Plant–insect interactions

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Recent research shows partially overlapping signal transduction pathways controlling responses to wounding, insects, and pathogens. Chemical and behavioral assays show that plants release herbivore-specific volatiles, and that parasitic wasps can distinguish between these emission patterns. QTL mapping and candidate gene studies are beginning to identify polymorphic resistance genes, and ecological analyses provide information on the physiological and fitness costs of resistance. Such multidisciplinary approaches can elucidate the physiological causes and ecological consequences of plant–herbivore interactions.

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Abbreviations

HR	hypersensitive response
LRR	leucine rich repeat
MAPK	mitogen-activated protein kinase
NBS	nucleotide binding site
PI	proteinase inhibitor
QTL	quantitative trait loci
WIPK	wound-induced protein kinase

Introduction

Plants and insects have coexisted for at least 100 million years, and have evolved a variety of beneficial and deleterious interactions. Pollination benefits both partners, with insects cross-fertilizing plants in the process of collecting nectar. Carnivorous plants trap insects in specialized organs, obtaining additional nitrogen and phosphorus in nutrient-poor environments. More often, however, plants are damaged by herbivorous insects. Mechanisms of defense against herbivores and microbial pathogens have been evolving for millions of years, and are, therefore, shared across many plant families [1]. This review focuses on recent progress in understanding plant defenses against insect attack.

Analysis of quantitative variation

Unlike the dichotomous patterns of resistance versus susceptibility that have been the foundation of recent advances in plant–pathogen interactions [2], resistance to insects is often continuously variable and controlled by segregation of quantitative trait loci (QTLs). Lee *et al.* [3•] mapped QTL controlling resistance to corn earworm (*Helicoverpa zea*) in maize, as well as levels of maysin and apimaysin, two flavonoid compounds that are implicated in antibiosis to corn earworm. They identified one QTL influencing insect antibiosis that was unrelated to flavonoid levels, as well as maysin and apimaysin QTLs tightly linked to insect resistance. However, they conclude that other compounds not measured in their study may actually be responsible for resistance to corn earworm. Clearly, greater understanding of secondary metabolism is required. This combined approach to mapping insect resistance QTLs and their physiological components is one of the fundamental tools that can elucidate the genetic basis of resistance to insects.

Glucosinolates are biologically active secondary metabolites that influence insect egg laying (oviposition) and feeding [4]. When plant tissue is damaged, they are hydrolyzed by myrosinase enzymes to nitriles, thiocyanates, and isothiocyanates. Glucosinolate and myrosinase levels are genetically variable in *Brassica* and *Arabidopsis*. Genetic manipulation of glucosinolate levels in *Brassica* cause changes in flea beetle herbivory in accordance with *a priori* predictions based on insect physiology [5]. QTLs controlling glucosinolates and myrosinase activity have been mapped in *Arabidopsis* [6,7•], and this will permit analysis of genetic causes and ecological consequences of this defense-related trait in a genetically tractable plant species.

Resistance genes

Hypersensitive cell death is a common resistance mechanism against microbial pathogens. Plants express resistance genes that trigger hypersensitive responses (HR) through their specific interactions with pathogen avirulence genes. In plant–insect interactions, this form of resistance has received less attention. However, there are reports of HR in *Brassica* following oviposition by *Pieris* butterflies [8] and of HR-based resistance against galling insects on a legume species [9]. Identification of HR against insects in several plant families suggests that such insect resistance mechanisms might be taxonomically widespread.

The first identification of a resistance/recognition gene that affects insects was fortuitous. The *Mi* gene of tomato was isolated due to its effect on root knot nematodes [10], but also confers resistance against the potato aphid, *Macrosiphum euphorbiae*. Both organisms penetrate plant tissue intercellularly and establish a feeding site in vascular tissue [11••]. The Mi protein belongs to the nucleotide binding site – leucine rich repeat (NBS–LRR) family of resistance genes and is located in the cytoplasm. The Mi protein could come into contact with signal molecules from aphids or nematodes upon cellular penetration. As aphids transmit viruses and also contain symbiotic bacteria, it is possible that this signal could be of microbial origin. It remains to be seen whether plants are able to specifically recognize chewing insects.

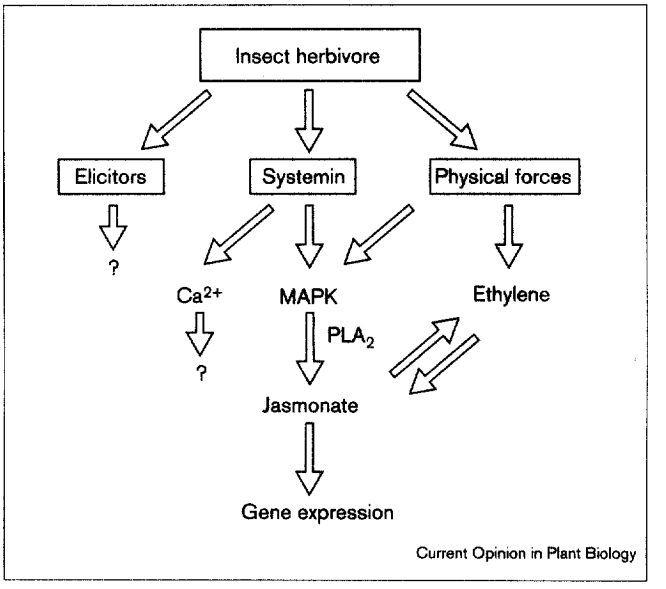
Proteinase inhibitors

Plant proteinase inhibitors (PIs) are small proteins that contribute to defense against insects. They inhibit digestive

