Molecular Interactions Between Plants and Insect Herbivores

Matthias Erb[1] and Philippe Reymond[2]

[1]Institute of Plant Sciences, University of Bern, 3000 Bern, Switzerland; e-mail: matthias.erb@ips.unibe.ch, ORCID 0000-0002-4446-9834

[2]Department of Plant Molecular Biology, University of Lausanne, 1015 Lausanne, Switzerland; e-mail: philippe.reymond@unil.ch, ORCID 0000-0002-3341-6200

Corresponding Authors: Matthias Erb (tel. +41 31 631 86 68) and Philippe Reymond (tel. + 41 21 692 42 29)

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Abstract

Molecular processes are at the heart of the interactions between plants and insect herbivores. Here, we review genes and proteins that are involved in plant-herbivore interactions, and discuss how their discovery has structured the current "standard model" of plant-herbivore interactions. Plants perceive damage-associated, and possibly herbivore-associated, molecular patterns (DAMPs and HAMPs) via receptors that activate early signaling components such as Ca^{2+}, reactive oxygen species (ROS) and map kinases (MPKs). Specific defense reprogramming proceeds via signaling networks that include phytohormones, secondary metabolites and transcription factors. Local and systemic regulation of toxins, defense proteins, physical barriers and tolerance traits protect plants against herbivores. Herbivores counteract plant defenses through biochemical defense deactivation, effector-mediated suppression of defense signaling and chemically controlled behavioral changes. The molecular basis of plant-herbivore interactions is now well established for selected model systems. Expanding molecular approaches to unexplored dimensions of plant-insect interactions should be a future priority.

INTRODUCTION

Plants face numerous abiotic and biotic stresses in nature. Insects are among the most important threats to plant survival due to their abundance and diversity. Millions of years of selection pressure generated by insect herbivores has resulted in the evolution of sophisticated plant defenses. The ecology and evolution of plant-insect interactions has been studied extensively (141). With the dawn of molecular biology, scientists have also begun to unravel the molecular mechanisms underpinning these interactions. Fifteen years after the acclaimed review by Kessler and Baldwin (70) on "the emerging molecular analysis of plant responses to herbivory", this review evaluates how the development of genomic tools and gene manipulation strategies has propelled the field forward and has resulted in a detailed mechanistic understanding of plant-herbivores interactions. We focus primarily on examples that demonstrate the functional role of plant and insect herbivore genes and molecules using molecular manipulative approaches and places the resulting insights into a general conceptual framework of plant defense. This approach complements a series of recent reviews on mechanisms, ecology and evolution of plant-herbivore interactions (1, 38, 160, 186).

PLANT PERCEPTION AND SIGNALING

An efficient defense response requires specific recognition of the herbivore and translation into defense signaling to reprogram cellular functions. Following the identification of numerous molecules that plants can use to detect herbivore attack, recent studies have unraveled downstream elements and their role in defense signaling. Substantial progress has also been made in identifying receptors that are involved in the recognition of damage- and, albeit to a lesser extent, herbivore-associated cues.

Recognition of herbivore- and damage-associated molecular patterns by pattern recognition receptors

The canonical model of plant herbivore perception states that plants perceive herbivory through the binding of herbivore- and damage-associated molecular patterns (HAMPs and DAMPs) to pattern recognition receptors (PRRs). Over the last years, many HAMPs have been isolated from insect herbivores (for review see (1, 154)) and information regarding their interaction with PRRs is emerging. Radiolabeled volicitin (17-hydroxylinolenoyl-L-Gln) from *Spodoptera exigua* oral secretions (OS) binds to an unidentified potential PRR in the maize plasma membrane with high affinity (159). Inceptin, a peptide fragment from a chloroplastic ATP synthase that is present in *S. frugiperda* OS, is a highly potent defense inducer in maize and cowpea (137). The discovery of a truncated form of inceptin that inhibits defenses suggests that inceptin may be recognized by a PRR to which the modified inceptin may bind as a competitive antagonist (138). The C-terminal region of a mucin-like protein (NlMLP) that is secreted by the brown planthopper (BPH) *Nilaparvata lugens* induces defense responses in rice (146). Interestingly, a cluster of three G-type lectin receptor kinases (RK) confers rice resistance against BPH (91), but whether they bind NlMLP or another ligand is unknown. Recently, a leucine-rich repeat LRR-RK from rice has been shown to be essential for perception and defense against the striped stemborer (SSB) *Chilo suppressalis* (59). Future investigation of these cell-surface localized orphan PRRs may unveil the nature of their respective ligands.

Defense activation by HAMPs may occur independently of PRRs. Glucose oxidase (GOX) for instance is found in saliva of different caterpillar species (1). GOX induces defenses in tomato (92) but suppresses them in tobacco and *Nicotiana attenuata* (30, 114). By oxidizing glucose, GOX produces the signaling molecule hydrogen peroxide (H_2O_2). Insect H_2O_2 modulation of defense may not require a PRR since H_2O_2 can diffuse through membranes or enter the cell via aquaporins. Also, the activity of OS from the grasshopper *Schistocerca gregaria* on the induction of OPDA in *Arabidopsis* is related to lipase activity of the OS, which may directly liberate defense hormone precursors from membrane lipids (134).

Chewing herbivores inflict mechanical damage that strongly modifies the extracellular space by releasing cell wall fragments and intracellular components. Collectively called DAMPs, these chemically diverse elicitors induce defense responses. Oligogalacturonides are pectic fragments perceived by wall-associated kinases (WAKs) in *Arabidopsis thaliana* (76). That cells can monitor cell wall modifications and respond by activating defense response has been observed in mutants impaired in cellulose synthesis. THESEUS1 (THE1) is a RK that senses cellulose-related cell-wall integrity (52). Like THE1, FERONIA (FER) contains two extracellular carbohydrate-binding malectin domains. FER monitors cell-wall integrity in response to salt stress by binding to pectin (39). Curiously, there is yet no information on the role of cell-wall integrity sensors like WAK1, THE1 or FER in defense against herbivores. However, it is tempting to speculate that perception of cell wall perturbation is a key factor in response to feeding. Testing insect performance on PRR mutants may unveil an additional role for these important components of the plant surveillance machinery.

Broken cells release numerous intracellular molecules in the apoplastic space. Exogenous treatment with ATP induces defense responses in *Arabidopsis* including an increase in cytosolic Ca^{2+} (22). A screen for ATP-insensitive mutants has identified DOES NOT RESPOND TO NUCLEOTIDES 1 (DORN1), a plasma membrane-localized L-type LecRK (LecRK-I.9) that binds ATP with high affinity and selectivity (22). *DORN1* overexpression increases plant response to wounding, implying a role in perception of mechanical damage (22). Furthermore, extracellular ATP induces a set of jasmonate (JA)-responsive genes, indicating that perception of ATP stimulates the JA pathway (158). NAD(P) has been identified in the extracellular space of wounded *Arabidopsis* leaves. When applied exogenously, NAD(P) induces defense gene expression in a Ca^{2+}-dependent manner (182). Recently, Wang et al. (164) have shown that LecRK-I.8 binds NAD^{+} and induces immune responses. Intriguingly, LecRK-I.8 is also implicated in *Arabidopsis* response to *Pieris brassicae* eggs (47). Whether this is linked to NAD^{+} perception or whether LecRK-I.8 recognizes another egg-derived ligand remains to be elucidated. It is however striking that two closely related LecRKs bind extracellular nucleotides. A G-type LecRK from *N. attenuata* also contributes to resistance against *Manduca sexta* but the corresponding ligand is unknown (43).

Wounding or insect feeding triggers the production of plant peptides that are released in the apoplastic space and are considered secondary endogenous danger signals (SDSs). The best characterized SDS is systemin from tomato, an 18 amino acid (aa) polypeptide that is cleaved from the precursor prosystemin, spreads throughout the plant, and induces JA-dependent accumulation of proteinase inhibitors that can negatively impact chewing herbivores (119). In tomato, LRR-RK SYR1 binds systemin with high affinity, and introgression lines that lack SYR1 are more susceptible to *S. littoralis* feeding, although local and distal induction of proteinase inhibitors is not affected (166). Systemin is restricted to Solanaceae but other wound peptides have been identified. In maize, a family of five related peptides induce emission of herbivore-related volatiles, with the 23 aa ZmPep3 triggering responses similar to those induced by *S. exigua* (63). ZmPeps are orthologous to *Arabidopsis* AtPeps, which were initially discovered as defense signals that amplify innate immunity (64). AtPep1 and its homologs trigger both JA- and salicylic acid (SA)-responsive genes and are perceived by two related LRR-RKs, PEPR1 and PEPR2 (174). *PEPR1*, *PEPR2* and *PROPEP3* expression is strongly activated by herbivore feeding and *pepr1 pepr2* double mutants are more susceptible to *S. littoralis* and *Pseudomonas syringae* pv. *tomato* (*Pst*) infection, consistent with a dual role for the AtPep-PEPR system in danger detection against microbes and insects (74, 174).

In summary, the first phase of insect feeding involves the perception of HAMPs and DAMPs that reach the apoplastic interface (**Figure 1**). HAMP PRRs have not yet been described, but recent breakthroughs have identified DAMP PRRs. Since insect feeding is accompanied by a mixture of HAMPs and DAMPs, the specific contribution of each molecule to plant defense response is difficult to assess and has led to controversy (14). The recent identification of DAMP receptors and receptor mutants such as *dorn1* and *lecRK-I.8* provides an excellent opportunity to revisit this issue. The identification of insect genes responsible for HAMP biosynthesis in combination with physical ablation of secretory structures could shed further light on the importance of HAMPs in plant-herbivore interactions (114).

