and regurgitate components. *Plant Physiol.* 134:1752–62
71. Maffei ME, Mithofer A, Arimura G, Uchtenhagen H, Bossi S, et al. 2006. Effects of feeding *Spodoptera littoralis* on lima bean leaves. III. Membrane depolarization and involvement of hydrogen peroxide. *Plant Physiol.* 140:1022–35
72. Maischak H, Grigoriev PA, Vogel H, Boland W, Mithofer A. 2007. Oral secretions from herbivorous lepidopteran larvae exhibit ion channel–forming activities. *FEBS Lett.* 581:898–904
73. Malone M, Alarcon JJ, Palumbo L. 1994. An hydraulic interpretation of rapid, long-distance wound signaling in the tomato. *Planta* 193:181–85
74. Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, et al. 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nat. Biotechnol.* 25:1307–13
75. Mattiacci L, Dicke M, Posthumus MA. 1995. beta-Glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proc. Natl. Acad. Sci. USA* 92:2036–40
76. McGurl B, Pearce G, Orozco-Cardenas M, Ryan CA. 1992. Structure, expression, and antisense inhibition of the systemin precursor gene. *Science* 255:1570–73

77. Meiners T, Hilker M. 1997. Host location in *Oomyzus gallerucae* (Hymenoptera: Eulophidae), an egg parasitoid of the elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). *Oecologia* 112:87–93
78. Meldau S, Wu JQ, Baldwin IT. 2009. Silencing two herbivory-activated MAP kinases, SIPK and WIPK, does not increase *Nicotiana attenuata*'s susceptibility to herbivores in the glasshouse and in nature. *New Phytol.* 181:161–73
79. Mendelsohn R, Balick MJ. 1995. The value of undiscovered pharmaceuticals in tropical forests. *Econ. Bot.* 49:223–28
80. Menke FL, Kang HG, Chen Z, Park JM, Kumar D, Klessig DF. 2005. Tobacco transcription factor WRKY1 is phosphorylated by the MAP kinase SIPK and mediates HR-like cell death in tobacco. *Mol. Plant-Microbe Interact.* 18:1027–34
81. Mewis I, Tokuhisa JG, Schultz JC, Appel HM, Ulrichs C, Gershenzon J. 2006. Gene expression and glucosinolate accumulation in *Arabidopsis thaliana* in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. *Phytochemistry* 67:2450–62
82. Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM. 1998. The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* 10:1307–19
83. Mithofer A, Boland W. 2008. Recognition of herbivory-associated molecular patterns. *Plant Physiol.* 146:825–31
84. Mithofer A, Wanner G, Boland W. 2005. Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiol.* 137:1160–68
85. Mori IC, Murata Y, Yang Y, Munemasa S, Wang YF, et al. 2006. CDPKs CPK6 and CPK3 function in ABA regulation of guard cell S-type anion- and $Ca^{2+}$-permeable channels and stomatal closure. *PLoS Biol.* 4:e327
86. Musser RO, Cipollini DF, Hum-Musser SM, Williams SA, Brown JK, Felton GW. 2005. Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. *Archives Insect. Biochem. Physiol.* 58:128–37
87. O'Donnell PJ, Calvert C, Atzorn R, Wasternack C, Leyser HMO, Bowles DJ. 1996. Ethylene as a signal mediating the wound response of tomato plants. *Science* 274:1914–17
88. Orians CM, Pomerleau J, Ricco R. 2000. Vascular architecture generates fine scale variation in systemic induction of proteinase inhibitors in tomato. *J. Chem. Ecol.* 26:471–85
89. Orozco-Cardenas M, Ryan CA. 1999. Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. *Proc. Natl. Acad. Sci. USA* 96:6553–57
90. Orozco-Cardenas ML, Narvaez-Vasquez J, Ryan CA. 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell* 13:179–91
91. Pare PW, Alborn HT, Tumlinson JH. 1998. Concerted biosynthesis of an insect elicitor of plant volatiles. *Proc. Natl. Acad. Sci. USA* 95:13971–75
92. Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF. 2007. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science* 318:113–16
93. Paschold A, Bonaventure G, Kant MR, Baldwin IT. 2008. Jasmonate perception regulates jasmonate biosynthesis and JA-Ile metabolism: the case of COI1 in *Nicotiana attenuata*. *Plant Cell Physiol.* 49:1165–75
94. Paschold A, Halitschke R, Baldwin IT. 2007. Co(i)-ordinating defenses: NaCOI1 mediates herbivore-induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. *Plant J.* 51:79–91
95. Pei ZM, Murata Y, Benning G, Thomine S, Klusener B, et al. 2000. Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. *Nature* 406:731–34
96. Piedras P, Hammond-Kosack KE, Harrison K, Jones JDG. 1998. Rapid, Cf-9- and Avr9-dependent production of active oxygen species in tobacco suspension cultures. *Mol. Plant-Microbe Interact.* 11:1155–66
97. Pohnert G, Jung V, Haukioja E, Lempa K, Boland W. 1999. New fatty acid amides from regurgitant of lepidopteran (Noctuidae, Geometridae) caterpillars. *Tetrahedron* 55:11275–80

98. Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, et al. 2003. EIN3-dependent regulation of plant ethylene hormone signaling by two arabidopsis F box proteins: EBF1 and EBF2. *Cell* 115:679–89
99. Qiu JL, Fiil BK, Petersen K, Nielsen HB, Botanga CJ, et al. 2008. *Arabidopsis* MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. *EMBO J.* 27:2214–21
100. Rayapuram C, Baldwin IT. 2007. Increased SA in NPR1-silenced plants antagonizes JA and JA-dependent direct and indirect defenses in herbivore-attacked *Nicotiana attenuata* in nature. *Plant J.* 52:700–15
101. Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE. 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell* 16:3132–47
102. Reymond P, Weber H, Damond M, Farmer EE. 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell* 12:707–19
103. Romeis T, Ludwig AA, Martin R, Jones JD. 2001. Calcium-dependent protein kinases play an essential role in a plant defense response. *EMBO J.* 20:5556–67
104. Romeis T, Piedras P, Zhang S, Klessig DF, Hirt H, Jones JD. 1999. Rapid Avr9- and Cf-9-dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. *Plant Cell* 11:273–87
105. Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, et al. 2004. Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum*. *Plant Cell* 16:616–28
106. Sagi M, Fluhr R. 2001. Superoxide production by plant homologues of the gp91(phox) NADPH oxidase. Modulation of activity by calcium and by tobacco mosaic virus infection. *Plant Physiol.* 126:1281–90
107. Schittko U, Preston CA, Baldwin IT. 2000. Eating the evidence? *Manduca sexta* larvae can not disrupt specific jasmonate induction in *Nicotiana attenuata* by rapid consumption. *Planta* 210:343–46
108. Schmelz EA, Alborn HT, Banchio E, Tumlinson JH. 2003. Quantitative relationships between induced jasmonic acid levels and volatile emission in *Zea mays* during *Spodoptera exigua* herbivory. *Planta* 216:665–73
109. Schmelz EA, Carroll MJ, LeClere S, Phipps SM, Meredith J, et al. 2006. Fragments of ATP synthase mediate plant perception of insect attack. *Proc. Natl. Acad. Sci. USA* 103:8894–99
110. Schmelz EA, Engelberth J, Alborn HT, Tumlinson JH 3rd, Teal PE. 2009. Phytohormone-based activity mapping of insect herbivore-produced elicitors. *Proc. Natl. Acad. Sci. USA* 106:653–57
111. Schmelz EA, LeClere S, Carroll MJ, Alborn HT, Teal PE. 2007. Cowpea chloroplastic ATP synthase is the source of multiple plant defense elicitors during insect herbivory. *Plant Physiol.* 144:793–805
112. Schuman MC, Heinzel N, Gaquerel E, Svatos A, Baldwin IT. 2009. Polymorphism in jasmonate signaling partially accounts for the variety of volatiles produced by *Nicotiana attenuata* plants in a native population. *New Phytol.* 183:1134–48
113. Seino Y, Suzuki Y, Sogawa K. 1996. An ovicidal substance produced by rice plants in response to oviposition by the whitebacked planthopper, *Sogatella furcifera* (HORVATH) (Homoptera: Delphacidae). *Appl. Entomol. Zool.* 31:467–73
114. Seo S, Katou S, Seto H, Gomi K, Ohashi Y. 2007. The mitogen-activated protein kinases WIPK and SIPK regulate the levels of jasmonic and salicylic acids in wounded tobacco plants. *Plant J.* 49:899–909
115. Shoji T, Nakajima K, Hashimoto T. 2000. Ethylene suppresses jasmonate-induced gene expression in nicotine biosynthesis. *Plant Cell Physiol.* 41:1072–76
116. Skibbe M, Qu N, Galis I, Baldwin IT. 2008. Induced plant defenses in the natural environment: *Nicotiana attenuata* WRKY3 and WRKY6 coordinate responses to herbivory. *Plant Cell* 20:1984–2000
117. Solano R, Stepanova A, Chao Q, Ecker JR. 1998. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes Dev.* 12:3703–14
118. Spiteller D, Boland W. 2003. N-(15,16-Dihydroxylinoleoyl)-glutamine and N-(15,16-epoxylinoleoyl)-glutamine isolated from oral secretions of lepidopteran larvae. *Tetrahedron* 59:135–39
119. Spiteller D, Oldham NJ, Boland W. 2004. N-(17-phosphonooxylinolenoyl)glutamine and N-(17-phosphonooxylinoleoyl)glutamine from insect gut: the first backbone-phosphorylated fatty acid derivatives in nature. *J. Org. Chem.* 69:1104–9

