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Disarming the defenses: Insect detoxification of plant defense-related specialized metabolites

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Abstract

The ability of certain insects to feed on plants containing toxic specialized metabolites may be attributed to detoxification enzymes. Representatives of a few large families of detoxification enzymes are widespread in insect herbivores acting to functionalize toxins and conjugate them with polar substituents to decrease toxicity, increase water solubility and enhance excretion. Insects have also developed specific enzymes for coping with toxins that are activated upon plant damage. Another source of detoxification potential in insects lies in their microbiomes, which are being increasingly recognized for their role in processing plant toxins. The evolution of insect detoxification systems to resist toxic specialized metabolites in plants may in turn have selected for the great diversity of such metabolites found in nature.

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Plant specialized metabolites, Insect herbivores, Detoxification, Insect microbiome.

Introduction

Plant specialized metabolites are often described as toxins and deterrents to insect herbivores, yet the literature is full of examples of insects feeding on plants rich in specialized metabolites without suffering ill effects. These insects are thought to employ various mechanisms to resist the effects of plant toxins, such as behavioral avoidance, rapid excretion, sequestration, detoxification and target site insensitivity [1]. The most widely studied of these mechanisms is detoxification, but we still know little about its importance in insect resistance to plant specialized metabolites relative to other mechanisms. In fact, in most cases we do not even know if insect metabolism of plant specialized compounds represents real detoxification, since the relative toxicity of plant-produced compounds and their insect metabolites have seldom been compared. In this review, we survey recent literature on several topics including the major types of detoxification reactions employed by insect herbivores for plant specialized metabolites, how activated plant defenses are detoxified, and the role of microbes in insect detoxification processes.

General detoxification reactions of insect herbivores (Box 1)

Detoxification reactions in mammals are frequently divided into Phase I (functionalization), Phase II (conjugation) and Phase III (excretion) processes, a classification that is also useful for insect herbivores. Phase I includes oxidation, reduction and hydrolysis of specialized metabolites to yield more polar derivatives, which usually reduces toxicity and facilitates eventual excretion. The best studied Phase I enzymes are the cytochrome P450s, an enzyme superfamily present in all kingdoms of life [2]. The typical reaction of this very large family of catalysts involves the hydroxylation of a lipophilic substrate (Figure 1a), but many other oxidations are also well known. A large body of work on the role of cytochrome P450s from Lepidoptera in the detoxification of furanocoumarins of the Apiaceae has been carried out over the years. Remarkably, these enzymes appear to vary in their degree of substrate specificity according to the diet breadth of the insect producing them [3,4]. Many genes encoding P450s have been shown to be induced by plant specialized metabolites in the diet. Such results help implicate the corresponding enzymes in detoxification processes and also show that their continued presence in the insect is likely to be costly.

Phase II detoxification enzymes conjugate plant specialized metabolites or their Phase I products to polar groups, such as sugar, phosphate, sulfate, malonate, amino acid or glutathione moieties, to form products that are less toxic and more easily excreted. Among the best-known Phase II enzymes are the UDP-glycosyltransferases (UGTs), which catalyze the

Box 1. Major detoxification enzymes in herbivorous insects.

Detoxification phase	Enzyme family	Reactions catalyzed	Plant specialized metabolites used as substrates	Size of gene family[1]
Phase I (Functionalization)	Cytochrome P450s (P450s) [2]	Oxidize lipophilic substrates via hydroxylation, epoxidation, dealkylation and rearrangement reactions	Many terpenes [e.g. 5,6], phenolics [e.g. 7,8] and alkaloids	92–130
	Carboxyl/ Cholinesterases (CCEs) [9]	Hydrolyze ester or amide bonds; well known for metabolizing organophosphate and pyrethroid pesticides	Salicinoids [10,11], aliphatic esters [12]	46–115
Phase II (Conjugation)	UDP-glycosyl-transferases (UGTs) [13,14]	Catalyze the conjugation of a sugar moiety to a broad range of metabolites	Terpenes [15], phenolics, benzoxazinoids [16], fatty acid amides (capsaicin)	26–80
	Glutathione *S*-transferases (GSTs) [17]	Conjugate the tripeptide glutathione to electrophilic sites on usually lipophilic acceptors, such as toxic glucosinolate hydrolysis products	Isothiocyanates from glucosinolates	26–46
Phase III (Excretion)	ABC transporters [18]	Actively transport plant toxins and other ligands across cell membranes (not covered in this review)		63–144