Early signaling events triggered by herbivory

Early signaling steps following insect perception include i) depolarization of the plasma transmembrane potential (V_m), ii) rise in cytosolic Ca^{2+}, iii) production of reactive oxygen species (ROS), and iv) mitogen-activated protein kinase (MAPK) activity. V_m variation occurs within seconds and $[Ca^{2+}]_{cyt}$ within minutes in lima bean and *Arabidopsis* leaves after wounding, application of HAMPs and DAMPs, and natural feeding by *S. littoralis* or the aphid *Myzus persicae* (104, 127, 134, 161, 163). The use of Ca^{2+} channel inhibitors suggests that the increase in $[Ca^{2+}]_{cyt}$ is driven by a membrane channel (104, 134, 175). So far, the only known channel with a demonstrated effect on herbivory is the vacuolar cation channel two-pore channel 1 (TPC1). The *Arabidopsis* gain-of-function mutant *fou2* possesses a hyperactive version of TPC1, displays strong JA pathway activation, and is more resistant to *S. littoralis* feeding (11, 83). Interestingly, local aphid-induced $[Ca^{2+}]_{cyt}$ elevation is greatly diminished (163). In addition, AtPep3 activates the plasma membrane cyclic nucleotide-gated cation channel 2 (CNGC2) and triggers $[Ca^{2+}]_{cyt}$ elevation through the guanylyl cyclase activity of AtPEPR1 (127). The role of CNGC2 in response to herbivory is unknown. There are at least 57 putative cation channels in the *Arabidopsis* genome and it is conceivable that a high level of functional redundancy explains the paucity of characterized candidates.

The decoding of Ca^{2+} signals is mediated by various Ca^{2+}-sensors, including calmodulins (CaMs), calmodulin-like proteins (CMLs), and calcium-dependent protein kinases (CDPKs). In a series of experiments, Yan et al. (175) have demonstrated that the *Arabidopsis* JA-biosynthesis regulator JAV1 (jasmonate-associated VQ motif 1) is phosphorylated upon wounding and interacts with CaM1, CaM4, and CaM7 in a Ca^{2+}-dependent manner. Furthermore, the *Arabidopsis* CML37 positively regulates *S. littoralis*-induced defense by activating the JA pathway (140), whereas CML42 acts as a negative regulator and *cml42* displays enhanced resistance to *S. littoralis* and higher $[Ca^{2+}]_{cyt}$ accumulation (161). In *N. attenuata*, the Ca^{2+}-sensor homologues *Na*CDPK4 and *Na*CDPK5 suppress JA biosynthesis and promote *M. sexta* herbivory through an unknown mechanism (176). Thus, available studies suggest that $[Ca^{2+}]_{cyt}$ elevation after wounding/feeding is decoded by different sensors that are part of a larger regulatory network.

ROS are induced by herbivory and have been associated with plant defense regulation. Plant-derived ROS are primarily produced by plasma membrane NADPH oxidases (respiratory burst oxidase homologs, RBOHs). Wounding causes a rapid local and systemic ROS burst that depends on RBOHD in *Arabidopsis* (109). The *rbohD* mutant is more susceptible to *M. persicae* infestation (109) and a silenced *rbohD* line in *N. attenuata* is more susceptible to *S. littoralis* (172). By contrast, Arabidopsis the *rbohD/F* mutant is more resistant to *S. exigua* and *T. ni* feeding (9). Thus, ROS can act as both positive or negative regulators of plant resistance. As RBOHs are activated by ROS-dependent Ca^{2+} influx and CDPKs they likely interact closely with Ca^{2+} in early defense signaling.

Herbivory and wounding rapidly activate MAPKs (57). Silencing genes encoding wound-induced protein kinase (WIPK/MPK3) and salicylic-acid induced protein kinase (SIPK/MPK6) in *N. attenuata* inhibits JA biosynthesis, expression of WRKY transcription factors, and accumulation of defense compounds (171). Silencing tomato *MPK6* orthologs *MPK1* and *MPK2* renders plants more susceptible to *M. sexta* and the potato aphid *Macrosiphum euphorbiae* (67, 85). In rice, SSB activates MPK3 and MPK6, which positively regulate JA accumulation. Consequently, the ir-*mpk3* line is more susceptible to SSB feeding (60, 88, 167). In contrast, *N. attenuata* MPK4 suppresses JA-dependent defenses and silencing *MPK4* increases resistance to the specialist *M. sexta* but not to the generalist *S. littoralis* (51).

In summary, molecular studies have provided clear evidence for the involvement of Ca^{2+}, ROS, and MAPKs in plant responses to DAMPs and HAMPs, thus supporting current models of early defense signaling. However, major open questions remain. It is for instance unclear how potential PRRs are connected to these early signaling events, how V_m variation is connected to Ca^{2+} influx, if there is a hierarchical or independent organization of the signaling network, how positive and negative regulatory steps are modulated, and to what extent the network topology is species-dependent (**Figure 2**). Understanding the topology of early signaling networks of plant responses to herbivory thus remains a major challenge which may require the integration of quantitative genetics and systems biology approaches to be overcome.

Jasmonate signaling as a conserved core pathway in herbivory-induced responses

Hormonal signaling networks connect perception and early signaling to broad transcriptional reorganization and defense induction. JA signaling is well established as the core pathway that regulates plant defense responses against herbivores, and intense research over the last two decades has revealed essential molecular components of the JA pathway (58). In brief, upon injury, acyl-lipid hydrolases release α-linolenic acid (18:3) from galactolipids in plastid membranes. Oxygenation by 13-lipoxygenases (LOX) is followed by epoxidation and cyclization reactions to generate cis-(+)-12-oxo-phytodienoic acid (OPDA). OPDA is transported into the peroxisome where it is reduced and undergoes three cycles of β-oxidation to form (+)-7-*iso*-JA. (+)-7-*iso*-JA is transported into the cytosol where it is conjugated to Ile by jasmonoyl amino acid conjugate synthase (JAR1). JA-Ile is the canonical bioactive jasmonate and is transported into the nucleus by the ABC transporter JAT1 where it binds to its receptor, a complex consisting of CORONATINE-INSENSITIVE1 (COI1), JASMONATE-ZIM DOMAIN (JAZ) and an inositol-polyphosphate cofactor. COI1 is a component of a SKP1–CUL1–F-box protein (SCF) E3 ubiquitin ligase complex (SCF^{COI1}) and, upon binding JA-Ile, interacts with JAZ repressors and triggers their degradation by the 26S proteasome. JAZ degradation releases repression of MYC transcription factors, resulting in expression of defense genes and resistance against a wide variety of herbivores (**Table 1** and **Table S1**). The JA signaling pathway was likely already present in early land plants such as liverwort. It has been recently shown that COI1 of *Marchantia polymorpha* is a functional protein that regulates resistance to herbivory (111). Strikingly, MpCOI1 harbors a single amino acid substitution that allows it to bind to two isomeric forms of dinor-OPDA, but not to JA-Ile, which is absent from *M. polymorpha* (111).

Although the JA pathway has been extensively studied, there are still some open questions. The connection between early defense signaling and the activation of acyl-lipid hydrolases in chloroplasts to initiate JA biosynthesis for instance remains largely unknown. A recent study by Yan et al. (175) in *Arabidopsis* has shown that $Ca^{2+/}$Cam-dependent phosphorylation of JAV1 leads to its degradation by the proteasome, disrupting a nuclear JAV1-JAZ8-WRKY51 repressing complex that inhibits expression of JA biosynthesis genes. This work provides the first coherent model that links herbivore-induced $[Ca^{2+}]_{cyt}$ accumulation with JA biosynthesis. Of note, JA accumulates within 30 seconds in wounded *Arabidopsis* tissues (44), suggesting that the first JA burst does not require transcriptional activation of JA biosynthesis genes and that JAV1-mediated regulation may rather be a secondary amplification step (**Figure 2**).

Signaling networks mediated by small metabolites

Plant responses to herbivory display a great deal of specificity, which is incompatible with the notion of a single hormonal pathway controlling all responses. Indeed, numerous other hormones are known to influence JA-dependent and JA-independent responses through hormonal cross-talk. Furthermore, inducible plant secondary metabolites are emerging as defense regulators that can modulate defense deployment, thereby increasing the specificity of the signaling networks underlying plant defense responses.

Salicylic acid (SA), ethylene (ET), and abscisic acid (ABA) are stress-related phytohormones that are induced upon herbivory and are well established modulators of plant resistance to herbivores. The impact of these hormones on resistance seems to be highly context-dependent. SA-deficient tomato plants for instance are more susceptible to the potato aphid, while SA-signaling mutants in *Arabidopsis* are not affected in their resistance to *M. persicae* (28, 85). Furthermore, mutants deficient in SA biosynthesis (*sid2-1*) or signaling (*npr1*) are more resistant to *S. littoralis* and *Bemisia tabaci* (10, 179). The SA pathway is known to antagonize JA signaling and can therefore act as a negative regulator of JA-dependent defenses in plants. Silencing a rice 1-aminocyclopropane-1-carboxylic acid (ACC) synthase reduces rice ET production and resistance to a chewing herbivore (SSB), but increases resistance to a phloem feeder (BPH) (93). *Arabidopsis* ethylene-insensitive mutants *ein2-1* and *ein3 eil1* on the other hand are more resistant to *S. littoralis* and *S. exigua* (10, 151). ET-stabilized transcription factors ETHYLENE-INSENSITIVE 3 (EIN3) and EIN3-LIKE1 (EIL1) interact with JA-activated MYC2 and inhibit JA-regulated defenses against herbivores, providing a molecular mechanism for such ET/JA antagonism (151). ABA-deficient *Arabidopsis* plants are more susceptible to *S. littoralis* (10). In *N. attenuata*, HERBIVORE ELICITOR REGULATED 1 (HER1) inhibits ABA catabolism. Lines with reduced *HER1* expression are more susceptible to *M. sexta* and accumulate less JA and defense metabolites (31). Because ABA is involved in drought stress, a response that often occurs after leaf damage, it is not surprising that ABA contributes and may even reinforce plant resistance to chewing herbivores. The recent finding that expression of *Arabidopsis* chloroplast-localized glycerolipid A_1 lipases *PLIP2* and *PLIP3* is induced by ABA and leads to JA accumulation provides an attractive mechanistic link between ABA accumulation and downstream JA-defense responses. Herbivore performance on *plip2/3* mutants has however not been tested (165). Furthermore, pea aphid performance is decreased on the ABA biosynthesis mutant *aba1-1* in *Arabidopsis* (55). In summary, SA, ET and ABA are well established modulators of plant defense and resistance, but their impact varies between plant species and herbivore feeding guilds.