proteinases, causing insect growth retardation and death from starvation. Therefore, much effort has been directed to genetic manipulation of PI genes to enhance insect resistance of crop plants. Some insects, however, can overcome this defense by increasing proteolytic activity [12], by inducing different proteolytic enzymes that are insensitive to the corresponding plant PIs [13], or by expressing proteinases that degrade PIs [14].

In addition, some insect populations are genetically variable for tolerance to plant PIs. For instance, when populations of the weevil *Ceutorynchus assimilis* were collected from two oilseed rape fields in France and reared on transgenic oilseed rape expressing a rice cysteine PI, one insect strain showed an increased growth rate whereas the other remained unaffected [15]. Thus, insects may rapidly evolve tolerance to transgenic PIs, even from novel sources that they have not previously encountered.

Figure 1



Insect-related signal transduction in plants. Chemical and physical cues relay information to the plant. Physical signals, such as hydraulic forces, may be primary in nature and endogenous chemical signals of plants, such as systemin, may be secondary. PLA_2 is assumed to mediate MAPK signaling leading to jasmonate synthesis. Besides jasmonate, insect elicitors and Ca^{2+} affect plant gene expression.

Can plants discriminate between a wound and a bite?

Systemin is a chemical messenger implicated in wound-signal transduction in tomato and related species [16]. It elicits a lipid-based signaling cascade involving jasmonate production via the octadecanoid pathway, leading to systemic defense against insect herbivores [17]. Early responses to systemin in tomato cells involve ion fluxes across the plasma membrane, a transient increase in cytoplasmic free calcium [18], and induction of mitogen-activated protein kinase (MAPK) activity [19]. It has recently been shown in tobacco that a wound-induced protein kinase (WIPK) is activated in response to wounding, leading to jasmonate-dependent expression of proteinase inhibitor II [20•]. It is possible that wound-related MAPKs activate the octadecanoid pathway via phospholipase A_2 (PLA_2), as in animal cells [19]. Indomethacin, an inhibitor of PLA_2, reduces expression of several jasmonate-induced genes upon wounding, but not their induction by jasmonate. Other wound-responsive genes are not repressed, providing further evidence for the existence of separate jasmonate-dependent and independent wound signal transduction pathways [21]. Apparently, protein phosphatases are also involved in these pathways [22]. Additional phytohormones also mediate wound responses: salicylic acid acts antagonistically to jasmonates [23,24], whereas ethylene has synergistic effects [25,26].

Insect herbivory inevitably involves wounding of the host plant. It is not surprising, therefore, that plants initiate wound-response pathways when challenged with insects. For instance, feeding of the tobacco hornworm, *Manduca sexta*, stimulates MAPK systemically within three minutes in tomato [19]. In addition, certain mutants in the octadecanoid pathway are more susceptible to insect herbivores and fungal pathogens than wild-type [27,28,29•]. Jasmonate, however, has multiple pleiotropic effects on plants [30], and is unlikely to provide general resistance against all insects. Also, some results suggest that plant signal transduction pathways respond differently to insects than to mechanical stresses. Volatile patterns released upon herbivory and mechanical wounding exhibit significant differences, both quantitatively and qualitatively [31,32,33••]. In addition, wound-related gene expression in potato is activated more rapidly by insect herbivores than by mechanical stimuli, and this acceleration is also evoked by heat-stable regurgitant fluids from *Manduca sexta* [34]. Further work is required to dissect the signaling pathways controlling responses to wounding, insects, and pathogens.

Tritrophic interactions: plants, herbivores, and parasitoids

In response to insect damage, plants emit a blend of volatile compounds, which are released from storage or synthesized *de novo* [32,35]. These compounds attract insect parasitoids, which attack herbivorous insects. As this reduces feeding on the plant, volatiles may be regarded as a component of an indirect defense system in plants. An elicitor of volatile production, volicitin or N-(17-hydroxylinolenoyl)-L-glutamine, has been isolated from oral secretions of beet armyworm [31]. When volicitin is applied to damaged maize leaves, it triggers plant emission of parasitoid-attracting volatiles. The fatty acid portion of this insect 'spit factor' is plant derived, and is subsequently modified in the insect gut, leading to induced volatile emission and attraction of insect parasitoids which may defend the plant [36•].