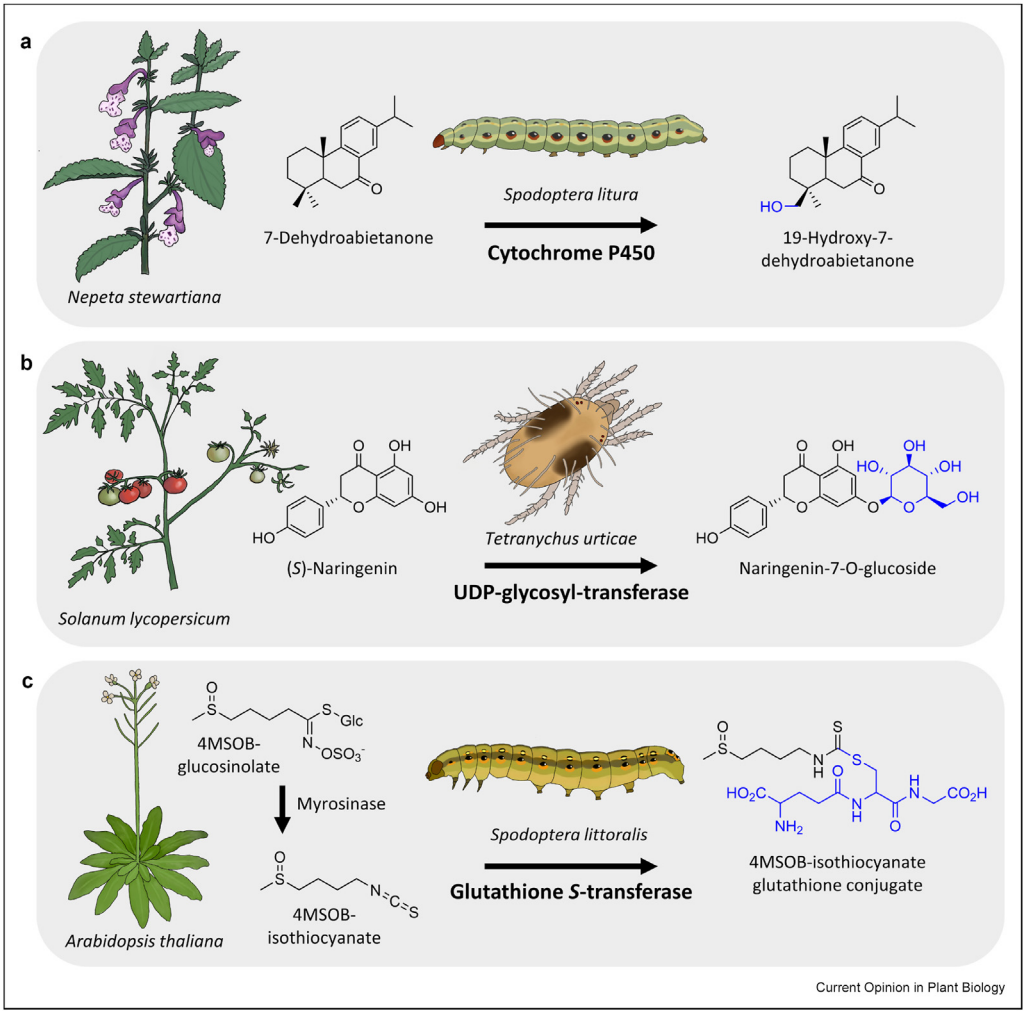
[1] Numbers refer to the range in size of gene families in four well-studied species of insect herbivores: *Bemisia tabaci* (Condylognatha, generalist), *Spodoptera litura* (Lepidoptera, generalist), *Plutella xylostella* (Lepidoptera, specialist), *Dendroctonus ponderosae* (Coleoptera, specialist). Data from Ref. [19].

formation of a glycosidic linkage between a sugar moiety and a broad range of lipophilic specialized metabolites in insects and other arthropods [13,14]. A UGT from the non-insect arthropod, the two-spotted spider mite (*Tetranychus urticae*), has been found to glucosylate a range of flavonoids [20] (Figure 1b). Analysis of the crystal structures of this enzyme complexed to different tomato flavonoids demonstrated a highly plastic and open-ended binding site for the sugar acceptor. The two-spotted spider mite possesses 80 genes encoding UGTs, which may be responsible for its ability to feed on over 150 different crops. Tomato flavonols that are already glucosylated are targets for an additional type of Phase II enzyme, one that transfers malonate residues in the whitefly *Bemisia tabaci* [22]. Malonyl transfer reduces the toxicity of these flavonol glucosides to the whitefly while increasing their polarity and ease of excretion. Interestingly, the whitefly gene for this malonyl-transferase, which is also active against a range of other phenolic glycosides, was acquired by horizontal gene transfer from a plant.