Growth hormones such as gibberellins (GAs), auxin and cytokinins (CKs) are also thought to be involved in the regulation of anti-herbivore defenses. GAs regulate growth via proteasome-mediated degradation of DELLA repressors. DELLAs modulate the JA pathway by physically interacting with JAZs, thereby preventing the negative effect of JAZs on MYC2-related defense expression. Thus, activation of the GA pathway leads to DELLA degradation and inhibition of JA responses. However, the *Arabidopsis quad della* mutant does not display dramatic changes in defense metabolite accumulation upon *S. exigua* feeding and insect performance has not been tested (82). In contrast, a rice GA-accumulating mutant increases resistance to BPH, suggesting that GAs positively regulate defense against phloem-feeding insects (88). Auxins and CKs have been explored in the context of systemic defense regulation and are discussed in more detail in the section “Systemic regulation of defenses”.

Apart from hormones, induced plant secondary metabolites are increasingly recognized as regulators of plant defense deployment. Glucosinolate (GS) breakdown products in *Arabidopsis* and benzoxazinoids in maize act as positive regulators of callose accumulation (24, 107), and various plant volatiles prime JA signaling and plant defense (97). Flavonols and the GS breakdown product indole-3-carbinol inhibit auxin transport and perception signaling and may thereby influence plant responses to herbivores (69). These recent findings blur the dichotomy between defense hormones and defense metabolites and suggest that plants can use a wide variety of molecules to specifically regulate their defenses. Understanding the evolutionary and ecological implications of this phenomenon will be greatly facilitated by the availability of secondary metabolite biosynthesis mutants in various plant species.

In addition, studies in *N. attenuata* suggest that small RNAs can modulate the JA pathway during herbivory. Insect feeding induces a significant change in the small-RNA transcriptome (120), and *N. attenuata* mutants in components of the small-RNA machinery are more susceptible to insects (**Table S1**). However, how is the reprogramming of plant defenses modulated by small RNAs and whether this regulation occurs in other plants will need further research.

Transcription factors as major players in early and late defense signaling

Transcription factors play a key role in regulating defenses both up- and downstream of phytohormone signaling. Basic helix–loop–helix (bHLH) MYCs form a transcriptional complex that regulates defense against herbivores in *Arabidopsis*. In absence of insect feeding, JAZs bind and repress MYCs. Activation of the JA pathway leads to JAZ degradation, subsequent binding of MYCs to MED25 of the mediator complex, and recruitment of RNA polymerase II (for review see (58)). Four closely related MYCs from the IIIe subgroup of bHLH factors act synergistically to control JA-dependent defenses. Indeed, single and higher order mutants of MYC2, MYC3, MYC4, and MYC5 display increasing susceptibility to *S. littoralis* and *S. exigua* feeding (40, 152). Furthermore, *myc234* is as susceptible as *coi1-1* to *S. littoralis* and both mutants show a similarly altered transcriptome in response to feeding, indicating that MYCs are the main contributors of defense against chewing herbivores in *Arabidopsis* (144). MYCs bind to G-box on promoters of target genes, including GS biosynthesis genes (40, 145). In addition to MYCs, GS biosynthesis requires coordinate activity of six MYB transcription factors. MYB28, MYB29, MYB76 and MYB34, MYB51, MYB122 regulate synthesis of methionine-derived aliphatic-GS and tryptophan-derived indole-GS, respectively (42). These MYBs interact directly with MYC2, MYC3 and MYC4, but not with JAZs, and are also necessary for GS biosynthesis and defense (145). For instance, *myb28myb29* lacks aliphatic-GS and is susceptible to several lepidopteran herbivores (115). Thus, MYCs and MYBs together constitute a functional regulatory module that controls expression of GS genes in *Arabidopsis*.

In addition, MYCs regulate expression of other anti-herbivore genes. In *Arabidopsis*, MYC2 binds to the promoter of *TPS11* and *TPS21* to regulate sesquiterpene biosynthesis (56). In *N.*

attenuata, MYC2 homologues directly control nicotine biosynthesis (170). In tomato, MYC2 regulates the expression of a large number of defense genes, including genes coding for known antiherbivore defense proteins like threonine deaminase and proteinase inhibitor 1 (33). Besides MYCs, other transcription factors with demonstrated anti-herbivore effect have been identified, including for instance WRKY18/40/53/70 or MYB75 (88, 118, 144) (**Figure 2**, **Table 1** and **Table S1**). How these proteins regulate defense is however unclear.

Evidence of a negative regulation of MYC activity has recently been demonstrated. Four members of IIId subgroup of *Arabidopsis* bHLHs (bHLH3, bHLH13, bHLH14 and bHLH17) act additively as transcription repressors. Also known as JA-ASSOCIATED MYC2-LIKE (JAMs), they competitively bind to target sequences of MYC2. Consequently, *JAM* single or multiple mutants have enhanced JA-dependent defenses and are more resistant to *S. exigua* (131, 153). As discussed above, WRKY51 inhibits JA biosynthesis through association with JAV1 and JAZ8. WRKY51 binds to a W-motif on the promoter of AOS and inhibits its expression, potentially preventing unnecessary JA biosynthesis in absence of feeding (175). Rice MPK3/MPK6 directly phosphorylate WRKY53 (178), which in turn interacts with MPK3/MPK6 and suppresses their activity (60). This creates a negative feedback loop that restricts overexpression of defense genes. Accordingly, ir-*wrky53* is more resistant to the stem borer *C. suppressalis* (60). These findings illustrate a tight regulation of different steps of the JA pathway by various transcription factors (**Figure 2**). Future work should elucidate whether and how different biotic or abiotic stresses interact with these negative regulators.

Systemic regulation of defenses

Herbivores are often mobile and move from attacked to non-attacked tissues. Upon herbivore attack, plants can use systemic signals to regulate defenses in systemic tissues in preparation of incoming attack. JA signaling is well established to be important for the activation of systemic defense regulation. Grafting experiments with *N. attenuata* show that silencing homologues of JA biosynthesis and perception genes such as *AOC* and *COI1* in the roots reduces the capacity to accumulate nicotine and to deploy it to the leaves, leading to enhanced aboveground herbivory by *M.*

sexta and *S. littoralis* (41). To what extent JAs are moving between local and systemic tissues to regulate defenses is still subject to debate. Through localized manipulation of JA biosynthesis and perception, the production of JAs in systemic tissues, but not in locally induced tissues, has been shown to be important for systemic defense deployment in *Arabidopsis* and *N. attenuata* (41, 77). In *Arabidopsis*, glutamate-receptor like genes (GLRs) are required for the induction of systemic JA synthesis. Silencing of *GLRs* has been associated with a reduction in wound-induced, systemic surface potential charges, suggesting that electrical potentials are important for the systemic induction of JAs (113). Ca^{2+} and ROS have also been implicated in rapid systemic signaling, most probably in interaction with membrane potentials (for review see (54)).

Recent work demonstrates that, apart from JA, other mobile hormones such as CKs and auxins may regulate systemic defense responses. Wounded *N. attenuata* plants silenced in homologues of the CK receptor CHASE-DOMAIN CONTAINING HIS KINASE 2 accumulate lower levels of caffeoylputrescine in systemic leaves (133). Furthermore, inhibiting auxin transport through application of the IAA biosynthesis inhibitor L-kynurenine, or the IAA transport inhibitor TIBA abolishes herbivore-induced systemic induction of stem anthocyanins (102). Further experiments with auxin and CK signaling mutants are required to understand the impact of these hormones on plant resistance to herbivores.

Plants can also use volatiles to regulate systemic defenses. Several volatile organic compounds, including C6 green-leaf volatiles, terpenes and indole can be emitted by herbivore-attacked leaves and can induce and/or prime defenses in systemic leaves (36). Using an *igl* mutant that does not produce indole, it has been shown that this volatile is required for the priming of monoterpenes in systemic maize leaves (37). *Arabidopsis* genes involved in root response to (*E*)-2-hexenal include the gamma-amino butyric acid (GABA) transaminase HER1, which degrades GABA, and the oxidoreductase HER2, which regulates the redox status in mitochondria (110, 132). Systemic signaling through plant volatiles may be particularly important when vascular constraints and long vascular distances between adjacent tissues limit the spread of internal signals.

Spatiotemporal control of plant defense deployment

Many constitutive and induced defenses show pronounced ontogenetic (i.e. developmental) patterns and vary with the age of plants and organs. While this variation has been mapped in great detail in many different plant species (6), the underlying molecular mechanisms are only beginning to be unveiled. In addition, secondary metabolite transporters have been identified as important determinants of defense distribution (see sidebar titled Secondary Metabolite Transport).