Recent research demonstrates that damaged plants emit different blends of volatile compounds in an herbivore-specific manner, and that parasitic wasps can distinguish between these volatile blends [33••]. Females of the parasitic wasp

Figure 2

Diamondback moth (*Plutella xylostella*) larvae feeding on *Arabidopsis thaliana*. This insect species attacks *Arabidopsis* in the field (T Mitchell-Olds, unpublished data), and is convenient to raise in the laboratory. It consumes *Arabidopsis* in a quantifiable and repeatable fashion, and discriminates among different plant genotypes [1]. Studies of insect resistance in genetically tractable model plant species should increase our understanding of the mechanisms of insect resistance.

Cardiochiles nigriceps significantly prefer tobacco or cotton plants infested by their host, *Heliothis virescens*, over those attacked by the closely related species *Helicoverpa zea* or over control plants, even when all damaged leaves were removed to exclude possible cues associated with the larvae themselves [33••]. Volatile signaling in these plant–herbivore–parasitoid interactions will yield exciting functional information on physiology and molecular biology, as well as ecological understanding of interactions among three trophic levels. Exploiting indirect defenses for agricultural purposes has a potential role in integrated pest management, but will require greater understanding of complex agroecosystems.

Ecology

Studies of resistance to insects in natural plant populations often reveal genetic variation for resistance [37]. What evolutionary factors can maintain widespread genetic variation for a presumably advantageous trait? Fitness costs of physiological investments in plant defense could maintain this heritable variation. In addition, inducible defenses may protect against attack by insect herbivores while avoiding physiological costs of defense when herbivores are absent. To test these hypotheses, Baldwin [38•] compared fitness of *Nicotiana attenuata* when herbivores were present or absent, and with defensive secondary compounds at basal or elevated levels. The wound-related plant hormone methyl jasmonate was used to induce plant defenses. Nicotine, an energetically expensive secondary compound with insect toxicity, was used to monitor the plant response. In populations with intermediate levels of herbivory, induced plants experienced less insect damage and had higher fitness than uninduced controls. In low herbivory treatments, however, induced plants had lower fitness than their uninduced counterparts. These results indicate that jasmonate-induced responses are favored when herbivory is high. Induced responses to jasmonates are costly, however, and reduce plant fitness when herbivory is low. Similarly, Mauricio [39] examined quantitative genetic variation in *Arabidopsis* populations growing in the field, and found that high trichome density is associated with greater resistance when herbivory levels are high, but low trichome density is favored when herbivores are excluded. This suggests that trichome production is expensive, and the benefit of this mechanical defense depends upon levels of herbivory.

Although physiological [38•] and quantitative genetic [39,40] approaches can offer suggestive evidence regarding costs of plant defense, they cannot prove that fitness costs actually arise from defense mechanisms. Jasmonates have extensive pleiotropic effects on growth and metabolism [41], so it is possible that defense against herbivores is cost-free, and costs result from other, pleiotropically induced aspects of plant growth and development. Likewise, genetically correlated traits may arise from spurious effects of linkage disequilibrium, especially when inbred *Arabidopsis* genotypes are combined from several populations [39]. Manipulative experiments with mutants [28], transgenic plants [42], or fine-scale mapping [7•] are required to assess costs and fitness consequences of plant defense.

Conclusions

To understand plant–insect interactions in natural and agricultural ecosystems, we need more information on insect physiology, genomics, and population genetics. This information will be especially important for efforts to reduce the rate at which herbivorous insects evolve tolerance to natural and transgenic mechanisms of plant defenses. Studies of digestive [43,44] and sensory physiology can suggest candidate genes that influence levels of insect damage. Entomologists should take advantage of genetic tools available in *Drosophila* (e.g., [45•,46•,47,48]), where odorant

receptor genes have recently been identified [49•,50•]. Although EST sequencing has been a productive and cost effective tool for functional genomics of several plant species, this approach has received little attention in studies of insect herbivores. Sequencing of the *Drosophila* genome may help promote dipteran pests as model genetic systems for understanding oviposition and feeding preferences.

In the past decade our understanding of the functional basis of plant defense against microbial pathogens has increased dramatically. These advances have benefited from progress in molecular biology and genetics, and from widespread use of model plant and pathogen species. In contrast, systematic molecular genetic analyses of plant defense against insect herbivores have lagged behind. Studies of plant–insect interactions are hindered by the inherent complexity of interactions between insects and plants. For example, in addition to plant chemical defenses that influence insect feeding, plant resistance is also determined by insect host choice and by predators and parasites that attack herbivorous insects. Furthermore, few studies of insect resistance have employed genetically tractable plant species, which allow identification of any mutant gene causing measurable phenotypic effects. Many aspects of pest recognition and signal transduction are conserved in a broad range of plant species, so results from model systems may be extended to plants of agricultural and ecological importance [1]. Genomics in model plant species should greatly increase understanding of the functional basis of insect resistance in the near future.

Genome-wide expression studies with model plant species will identify candidate genes potentially responsible for induced plant defences against insects. Putative resistance genes can be characterized by analyzing the phenotypic effect of knock-outs in T-DNA and transposon insertion lines. Comparative genomics with related plant species will help understand naturally occuring genetic variation for defense physiology and insect resistance.

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