An enzyme family that may detoxify plant specialized metabolites by phosphorylation has recently been discovered by phylogenetic analyses of genes encoding a set of kinases, some of which are known to be active on the insect molting hormones, the ecdysteroids [23]. Genes encoding these enzymes, known as ecdysteroid-like kinases, are found in many insect genomes with increased abundance correlated with the chemical complexity of the diet. While no detoxification enzymes have yet been characterized from this family, the phosphorylated salicinoids reported from the spongy moth (*Lymantria dispar*) feeding on poplar [10] and the phosphorylated cyanogenic glycosides reported from the honeydew of *B. tabaci* feeding on cassava [24] could conceivably arise from kinases in this enzyme family.

Whether detoxification enzymes are the major mechanism by which insect herbivores resist plant specialized metabolites is not yet known. However, it is clear that detoxification enzymes are much more widespread in insect herbivores than once thought. The vast increase of available insect genomic sequences indicates that families of detoxification genes, including those encoding cytochrome P450s, UDP-glycosyltransferases, carboxyl/cholinesterases and glutathione-*S*-transferases, are among the largest gene families present, with as many as 200 genes (Box 1), even if very few individual members have been functionally

Figure 1



Examples of detoxification reactions in herbivorous arthropods. (**a**) The abietane diterpene 7-dehydroabietanone from the catmint *Nepeta stewartiana* is oxidized into a less toxic product via hydroxylation by a cytochrome P450 from the tobacco cutworm (*Spodoptera litura*) [5]. (**b**) The tomato (*Solanum lycopersicon*) flavonoid (*S*)-naringenin is glucosylated by the two-spotted spider mite (*Tetranychus urticae*) by a UDP-glycosyltransferase [20]. (**c**) *Arabidopsis thaliana* produces 4-MSOB (4-methylsulfinylbutyl) glucosinolate, a non-toxic compound that is converted into toxic 4-MSOB isothiocyanate after herbivory. Herbivory disrupts the spatial separation of glucosinolates and the plant myrosinases, thereby activating toxic defenses. The African cotton leafworm (*Spodoptera littoralis*) conjugates glutathione to 4-MSOB isothiocyanate via a glutathione-*S*-transferase (GST), and further hydrolysis via the mercapturic acid pathway leads to an *N*-acetylcysteine derivative of the isothiocyanate [21].

characterized. Detoxification genes are abundant in all major orders of herbivorous insects [19], in pollinators [25,26], and even in the sensory organs of insects to protect receptors from toxins [27]. They are present in both generalist and specialist feeders [28–30], although they are found in larger amounts in generalists than specialists [31,32]. Detoxification gene families are not only large, but the encoded enzymes frequently exhibit broad substrate specificity. Evidence for this comes not only from surveys of plant toxins, but also from the abilities of many detoxification enzymes to metabolize synthetic insecticides to which they have never been previously exposed [33–35].

Detoxifying activated defenses

Certain plant specialized metabolites are not directly toxic to insect herbivores but are stored as glycosides to avoid autotoxicity to the plant itself. After herbivore damage, the glycoside protoxins are hydrolyzed by specific glycohydrolases to form active toxins and deterrents. The list of such activated or two-component defenses includes several classes of plant specialized metabolites, including benzoxazinoids, glucosinolates, cyanogenic glycosides and iridoid glycosides.

Activated defenses present special challenges for detoxification systems. In the case of glucosinolates of

the Brassicaceae, some insects metabolize the glucosylated protoxin first before the glucohydrolases (myrosinases) can hydrolyze it to toxic products, such as isothiocyanates. For example, caterpillars of the diamondback moth, *Plutella xylostella*, employ glucosinolate sulfatases (GSSs) to catalyze the desulfation of glucosinolates to products that can no longer be converted to isothiocyanates [36,37]. To determine if this process works as a genuine detoxification, silencing of the diamondback moth GSS was carried out [38,39], which resulted in a many-fold increase in isothiocyanates and a consequent decrease in insect growth, survival and reproduction, thus confirming the value of this detoxification process. The success of desulfation may be partially due to the expression of the gene and encoded protein not only in the diamondback moth caterpillar gut, but also in its salivary glands [40,41] so that the metabolism of glucosinolates starts promptly when feeding is initiated. The diamondback moth possesses three GSSs, each specific for different types of glucosinolates [42]. GSSs are known from other insects that specialize on glucosinolate-containing plants of the Brassicaceae [43–47], but these have been independently recruited from arylsulfatase-like genes in each lineage [45–47].