In maize, the decline of benzoxazinoids in older plants has been mapped to the *Bx* gene cluster containing major benzoxazinoid biosynthesis genes (183). Expression of *Bx1*, the gene encoding for an indole-3-glycerol-phosphate lyase which provides indole as a benzoxazinoid precursor, is associated with benzoxazinoid levels, and overexpression of this gene enhances benzoxazinoid levels in older maize plants (183), thus suggesting that the decrease of these metabolites in older plants is the result of transcriptional regulation of biosynthesis genes (96). Transcriptional regulation is also thought to regulate the tissue specific production of alkaloids and other secondary metabolites in plants (84). For instance, a cluster of ERF transcription factors regulate the expression of nicotine biosynthesis genes in tobacco. Although nicotine accumulates specifically in roots, expression of some of these ERFs is not restricted to roots and is also observed in leaves, suggesting that another factor is crucial for root-specific biosynthesis or that ERFs are inhibited in the leaf (150). In *N. attenuata*, CKs co-vary with caffeoylputrescine inducibility, which is higher in young than old leaves. Increasing CK levels through dexamethasone-inducible expression of the CK biosynthesis isopentenyltransferase is sufficient to restore the inducibility of caffeoylputrescine in old leaves (17). In *N. attenuata* flowers, only one member of the JAZ family of repressors, NaJAZi, regulates constitutive accumulation of defense compounds and silenced *NaJAZi* lines show reduced feeding by the florivore *H. virescens* (87). The capacity to manipulate ontogenetic patterns of defense expression will eventually allow to test long-standing ecological hypotheses such as the optimal defense theory, which predicts that ontogenetic patterns reflect costs and benefits of defense production (108).

Defense metabolites also show diurnal fluctuations that may be tailored to insect feeding behavior. For instance, *Arabidopsis* displays circadian accumulation of JA and GS that correlates with circadian feeding of *T. ni*. Plants exposed to experimentally desynchronized *T. ni* larvae are significantly more eaten than under normal conditions (46). In *N. attenuata*, many herbivore-induced metabolites show diurnal rhythmicity in a tissue-specific manner (72). Silencing the clock component *ZEITLUPE* (*ZTL*) in *N. attenuata* renders plants more susceptible to *S. littoralis*. ZTL interacts with JAZs, and therefore enhances MYC2-dependent expression of nicotine biosynthesis genes (86). Tobacco plants emit herbivore-induced volatiles during the night to repel oviposition by nocturnal female moths of the pest *Heliothis virescens* (27). Similarly, *N. attenuata TPS38* is expressed in flowers during the night and produces (*E*)-α-bergamotene that attracts *M. sexta* moths for pollination, whereas the same gene is expressed in leaves upon *M. sexta* feeding and triggers production of (*E*)-α-bergamotene during the day to attract predators of *M. sexta* larvae (185).

PLANT DEFENSE TRAITS

Physical barriers, secondary metabolites and defense proteins directly determine herbivore resistance by interacting with the feeding, digestive system and physiology of the attacker (38, 57). Over the last years, molecular studies have been instrumental in providing evidence for the functional importance of a number of putative defense traits in vivo.

Toxic secondary metabolites

Plants produce hundreds of thousands of different specialized metabolites, and many of them are assumed to function as defenses by reducing the digestibility of plants. In *Arabidopsis*, mutations in genes involved in GS biosynthesis, regulation, or activation render plants highly susceptible to a wide range of chewing herbivores (**Table 1** and **Table S1**). For specialist insects adapted to GS, metabolites from the phenylpropanoid pathway provide some level of resistance. *Arabidopsis fah1-7* lacks sinapoyl malate and shows increased susceptibility to *P. brassicae* (117). In addition, reduced levels of kaempferol 3,7-dirhamnoside in OE-MYB75 correlates with increased *P. brassicae* performance (118). In tobacco, reducing nicotine content in silenced *N. attenuata* lines increases performance of *M. sexta*, *S. exigua* and other native herbivores, an effect that can be reversed by the application of nicotine (99, 155). In maize, a *bx1* mutant devoid of benzoxazinoids DIMBOA-Glc and HDMBOA-Glc allows increased feeding by *S. exigua* and *S. littoralis* (98). In tomato, production of the sesquiterpene 7-epizingiberene by expression of a terpene synthase from a wild variety increases resistance to *M. sexta* (8). The diterpene rhizathalene A is produced in *Arabidopsis* roots by terpene synthase TPS08 and feeding by the root herbivore *Bradysia* sp. significantly increases in *tps08-1* (162). In dandelion (*Taraxacum officinale*), silencing the germacrene A synthase *ToGAS1*, which catalyzes formation of sesquiterpene lactone taraxinic acid β-D-glucopyranosyl ester in root latex, enhances the attractiveness of roots to the common cockchafer *Melolontha melolontha* (62).

For phloem-feeding insects, the role of secondary metabolites is less clear. Mutants with altered indole-GS levels for instance display contrasting effects on aphid performance (5, 124). The camalexin-deficient *pad3-1* on the other hand is susceptible to aphids (81). In maize, benzoxazinoids are directly toxic and regulate callose inducibility, which allow them to operate against chewing and phloem feeding insects (107). A *bx13* mutant defective in the conversion of the benzoxazinoid DIMBOA-Glc to DIM$_2$BOA-Glc and HDM$_2$BOA-Glc slightly enhances the performance of the corn leaf aphid, *Rhopalosiphum maidis* (50).

Defense proteins

Overexpression of proteinase inhibitor (PIs) genes in transgenic plants has provided the first evidence that PIs are efficient antiherbivore proteins (66). Further work with knock-out lines has confirmed the importance of PIs in several plant species against various herbivores (**Table S1**). Overexpression of a cysteine protease in maize disrupts insect peritrophic matrix and severely retards growth of *S. frugiperda* (122). Depletion of essential amino acids from insect diet is another efficient defense strategy. Transgenic tomato lines deficient in a threonine deaminase (TD2) are more susceptible to *S. exigua* and *T. ni* (45). Interestingly, proteolytic activation in insect midgut releases feedback inhibition by Ile and enhances TD efficiency (20). Similarly, TD-deficient *N. attenuata* lines are more susceptible to *M. sexta* (68). Finally, overexpression of arginase in tomato significantly reduces Arg in larval midgut and renders plants more resistant to *M. sexta* (20).

To our knowledge, there are only three reports demonstrating a role for defense proteins against phloem-feeding insects. Overexpression of *PP2-A1*, a member of the multigene family of *Arabidopsis* phloem lectins, reduces *M. persicae* infestation, presumably by clogging sieve pores (180). SLI1 is a recently identified membrane-anchored chaperone that is postulated to prevent stylet from piercing the parietal membrane of sieve tubes. *Arabidopsis sli1* shows enhanced feeding and infestation by *M. persicae* (75). In rice, Bph6 is an unknown protein that interacts with exocyst subunit EXO70E1. *Bph6*-carrying cultivars provide resistance to BPH and *S. furcifera* by increased exocytosis and cell wall reinforcement (48).

Volatiles as attractants of herbivore natural enemies

Plants that are attacked by herbivores release distinct volatile blends, which affect herbivores and increase the attraction of herbivore natural enemies. Over the last years, molecular approaches have helped to understand how these effects affect plant-herbivore interactions. Overexpressing a hydroperoxide lyase gene (*HPL*) in *Arabidopsis* enhances the production of green leaf volatiles, renders plants more attractive to the *Cotesia glomerata* parasitoid and increases *P. rapae* larval mortality (147). Silencing *HPL* in *N. attenuata* significantly reduces the attraction of the egg predator *Geocoris punctipes* in the field (49), and silencing *LOX2*, which is involved in green-leaf volatile biosynthesis, reduces predator recruitment and increases damage by *M. sexta* (142). Transgenic *Arabidopsis* lines that overexpress the maize terpene synthase *TPS10* emit more herbivore-induced sesquiterpenes and are more attractive to *Cotesia marginiventris* (139). Interestingly, expression of the maize AP2/ERF *EREB58* is induced by JA treatment and this factor binds to the promoter of *TPS10* to control the production of volatile sesquiterpenes (89). In rice, silencing *S*-linalool synthase diminishes attraction of the parasitoid *Anagrus nilaparvatae*, leading to enhanced BPH performance in laboratory and field conditions (173). In maize, overexpression of the terpene synthase *TPS23* increases the recruitment of entomopathogenic nematodes, the western corn rootworm and *S. frugiperda* in the field (29), resulting in neutral effects on plant yield (130). Overexpressing the terpene synthase *Eβf* in wheat repels aphids and increases parasitoid recruitment in the laboratory, but has not resulted in clear effects in a two-year field trial (15). A recent study with an indole deficient *igl* maize mutant demonstrates that indole attracts parasitoids to plants, but reduces the attractiveness of the caterpillars themselves, thereby reducing overall parasitoid recruitment (177). Together, these manipulative studies reveal that the effects of plant-volatiles on plant-herbivore interactions can be multifaceted and can result in unexpected patterns, which complicates their use as strengtheners of biological control (160).

Physical barriers

Physical barriers of plants such as trichomes, cuticule, epidermis and bark tissues are important to stop herbivores from attaining nutritious plant tissues. *Arabidopsis lyrata* genotypes with mutation in *GLABROUS1* suffer from a higher abundance of leaf-chewing insects on the leaves in the field (73), whereas *Arabidopsis gl1* is more susceptible to *S. littoralis* in the laboratory (128). Surprisingly, an *Arabidopsis* mutant with reduced cuticular wax and cutin is more resistant to feeding by the generalist *S. littoralis* (7). Apart from these studies, there are surprisingly few reports on the impact of molecular manipulation of physical defenses on plant-herbivore interactions.