120. Spoel SH, Koornneef A, Claessens SM, Korzelius JP, Van Pelt JA, et al. 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *Plant Cell* 15:760–70
121. Stankovic B, Davies E. 1997. Intercellular communication in plants: electrical stimulation of proteinase inhibitor gene expression in tomato. *Planta* 202:402–6
122. Staswick PE, Tiryaki I. 2004. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. *Plant Cell* 16:2117–27
123. Steinite I, Gailite A, Ievinsh G. 2004. Reactive oxygen and ethylene are involved in the regulation of regurgitant-induced responses in bean plants. *J. Plant Physiol.* 161:191–96
124. Stintzi A, Weber H, Reymond P, Browse J, Farmer EE. 2001. Plant defense in the absence of jasmonic acid: the role of cyclopentenones. *Proc. Natl. Acad. Sci. USA* 98:12837–42
125. Stork W, Diezel C, Halitschke R, Galis I, Baldwin IT. 2009. An ecological analysis of the herbivory-elicited JA burst and its metabolism: plant memory processes and predictions of the moving target model. *PLoS One* 4:e4697
126. Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, et al. 2000. Induced plant defense responses against chewing insects. Ethylene signaling reduces resistance of *Arabidopsis* against Egyptian cotton worm but not diamondback moth. *Plant Physiol.* 124:1007–18
127. Suza WP, Staswick PE. 2008. The role of JAR1 in jasmonoyl-L-isoleucine production during *Arabidopsis* wound response. *Planta* 227:1221–32
128. Suzuki Y, Sogawa K, Seino Y. 1996. Ovicidal reaction of rice plants against the whitebacked planthopper, *Sogatella furcifera* HORVATH (Homoptera: Delphacidae). *Appl. Entomol. Zool.* 31:111–18
129. Tatsuki M, Mori H. 2001. Phosphorylation of tomato 1-aminocyclopropane-1-carboxylic acid synthase, LE-ACS2, at the C-terminal region. *J. Biol. Chem.* 276:28051–57
130. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, et al. 2007. JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signaling. *Nature* 448:661–65
131. Torres MA, Dangl JL, Jones JD. 2002. *Arabidopsis* gp91phox homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proc. Natl. Acad. Sci. USA* 99:517–22
132. Truitt CL, Wei HX, Pare PW. 2004. A plasma membrane protein from *Zea mays* binds with the herbivore elicitor volicitin. *Plant Cell* 16:523–32
133. Vijayan P, Shockey J, Levesque CA, Cook RJ, Browse J. 1998. A role for jasmonate in pathogen defense of *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 95:7209–14
134. von Dahl CC, Winz RA, Halitschke R, Kuhnemann F, Gase K, Baldwin IT. 2007. Tuning the herbivore-induced ethylene burst: the role of transcript accumulation and ethylene perception in *Nicotiana attenuata*. *Plant J.* 51:293–307
135. Walling LL. 2000. The myriad plant responses to herbivores. *J. Plant Growth Regul.* 19:195–216
136. Wang KL, Li H, Ecker JR. 2002. Ethylene biosynthesis and signaling networks. *Plant Cell* 14(Suppl.):S131–51
137. Wang L, Allmann S, Wu J, Baldwin IT. 2008. Comparisons of LIPOXYGENASE3- and JASMONATE-RESISTANT4/6-silenced plants reveal that jasmonic acid and jasmonic acid-amino acid conjugates play different roles in herbivore resistance of *Nicotiana attenuata*. *Plant Physiol.* 146:904–15
138. Wasternack C. 2007. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann. Bot. (Lond.)* 100:681–97
139. Weigel D, Mott R. 2009. The 1001 genomes project for *Arabidopsis thaliana*. *Genome Biol.* 10:107
140. Windsor AJ, Reichelt M, Figuth A, Svatos A, Kroymann J, et al. 2005. Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae). *Phytochemistry* 66:1321–33
141. Winz RA, Baldwin IT. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. IV. Insect-induced ethylene reduces jasmonate-induced nicotine accumulation by regulating putrescine N-methyltransferase transcripts. *Plant Physiol.* 125:2189–202
142. Wu J, Hettenhausen C, Meldau S, Baldwin IT. 2007. Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata*. *Plant Cell* 19:1096–122