Other insect herbivores take a very different approach to circumventing activated defenses: they first allow protoxin cleavage to take place and then deactivate the toxic hydrolysis products formed. The best known of these mechanisms avoids glucosinolate toxicity by converting the toxic isothiocyanate hydrolysis products to conjugates with the tripeptide glutathione [48] (Figure 1c). This process is carried out by glutathione-*S*-transferases (GSTs), another large family of Phase II detoxification enzymes, which catalyzes the conjugation of glutathione to electrophilic sites on molecules, such as isothiocyanates, creating water-soluble derivatives [17]. Following initial conjugation of glutathione, the glutamate and glycine moieties are usually recovered via the mercapturic acid pathway leaving an *N*-acetylcysteine derivative of the original isothiocyanate. Glutathione transfer can be a costly mechanism of detoxification. When caterpillars of the generalist-feeding lepidopteran *Spodoptera littoralis* were fed for ten days on diets containing isothiocyanates at a concentration naturally found in damaged Arabidopsis foliage, there was up to a 90% decline in glutathione content and a 50% decline in the level of the glutathione precursor cysteine [21]. The result was a significant decrease in protein content and body weight, indicating the long-term risks of glutathione-mediated detoxification. Moreover, this process exposes the insect to the toxic isothiocyanates prior to conjugation with glutathione.

Benzenic and indolic glucosinolates have been recently reported to be transformed to amino acid conjugates by various species of beetles, likely after degradation to isothiocyanates [49,50]. These may also represent detoxification reactions since the lipophilic isothiocyanates end up becoming conjugated to a polar moiety, which should decrease their toxicity and increase their solubility for excretion. Flea beetles and certain flies can also convert isothiocyanates to their corresponding amines [51,52], a reaction originally identified in microbes [53] and more recently in microbial symbionts of an insect [54]. In addition to representing a detoxification product, the amine may also provide insects with a readily available source of nitrogen.

Among phloem-feeding insects, some are known to trigger activated defenses [27], which then require detoxification. For example, cyanogenic glycosides are subject to conjugation with an additional glucose residue, which prevents their hydrolysis to release toxic hydrogen cyanide [24]. If hydrogen cyanide is formed, some insects, such as *B. tabaci*, are able to detoxify this respiratory poison by converting it to β-cyanoalanine [24]. A β-cyanoalanine synthase has recently been described from experimental populations of the non-insect arthropod, *T. urticae*, that are adapted to feed on Arabidopsis [55]. This enzyme is likely deployed to detoxify hydrogen cyanide formed upon indolic glucosinolate hydrolysis.

Thus, as described above, a number of insect herbivores have developed strategies to metabolize activated defenses to minimize the release or persistence of toxic hydrolysis products. These mechanisms differ extensively depending on the herbivore and compound class under consideration, suggesting they have arisen as a result of independent evolutionary events. Additional study of the genes and enzymes involved and their experimental manipulation will shed more light on the evolution of these herbivore strategies as well as their costs and benefits (Box 2).

Microbiome-mediated detoxification

Much of the recent work on insect detoxification of plant specialized metabolites has focused on the potential involvement of the insect microbiome. A well-demonstrated example of this process is the detoxification of caffeine by the coffee berry borer (*Hypothenemus hampei*) [59]. This beetle species lives its entire life cycle in alkaloid-rich coffee beans, but releases no free caffeine in its feces. However, after feeding on an antibiotic-amended diet, these insects had similar levels of caffeine in their feces as found in coffee beans themselves, indicating that the gut microbiome may be involved in a detoxification process. Gut bacteria that could survive on caffeine as a sole carbon and nitrogen source were then cultured, and one isolate (*Pseudomonas fulva*) was found to possess a caffeine demethylase gene. When antibiotic-treated insects were inoculated with *P. fulva*, they regained their capacity to degrade caffeine [59].

Box 2. Demonstrating detoxification in herbivorous insects: key experimental components[1].