Tolerance strategies

The capacity to regrow and reproduce after herbivore attack is important for plant survival and represents a complementary strategy to resistance. However, the molecular basis of plant tolerance to herbivory remains poorly studied. Herbivore attack depletes carbohydrates in the roots of *N. attenuata* and reduces the regrowth capacity of defoliated plants. Both effects are absent in JA-deficient ir-*AOC* plants and can be recovered by JA application (101). Solanaceae species that display a lower JA burst upon herbivory have greater defoliation tolerance (103), suggesting that JA signaling regulates both processes in opposite directions, most likely by antagonizing GA signaling (100). Furthermore, silencing the SNF1-related kinase *SnRK1*, which is also downregulated by herbivory, increases carbon transport to the roots and prolongs flowering in *N. attenuata* and may thus help to buffer root carbohydrate depletion and increase tolerance (143).

Progress with genomics approaches

Whole-genome analyses of herbivore-attacked plants have generated an unbiased view on transcriptional investment in defense, associated biological functions and quantitative contribution of signaling pathways. Generally, insects from the same feeding guild trigger overlapping transcriptome signatures, whereas these are clearly distinct between chewing larvae and phloem-feeding insects (81, 128). In all these experiments, the dominating biological functions activated by herbivory are responses to biotic and abiotic stress, production and response to ROS, calcium signaling, cell wall modification, secondary metabolism, hormone metabolism and transcriptional regulation. Consistent with the prominent role of the JA pathway, induction of a large majority of insect-responsive genes depends on a functional COI1 (128, 144).

Because forward genetic screens are not easily amenable for plant-insect interactions, genetic approaches such as quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) hold promise to uncover novel molecular players. Recording aphid behavior on 350 *Arabidopsis* accessions for instance has led to the identification of SLI1, a novel protein that is postulated to impair phloem ingestion (75). GWAS has also been used in *Arabidopsis* to determine loci that control variation in GS profiles in various environmental or developmental conditions (13, 18), or loci that are linked to the combined response to *P.rapae* feeding and drought (26). In maize, QTL-mapping led to the identification of a benzoxazinoid O-methyl transferase whose deactivation by a transposon is associated with increased callose accumulation and aphid resistance (107). Through a similar approach, an α-bergamotene synthase involved in pollinator and herbivore natural enemy attraction was identified in *N. attenuata* (185). As genomes and mapping populations become available for other plant model species, such strategies may prove valuable to exploit natural genetic diversity and discover novel important defense genes.

HERBIVORE ADAPTATIONS TO PLANT DEFENSES

Herbivore adaptations to plant defenses are as diverse and fascinating as plant defenses themselves. Technological advances such as next generation sequencing and the development of RNAi have begun to yield novel insights into the molecular biology of plant-insect interactions from the insect's perspective.

Detoxification and neutralization of plant defenses

Herbivores possess a variety of detoxification systems that allow them to deal with host toxins. Several herbivore genes have been identified that allow them to cope with plant defenses. Silencing the cytochrome P450 *CYP6AE14* in the cotton bollworm decreases larval growth, and the effect is more dramatic in the presence of gossypol (106). However, additional studies have not confirmed a role of CYP6AE14 in gossypol detoxification and this enzyme may thus be involved in general stress responsiveness (79). In *M. sexta*, silencing *CYP6B46* reduces nicotine levels in the hemolymph and renders *M. sexta* caterpillars more susceptible against wolf spiders (80). Excreted nicotine levels remain the same, however, and the exact function of CYP6B46 remains to be elucidated. *M. sexta* caterpillars with reduced β-glucosidase (BG1) levels no longer deglycosylate the hydroxygeranyllinalool diterpene glycoside lyciumoside IV and show developmental effects when feeding on lyciumoside IV-containing diets (126). Herbivores may also be able to deactivate structural defenses of their host plants. BPH secretes an enzyme with cellulose-degrading endo-β-1,4-glucanase activity (NlEG1) into rice plants. Silencing *NlEG1* decreases the capacity of BPH to reach the phloem with its stylet and reduces its fitness, suggesting that this enzyme helps BPH to break down plant cell walls (65). Together, these examples illustrate the capacity of insects to deal with plant defenses. They also illustrate the limits of inferring function from sequence homology and call for orthogonal biochemical experiments to distinguish between herbivore genes that are specifically involved in detoxification and host plant adaptation, and genes that are part of general stress-coping mechanisms. Many other studies have associated biochemical features of insect herbivores with adaptation to plant defense metabolites (90), including for instance highly conserved molecular changes that render sodium-potassium pumps resistant to toxic cardiac glycosides (32), glycosylation of benzoxazinoids to avoid the production of toxic hemiacetals (95), the diversion and detoxification of GS through a series of different enzymes (169), and the deactivation, sequestration and reactivation of various plant toxins as a potential means of self-defense (123, 129). Testing for the importance of these traits through molecular manipulation of insect genes is an exciting future prospect.

Plant defense suppression

Insect herbivores may be able to inject salivary molecules into plants to suppress defense induction. Putative defense suppressors (so-called effectors) have mainly been described in phloem-feeders. For instance, when transiently overexpressed in *N. benthamiana* using *Agrobacterium tumefaciens*, *Me10* and *Me23* from the potato aphid *M. euphorbiae* increase aphid performance, and *Me10* had similar effects in tomato when delivered through *Pst* (4). Furthermore, stable expression of *C002*, *Mp*1, and *Mp2* from *M. persicae* in *Arabidopsis* increase aphid performance, while silencing *C002* and *Mp2* through plant-mediated RNAi reduces aphid performance (34, 125). The molecular mechanisms by which these putative effectors increase aphid performance are currently unclear. Me47 from *M. euphorbiae* has glutathione-S-transferase (GST) activity against isothiocyanates and may thus have a role in detoxification. However, Me47 suppresses rather than enhances *M. euphorbiae* performance on *Arabidopsis*, which produces isothiocyanates during GS breakdown (71).

Much less is known about effectors from lepidopteran insects. GOX in *Helicoverpa zea* salivary glands inhibits nicotine production (114). OS from *Anticarsia gemmatalis* contain a modified inceptin that inhibit plant defenses. Strikingly, deletion of only one amino acid from inceptin transforms a defense-inducing HAMP into an effector (138). Unknown proteins or peptides from lepidopteran OS suppress defenses in *Arabidopsis* and *N. attenuata* (25, 136). Clearly, more studies are needed to identify effectors from chewing herbivores and demonstrate their function by gene knock-out methodologies.

Apart from injecting their own effectors during feeding, insect herbivores may use additional molecular strategies to suppress plant defenses. The capacity of the Colorado potato beetle to suppress plant defenses is abolished in individuals that are treated with antibiotics (23). The beetle saliva contains numerous bacteria, and reinoculating antibiotic-treated individuals with bacterial isolates or treating plants with flagellin from one group of isolates (*Pseudomonas* sp.) restores defense suppression, suggesting that beetles use bacterial flagellin as an effector (23). JA-dependent plant defenses are also suppressed following egg deposition through activation of the SA pathway that triggers SA/JA cross-talk, but the involved effector molecules remain to be discovered (16).

In response to bacterial and fungal effectors, some plant genotypes have evolved numerous resistance genes that detect effector activity and trigger an hyperactivation of defenses. Resistance genes code for intracellular nucleotide binding and leucine rich repeat domain-containing proteins (NB-LRRs). Strikingly, very few insect NB-LRRs have been characterized and all of them are efficient against phloem-sucking insects (**Table S1**). The reason for such bias is unknown and merits further investigation.

Behavioral adaptations

The onset and outcome of plant-herbivore interactions often depends on behavioral responses of herbivores. Many plant defenses for instance do not primarily operate through intoxication, but through deterrence or repellency (12, 99). The capacity of herbivores to choose the right plants and tissues or to avoid defenses by behavioral strategies are key adaptations that allow them to survive within diverse plant communities that include toxic or unsuitable individuals. So far, studies addressing the molecular underpinning of host plant choice and herbivore behavior are surprisingly rare. Potential chemosensory receptor proteins have been identified in different herbivores (35), and gene editing approaches for major herbivore pests are becoming available (181). In a proof-of-concept study using *S. littoralis*, mutation of olfactory receptor co-receptor (*Orco*) genes by CRISPR/Cas9 shows that antennae of *Orco* moths no longer respond to plant volatiles (78). Thus, functional studies involving the molecular manipulation of herbivore behavior are becoming possible and have the potential to substantially enhance our understanding of how insects detect and respond to plant cues.

FASCINATING BUT UNEXPLORED INTERACTIONS

Most molecular insights into plant-herbivore interactions are derived from a few model systems. We argue that the time is ripe to leave the beaten track and to address unexplored but fascinating examples of plant-insect interactions in model and non-model species (**Figure 3**). Below, we discuss a few selected examples.

- Some specialized herbivores induce striking changes in plant leaf morphology to produce galls. These diverse and elaborate structures generally provide shelter against natural enemies (156). How insect-derived cues prevent plant defense and dramatically alter plant development is largely unknown.
- As a stunning example of a potential plant defense mechanism that targets insect behavior, cyanogenic glycoside-containing plants of the *Passiflora* genus display egg-like structures evenly dispersed on leaves, possibly to repel oviposition by the adapted herbivore *Heliconius cydno* (168). The evolution, genetic basis and exact ecological consequences of this phenomenon is unknown.
- Acacia trees provide food in the form of extrafloral nectar and modified leaflet tips (Beltian bodies), and swollen thorns as home. Ants constantly patrol the tree to remove unwanted insect herbivores and parasitic plants. The origin, establishment and stability of this type of tritrophic interaction has rarely been studied at the molecular level.
- Insect- and plant-associated microbes strongly influence plant-insect interactions. Remarkable examples include for instance larvae of the Colorado potato beetle that contain defense-suppressing bacteria in their oral secretions (23) and effector proteins from insect-vectored phytoplasma that inhibit JA-defenses (157). For plant-associated microbes, high-throughput sequencing has allowed the identification of numerous leaf-inhabiting microbes but how they influence plant-insect interactions is poorly studied.