143. Wu J, Hettenhausen C, Schuman MC, Baldwin IT. 2008. A comparison of two *Nicotiana attenuata* accessions reveals large differences in signaling induced by oral secretions of the specialist herbivore *Manduca sexta*. *Plant Physiol.* 146:927–39

144. Yan J, Zhang C, Gu M, Bai Z, Zhang W, et al. 2009. The *Arabidopsis* CORONATINE INSENSITIVE1 protein is a jasmonate receptor. *Plant Cell* 21:2220–36

145. Yoo SD, Cho YH, Tena G, Xiong Y, Sheen J. 2008. Dual control of nuclear EIN3 by bifurcate MAPK cascades in C2H4 signaling. *Nature* 451:789–95

146. Yoshinaga N, Aboshi T, Abe H, Nishida R, Alborn HT, et al. 2008. Active role of fatty acid amino acid conjugates in nitrogen metabolism in *Spodoptera litura* larvae. *Proc. Natl. Acad. Sci. USA* 105:18058–63

147. Yoshinaga N, Aboshi T, Ishikawa C, Fukui M, Shimoda M, et al. 2007. Fatty acid amides, previously identified in caterpillars, found in the cricket *Teleogryllus taiwanemma* and fruit fly *Drosophila melanogaster* larvae. *J. Chem. Ecol.* 33:1376–81

148. Zavala JA, Patankar AG, Gase K, Baldwin IT. 2004. Constitutive and inducible trypsin proteinase inhibitor production incurs large fitness costs in *Nicotiana attenuata*. *Proc. Natl. Acad. Sci. USA* 101:1607–12

149. Zhu SY, Yu XC, Wang XJ, Zhao R, Li Y, et al. 2007. Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in *Arabidopsis*. *Plant Cell* 19:3019–36

150. Zimmermann MR, Maischak H, Mithofer A, Boland W, Felle HH. 2009. System potentials, a novel electrical long-distance apoplastic signal in plants, induced by wounding. *Plant Physiol.* 149:1593–600

**Annual Review of Genetics**

Volume 44, 2010

# Contents

## Errata

An online log of corrections to *Annual Review of Genetics* articles may be found at http://genet.annualreviews.org/errata.shtml