1.	Chemical analysis	Precise identification of the chemical structures of the specialized plant metabolites involved and their insect transformation products formed is essential. Transformation products can be extracted from fecal or gut contents with purification often required before spectral identification is possible. Untargeted metabolomics can be useful in selecting candidate insect transformation products correlated with ingestion of a particular plant specialized metabolite [49,50]. Quantification of both plant precursors and insect products is necessary to know which routes of processing are most important in the insect. Knowledge of the chemical structures of insect transformation products gives initial insight into the pathways and enzymes that may participate in detoxification.
2.	Toxicity tests	To determine whether insect metabolism constitutes a detoxification process, it is crucial to evaluate the toxicity of both the plant metabolite and the insect product. Insect survivorship, growth rate, or other performance markers can be used for comparing the biological activity of plant and insect compounds [56,6].
3.	Enzyme and gene characterization	Detoxification pathways can be determined through the identification and characterization of the genes and enzymes involved. For more complex pathways, feeding of isotopically-labeled precursors may also be necessary to elucidate the intermediates [57]. Heterologous expression of enzymes allows determination of their properties, such as substrate specificity [3]. Analysis of gene expression gives insights into the regulation of detoxification [46], while gene sequences can be used in phylogenetic analyses to explore the evolutionary origin of the process [42,52].
4.	Genetic manipulation of detoxification	Knocking down or silencing candidate genes involved in detoxification is the most rigorous way to prove their involvement in the detoxification process [5,7,8,16]. Performance and fitness tests on silenced lines can also identify the costs and benefits of this process for the insect [38,39].

[1] For a detailed review of these and other emerging techniques in this field, see Ref. [58].

Proving microbial symbiosis in the context of detoxification is difficult due to the intertwined nature of animals and their microbiomes. Hence, it is especially important to test a system where insect performance and fitness can be examined with and without a full microbiome and with and without the plant toxin of interest. Antibiotics are not necessarily required. Xia et al. [60] tested the interactions between the diamondback moth, *P. xylostella*, and its microbiome in connection with the radish flavonol kaempferol by surface sterilizing insect eggs and raising them under sterile conditions. This resulted in a bacteria-free gut based on 16S rRNA amplification and culturing. Larvae lacking gut bacteria suffered reduced growth when fed with kaempferol, but larval growth improved upon reintroduction of the gut bacterial community or a strain of *Enterobacter*, the most abundant microbe in the *P. xylostella* gut, which could degrade kaempferol.

To gain a complete understanding of the detoxification capabilities of insect-associated bacteria, it is imperative to know the chemical structures of the plant toxins and their microbial metabolites. For example, the detoxification of prenylated isoflavones from *Cudrania tricuspidata* (Moraceae) leaves by the gut microbiota of silkworm *Bombyx mori* larvae was studied [56]. Various glucosylated prenylated isoflavones were isolated from the feces and structurally characterized via NMR and other spectral data. These compounds were found to be significantly less toxic to insects than their prenylated isoflavone precursors. Bacterial strains cultured from *B. mori* guts were found to transform these specialized metabolites to the same products found in the silkworm.

Knowing the genes of the microbial toxin processing pathway allows experimental verification of microbial involvement in insect detoxification. An excellent study of this type was carried out on the red turpentine beetle, a North American bark beetle that has recently invaded China [61]. This insect is deterred by D-pinitol, an *O*-methylated inositol derivative that accumulates in its Chinese host pine, but this carbohydrate natural product is degraded by free-living bacteria and a fungus associated with the beetle. The authors elucidated the pathways of D-pinitol degradation in these external microbial symbionts and showed that mutant lines that

could not degrade D-pinitol did not support the growth of red turpentine beetles as effectively as microbes with intact D-pinitol detoxification pathways.

In our current state of knowledge, the overall significance of microbial detoxification for insect herbivores is still uncertain. Future studies combining chemical and molecular approaches with host phenotype manipulation are needed to assess its importance compared to the insect's own detoxification machinery and other toxin resistance mechanisms [62]. The role of bacteria may differ among insect groups, potentially being more important in Coleoptera and Hymenoptera than Lepidoptera based on evidence to date. The gut microbial communities of Lepidoptera are reported to be less dense and more variable in composition, with much influence ascribed to diet rather than maternal transmission [63].

Conclusion

Our expanding knowledge of the detoxification reactions employed by insects has interesting implications for research on plant specialized metabolites. Understanding of detoxification processes allows direct inferences as to which specialized metabolites will be effective defenses against particular insects as well as which ones will be readily broken down. In addition, the widespread occurrence of insect detoxification furnishes a plausible explanation for some of the chemical diversity of plant specialized metabolites observed in nature, as these compounds have likely been evolutionarily selected not just for their inherent toxicity, but also for their ability to avoid being easily detoxified by herbivore enzymes [64]. Further research progress on insect detoxification should provide more context on the roles of specialized metabolites in plant defense.

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Author contributions

KK and JG conceptualized, wrote, and revised the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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