CONCLUSIONS

Two decades of intense research on molecular plant-herbivore interactions in monocot and dicot plant species has unveiled a significant number of conserved molecular players involved in plant responses to insects. The rich catalogue of defense genes allows for a better understanding of i) the intricate signaling network underlying plant defenses ii) the genetic basis of defense traits and iii) mechanisms of herbivore adaptations to plant defenses. Challenges for the future will be to fill the existing gaps in our understanding of how perception is connected to downstream steps, how specificity is obtained with common signaling modules between herbivory and pathogenesis, and how insect perceive and respond to plant toxins. Leaving the "beaten track" and exploring the diversity of interactions between plants and herbivorous insects will also be essential. Herbivores have established specific interactions with plants over evolutionary time, including both biochemical and behavioral adaptations, which in turn have shaped the evolution of specific plant defense regulation and expression patterns. Understanding the molecular mechanisms underlying these phenomena holds the key to fully appreciate the beauty and diversity of plant-herbivore interactions.

SUMMARY POINTS

1. Plants detect and respond to damage and herbivore-associated molecular patterns (HAMPs and DAMPs) by activating defense responses. DAMP receptors have recently been identified, thus paving the way to understand the relative importance of DAMPS and HAMPS for plant-herbivore interactions.

2. Early signaling events in plant defense responses involve Ca^{2+}, ROS and MAPK signaling. Each layer has been shown to be important for plant defense and often includes both positive and negative regulators. Species-specific topologies of early defense signaling networks are emerging and require further investigation.

3. The JA pathway is an essential component of defense against chewing herbivores. In addition, several other small molecules, including growth and stress hormones as well as volatile and non-volatile secondary metabolites can regulate plant defenses. Plants thus have a substantial arsenal of

conserved and specific signaling molecules at their disposal to specifically regulate defenses against herbivores.

4. Transcription factors, including MYCs and WRKYs, play a crucial role in regulating defense signaling and activating the transcription of defense genes. Recent work shows how transcription factors mechanistically link early defense signaling, hormone signaling and defense deployment.

5. Plants display strong spatiotemporal variation in the expression of defense metabolites and proteins as well as physical barriers and tolerance traits. Transcriptional regulation of the underlying defense genes by developmental hormones and secondary metabolite transport are emerging as mechanisms by which defenses are regulated in space and time.

6. Insects counter plant defenses through behavioral and biochemical adaptations, including the production of defense-suppressing effectors. The (simultaneous) molecular manipulation of plant defenses and insect counter defenses allows for unprecedented insights into plant-herbivore interactions and connects plant defense and herbivore defense/immunity networks.

7. The current standard model of molecular interactions between plants and insect herbivores is derived from a handful of model species. Expanding molecular research to include more diverse and fascinating interactions will be essential to complement our current knowledge.

DISCLOSURE STATEMENT

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SIDEBAR: SECONDARY METABOLITE TRANSPORT

Transport allows plants to partition biosynthesis and accumulation of defensive secondary metabolites and to control their local and systemic accumulation. Alkaloids are often synthesized in belowground tissues and then transported to the leaves. In tobacco, NtMATE1 and NTMATE2 transport nicotine from the cytoplasm into the vacuole of root cells, while Nt-JAT1 and Nt-JAT2 have a similar function in leaves (112, 149). Furthermore, the tobacco permease NUP1 supports uptake of nicotine from the apoplast into root cells (53). In *Coptis japonica*, two ABC transporters, CjABCB1 and CjABCB2, function as berberine importers from the xylem into the rhizome (148). Finally, the tonoplast localized nitrate/peptide family transporter CrNPF2.9 from *Catharanthus roseus*, exports strictosidine from the vacuole to the cytosol (121). Most transporters identified so far function in metabolite uptake or subcellular localization, and much less is known about the molecular basis of systemic secondary metabolite transport. In *Arabidopsis*, two proton-dependent transporters, GTR1 and GTR2, transport GS from leaves and silique walls to the seeds (116). In *Petunia hybrida*, the ABC transporter PhABCG1 releases benzenoid volatiles from flowers (2). These recent discoveries pave a way to understand the contribution of systemic secondary metabolite transport through the vasculature and the headspace for plant-herbivore interactions.

TERMS AND DEFINITIONS

1. **Herbivore-associated molecular pattern (HAMP):** a component of insect oral secretions that is detected by a plant cell surface receptor and induces defense responses.
2. **Damage-associated molecular pattern (DAMP):** a plant cytosolic or apoplastic molecule that is freely released upon tissue injury. DAMPs are perceived by cell surface receptors and induce defense responses.
3. **Pattern recognition receptor (PRR):** cell surface receptor with a ligand-binding ectodomain, a transmembrane domain and an intracellular kinase domain. Some PRRs lack the kinase domain.
4. **Oral secretions (OS):** a mixture of insect saliva and regurgitant that can contain defense-inducing HAMPs or effectors.
5. ***Nicotiana attenuata:*** a wild relative of tobacco that has become an important model to identify molecular players of resistance to insects, both in the laboratory and in nature.
6. **Jasmonate (JA):** a generic term that refers to jasmonic acid or the bioactive form jasmonoyl-L-isoleucine (JA-Ile).
7. **Secondary endogenous danger signal (SDS):** a peptide that derives from a processed pro-protein and is secreted in the apoplast upon perception of a primary danger signal.
8. **CORONATINE-INSENSITIVE1 (COI1):** an F-box protein that is the receptor of JA-Ile and the major regulator of plant defense against insects.
9. **JASMONATE-ZIM DOMAIN (JAZ):** a family of repressors that inhibit transcription of defense genes, including bHLH MYC factors.
10. **WRKY transcription factors:** transcription factors involved in defense against insects. They target JA biosynthesis genes. Other target genes are still unknown.

11. **MYC transcription factors:** basic helix-loop-helix (bHLH) transcription factors. *Arabidopsis* MYC2,3,4,5 are target of JAZs and regulate transcription of defense genes.

12. **Feeding guild:** a group of unrelated insect species that have a similar feeding behavior, e.g. leaf chewer, phloem feeder, cell content feeder, leaf miner.

13. **Glucosinolates:** sulfated secondary metabolites that are essential for defense against insects in Brassicaceae.

14. **Benzoxazinoids**: indole-derived defense compounds in grasses that are effective against insects and plant pathogens.

15. **Effectors:** proteins or molecules that are released by an attacker to suppress plant defenses.

TABLES

Table 1 Examples of plant defense genes with a demonstrated role in plant-insect interactions.

FIGURE LEGENDs

Figure 1

Perception of known or putative ligands associated with insect attack. HAMPs from oral secretions of attacking insect herbivores are perceived by yet unknown plant cell-surface pattern recognition receptors (PRRs) and trigger downstream defenses against insects. Cell damage releases DAMPs consisting of cell wall fragments or intracellular metabolites that reach the apoplast. Herbivory also triggers the production and release of endogenous secondary danger signals like AtPeps and systemin that bind to PRRs and activate defense. Solid arrows indicate demonstrated effect on insect performance. Dashed arrows indicate absence of direct evidence for a role in defense against insects. Abbreviations: ATP, extracellular adenosine 5'-triphosphate; Cel, cellulose; DAMPs, danger-associated molecular patterns; DORN1, DOES NOT RESPOND TO NUCLEOTIDES 1; FACs, fatty-acid amino-acid conjugates; EGF, epidermal growth factor; FER, FERONIA; HAMPs, herbivore-associated molecular patterns; In, inceptin; PEPR1/2, LecRK1/2/3, lectin-like receptor 1, 2 and 3; Lec-RK-I.8, lectin-like receptor kinase I.8; LRR, leucine-rich repeat; NAD, extracellular nicotinamide adenine dinucleotide; OGs, oligogalacturonides; OS, oral secretions; Pect, pectin; Peps, At-PEP1-6; PEP-Receptor 1 and 2; RLK1, leucine-rich repeat receptor-like kinase 1; SDSs; secondary danger signals; SYR1, systemin receptor 1; THE1, THESEUS1; WAK1, Wall-associated kinase 1.

Figure 2

Current model of plant defense signaling network in response to herbivory. HAMPs from oral secretions, DAMPs from damaged cells or SDSs are perceived by PRRs at the plasma membrane. Membrane depolarization (V_m) (magenta), Ca^{2+} signaling (yellow), ROS signaling (green) and downstream MAPK signaling (orange) steps are activated and trigger the biosynthesis of JA-Ile, the bioactive form of JA. Chloroplast- and peroxisome-located enzymes (not shown) are rapidly activated to generate the primary JA burst through unknown mechanisms (dashed arrow). Binding of JA-Ile to the SCF^{COI1} complex leads to degradation of JAZ repressors, resulting in activation of transcription factors (emerald) that regulate the production of defense metabolites and proteins. H_2O_2 accumulation can be stimulated by Ca^{2+}-activated NADPH oxidases (RBOHD and F) or by glucose oxidase (GOX) in oral secretions. Several negative regulators have been identified and contribute to the fine-tuning of the JA pathway (red lines). Higher level of regulation by other hormonal pathways and plant secondary metabolites is not shown. For more information, see text. Abbreviations: CDPK, calcium-dependent protein kinase; CaM, calmodulin; CML, calmodulin-like proteins; DAMPs, damage-associated molecular patterns; EREB58, AP2/ERF; HAMPs, herbivore-associated molecular patterns; JAMs, bHLH3/13/14/17; JAV1, jasmonate-associated VQ motif 1; MAPK, mitogen-activated protein kinase MPK3/6, SIPK, WIPK; MYBs, MYB8/12/28/29/34/51/75/76/122; MYCs, MYC2/3/4/5; SDSs, secondary danger signals (e.g. systemin, AtPeps); WRKYs, WRKY3/6/18/40/53/70/72/89.

Figure 3

Examples of fascinating plant-insect interactions that await molecular characterization. (*a*) Members of the *Passiflora* genus develop egg-like structures (inset) that repel oviposition by *Heliconius* butterflies (*b*) A leaf mining insect drills tunnels underneath epidermis of a hazelnut leaf. (*c*) Gall from *Mikiola fagi* on a beach leaf. (*d*) Transverse cut of beach gall uncovers *M. fagi* larva (arrowhead). (*e*) Mutualistic interaction between bullhorn acacia (*Vachellia cornigera*) and *Pseudomyrmex ferruginea* ant. Ants feed from extrafloral nectar-producing structures (arrowhead) and live in hollowed-out thorns. Panels *a, b, c, d* from Zigmunds Orlovskis. Panel *e*, image number 476197198 from www.shutterstock.com.

SUPPLEMENTAL MATERIAL

Table S1 Plant genes with a demonstrated role in plant-insect interactions

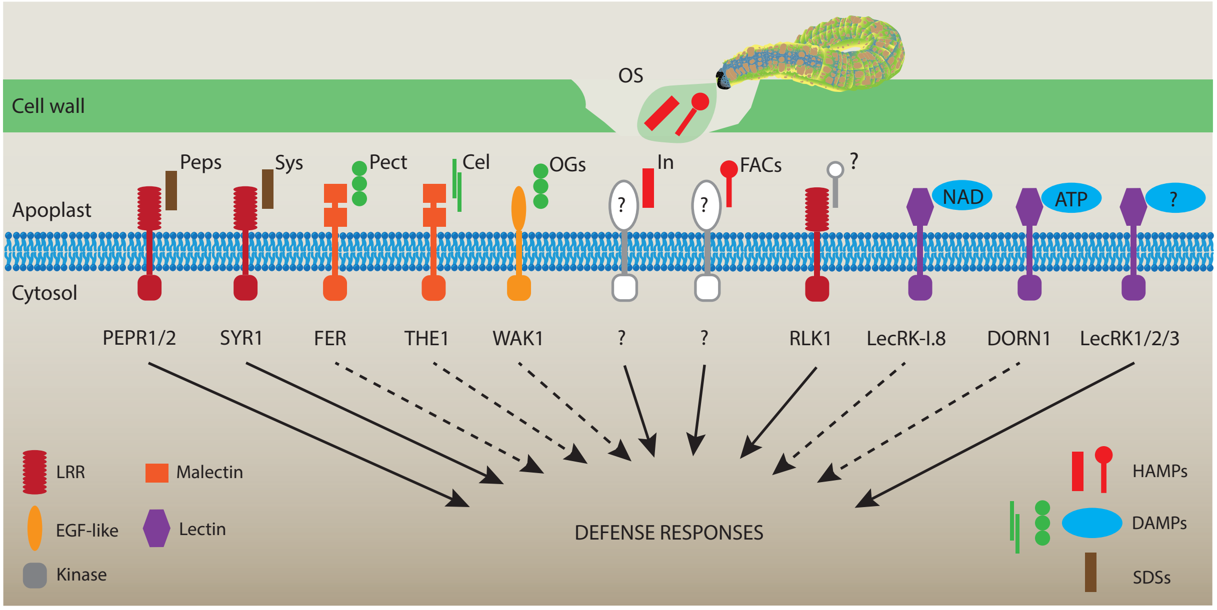
Table S2 Description of insect and nematode species mentioned in this review

Table 1 Examples of plant genes with a demonstrated role in plant-insect interaction

Gene	Description[a]	Plant	Genotype[b]	Insect performance on altered genotype[c]	Reference(s)
Perception					
LecRK1-3	Lectin-like RK	Rice, *N. attenuata*	OE *lecRK1-3,* ir-*lecRK1*	*N. lugens* ↓, *M. sexta* ↑	(43, 91)
LRR-RLK1	LRR-RK	Rice	ir-*LRR-RLK1*	*C. suppressalis* ↑	(59)
PEPR1/R2	LRR-RK, Pep receptor	*Arabidopsis*	*pepr1/2*	*S. littoralis* ↑	(74)
Prosystemin	Systemin precursor	Tomato	as-*prosystemin*	*M. sexta* ↑	(119)
SYR1	LRR-RK, systemin receptor	Tomato	*syr1*	*S. littoralis* ↑	(166)
Ca^{2+}, ROS, MAPK signaling					
TPC1	Vacuolar cation channel	*Arabidopsis*	*fou2*	*S. littoralis* ↓, *M. persicae* ↓	(83, 163)
JAV1	JA-associated VQ motif	*Arabidopsis*	RNAi *JAV1*	*S. exigua* ↓, *M. persicae* ↓	(61)
CML37/42	CaM-like protein	*Arabidopsis*	*cml37, cml42*	*S. littoralis* ↑, *S. littoralis* ↓	(140, 161)
CDPK4/5	Ca^{2+}-dependent protein kinase	*N. attenuata*	ir-*CDPK4/5*	*M. sexta* ↓	(176)
RBOHD/F	NADPH oxidase	*Arabidopsis,*	*rbohD/F*	*S. exigua* ↓, *M. persicae* ↑	(9, 109)
MPK1/2/3	MAPK	Tomato, rice	*mpk1/2,* ir-*MPK3*	*M. sexta* ↑, *C. suppressalis* ↑	(67, 167)
MPK4	MAPK	*N. attenuata*	ir-*MPK4*	*M. sexta* ↓	(51)
JA pathway					
LOX2/3/4/6	13-lipoxygenase	*Arabidopsis, rice*	*lox2/3/4/6,* as-*HI-LOX*	*S. littoralis* ↑, *N. lugens* ↓	(19, 184)
AOS	Allene oxide synthase	*Arabidopsis*	*aos*	*T. ni* ↑	(46)
AOC	Allene oxide cyclase	Rice, *N. attenuata*	*hebiba,* ir-*AOC*	*D. balteata* ↑, *M. sexta* ↑	(41, 94)
OPR3	OPDA reductase	*Arabidopsis*	*opr3-3*	*S. littoralis* ↑	(21)
ACX1	Acyl-CoA oxidase	*Arabidopsis*	*acx1/5*	*T. ni* ↑	(135)
JAR1	JA-Ile synthase	*Arabidopsis*	*jar1*	*T. ni* ↑	(46)
COI1	F-box protein	*Arabidopsis,* *N. attenuata*	*coi1-1*	*S. littoralis* ↑, *B. tabaci* ↑, *M. sexta* ↑	(10, 41, 179)
JAZ	Jasmonate ZIM domain	*Arabidopsis,*	*jaz*1/3/4/9/10	*T. ni* ↓	(105)
Transcription factors					
MYC2/3/4/5	bHLH TF	*Arabidopsis,*	*myc2/3/4, myc2/3/4/5*	*S. littoralis* ↑	(145, 152)
JAMs	bHLH TF	*Arabidopsis,*	*bhlh3/13/14/17*	*S. exigua* ↓	(153)
WRKY18/40	WRKY TF	*Arabidopsis*	*wrky18/40*	*S. littoralis* ↑	(144)
WRKY53	WRKY TF	Rice, wheat	ir-*WRKY53*	*C. suppressalis* ↓	(60)
WRKY70	WRKY TF	Rice, *Arabidopsis*	ir-*WRKY70, wrky70*	*N. lugens* ↓, *P. brassicae* ↓	(88, 117)
MYB28/29	MYB TF (aliphatic-GS)	*Arabidopsis*	*myb28/29*	*M. sexta* ↑, *S. exigua* ↑	(115)
MYB75	MYB TF (phenylpropanoids)	*Arabidopsis*	OE *MYB75,*	*P. brassicae* ↑	(118)
Defense regulators (hormones and secondary metabolites)					
ICS1	Isochorismate synthase (SA)	*Arabidopsis*	*sid2-1*	*S. littoralis* ↓, *P. brassicae* ↓	(10, 117)
NPR1	Non expressor of *PR-1* (SA)	*Arabidopsis*	*npr1*	*S. littoralis* ↓, *B. tabaci* ↓	(10, 179)
ACS2	Ethylene biosynthesis	Rice	as-*ACS2*	*C. suppressalis* ↑, *N. lugens* ↓	(93)
EIN2	Ethylene insensitive	*Arabidopsis*	*ein2-1*	*S. littoralis* ↓	(10)
EIN3/EIL1	TF, ethylene signaling	*Arabidopsis*	*ein3 eil1*	*S. exigua* ↓	(151)
ABA1	Zeaxanthin epoxidase	*Arabidopsis*	*aba1-1*	*M. persicae* ↓	(55)
ABA2	Xanthoxin dehydrogenase	*Arabidopsis*	*aba2-1*	*S. littoralis* ↑	(10)
HER1	Inhibitor of ABA catabolism	*N. attenuata*	ir-*HER1*	*M. sexta* ↑	(31)
CYP714D1	CYP450 (GA catabolism)	Rice	*eui*	*N. lugens* ↓	(88)
BX1	Indole-3-glycerol P lyase	Maize	*bx1*	*R. padi* ↑	(3)
Direct defense traits					
CYP79B2/B3	CYP450 (indole-GS)	*Arabidopsis*	*cyp79b2/b3*	*S. exigua* ↑	(115)
TGG1/2	Myrosinase (GS activation)	*Arabidopsis*	*tgg1/2*	*M. sexta* ↑, *T. ni* ↑	(5)
CYP81F2	CYP450 (indole-GS)	*Arabidopsis*	*cyp81F2*	*M. persicae* ↑	(124)
PAD3	Camalexin biosynthesis	*Arabidopsis*	*pad3-1*	*B. brassicae* ↑	(81)
F5H	Ferulate hydroxylase	*Arabidopsis*	*fah1-7*	*P. brassicae* ↑	(117)
BX1/13	Benzoxazinoid biosynthesis	Maize	*bx1*, *bx13*	*S. littoralis* ↑, *R. maidis* ↑	(50, 98)
PMT	Nicotine biosynthesis	*N. attenuata*	ir-*PMT*	*M. sexta* ↑	(155)
TPS08	Terpene synthase	*Arabidopsis*	*tps08-1*	*Bradysia* sp. ↑	(162)
ShZIS	Sesquiterpene synthase	Tomato	OE *ShZIS*	*M. sexta* ↓	(8)
GAS1	Germacrene synthase	*Taraxacum officinale*	RNAi *GAS1*	*M. melolontha* ↑	(62)
PIN I/II	Proteinase inhibitors	Tobacco	OE *PIN I/II*	*M. sexta* ↓	(66)
Mir1-CP	Cysteine protease	Maize	OE *Mir1-CP*	*S. frugiperda* ↓	(122)
TD	Threonine deaminase	Tomato	as-*TD2,*	*S. exigua* ↑, *T. ni* ↑	(45)
ARG2	Arginase	Tomato	OE *ARG2*	*M. sexta* ↓	(20)
PP2-A1	Phloem protein	*Arabidopsis*	OE *PP2-A1*	*M. persicae* ↓	(180)
SLI1	Chaperone	*Arabidopsis*	*sli1-1*	*M. persicae* ↑	(75)
GL1	Trichome formation	*Arabidopsis*	*gl1*	*S. littoralis* ↑	(128)
ATL2	Wax and cutin formation	*Arabidopsis*	*eca*	*S. littoralis* ↓	(7)
Putative indirect defense traits					
HPL	Hydroperoxide lyase	*N. attenuata*	as-*HPL*	*G. punctipes* ↓↓	(49)
TPS10	Terpene synthase	*Arabidopsis*	OE *TPS10*	*C. marginiventris* ↑↑	(139)
TPS23	(*E*)-β-caryophyllene synthase	Maize	OE *E–βC*	*H. megidis* ↑↑, *D. virgifera* ↓	(29)
LIS	*S*-linalool synthase	Rice	ir-*LIS*	*N. lugens* ↑, *A. nilaparvatae* ↓↓	(173)
Others					
ZTL	Zeitlupe, clock component	*N. attenuata*	ir-*ZTL*	*S. littoralis* ↑	(86)
BPH6	Unknown, binds EXO70E1	Rice	*Bph6*-carrying lines	*N. lugens* ↓, *S. furcifera* ↓	(48)

[a]Abbreviations: CaM, calmodulin; LRR, leucine-rich-repeat; MAPK, mitogen-activated protein kinase; NB-LRR, nucleotide binding leucine-rich repeat; PRR; pattern recognition receptor; RK, receptor kinase; RLCK, receptor-like cytoplasmic kinase; TF, transcription factor. [b]Abbreviations: as, antisense line; OE, overexpression line; ir, inverted repeats line; RNAi, RNA interference line; VIGS, virus-induced gene silencing. [c]↑, enhanced insect

performance; ↓, reduced insect performance; ↑↑, enhanced attraction of natural enemy; ↓↓, reduced attraction of natural enemy; for a more complete list of genes and a description of insect and nematode species, see **Table S1 and Table S2**.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Cell wall
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OGs
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FACs
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NAD
ATP
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Apoplast
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PEPR1/2
SYR1
FER
THE1
WAK1
?
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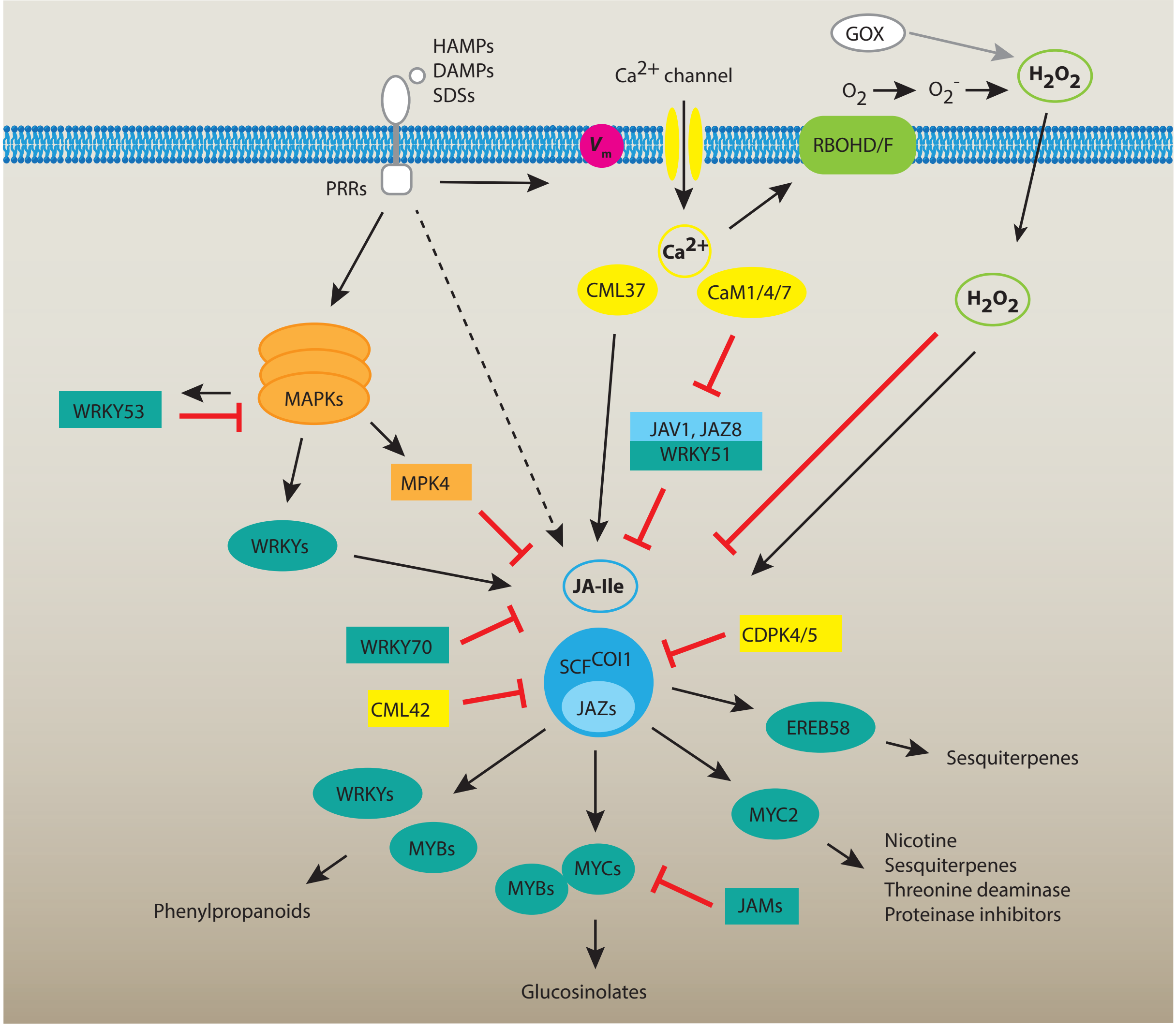
RLK1
LecRK-I.8
DORN1
LecRK1/2/3
LRR
Malectin
EGF-like
Lectin
Kinase
DEFENSE RESPONSES
HAMPs
DAMPs
SDSs

Figure 1



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HAMPs
DAMPs
SDSs
Ca^{2+} channel
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O_2^-
V_m
RBOHD/F
PRRs
Ca^{2+}
CML37
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MPK4
JAV1, JAZ8
WRKY51
WRKYs
JA-Ile
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JAZs
CML42
EREB58
Sesquiterpenes
WRKYs
MYBs
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Proteinase inhibitors
Phenylpropanoids
Glucosinolates

Figure 2

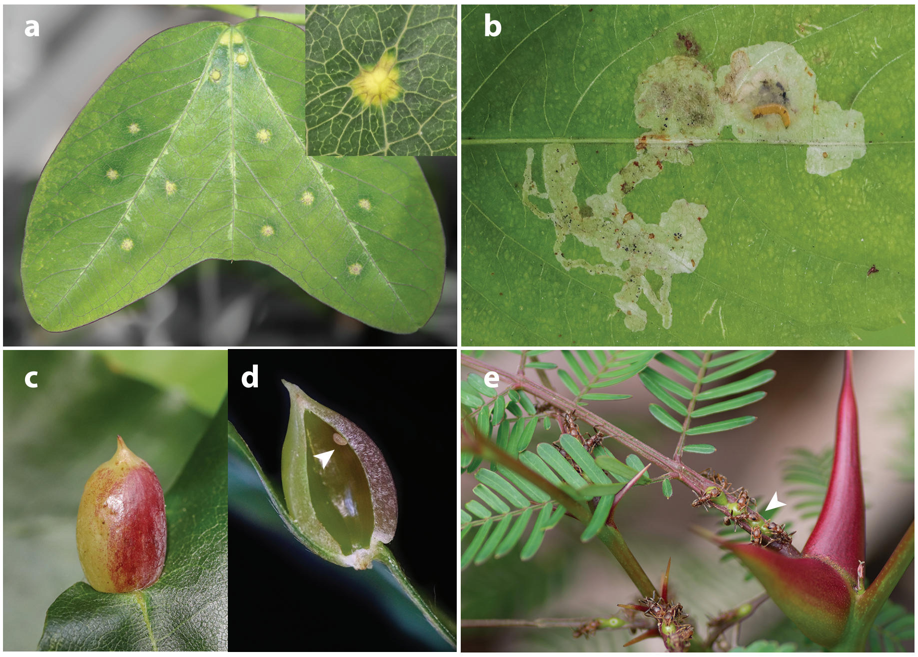

Figure 3