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The molecular machinery of insect herbivores for detoxifying plant chemical defenses

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ABSTRACT

Insect herbivores have evolved a sophisticated molecular machinery to survive and thrive on chemically defended plants. They employ coordinated, multi-layered detoxification strategies that include host-encoded enzymes, transport systems, and context-dependent contributions from symbiotic microorganisms. This review synthesizes current mechanistic and evolutionary understanding of how insect herbivores deploy their integrated detoxification "machinery" that enables survival and feeding on chemically defended hosts. At the molecular level, detoxification is performed by large, evolutionarily dynamic families of cytochrome P450 monooxygenases, glutathione S-transferases, carboxylesterases, and UDP glycosyltransferases, which functionalize, conjugate, and excrete plant allelochemicals. This biotransformation architecture is functionally analogous to xenobiotic metabolism but operates as a network phenotype, subject to pronounced tissue-specific, developmental, and dietary inducibility. Comparative genomics and functional genetics have shown that host specialization and rapid adaptation often result from gene family expansion, neofunctionalization, and tissue-specific expression, driven by regulatory rewiring and frequently shaped by transposable elements and structural variation. Importantly, the same molecular modules that detoxify plant allelochemicals also intersect with insecticide resistance, creating predictable and mechanistically grounded cross-resistance risks in agroecosystems. This review provides a systems-level mechanistic understanding of insect detoxification across major phytochemical classes, highlights regulatory, spatial, and evolutionary principles, and proposes an integrative framework that links metabolic networks, ecological context, and spatially resolved multi-omics to prioritize future research.

Keywords: Herbivore detoxification enzymes | Insect detoxification | Insect-plant interactions | Plant secondary metabolites | Xenobiotic metabolism

1 | INTRODUCTION

Plants and insects are engaged in a long-standing evolutionary arms race, where chemical innovations from one side are met with biochemical and physiological countermeasures from the other [1]. Insect herbivores have evolved sophisticated mechanisms to cope with plant secondary metabolites (PSMs) that serve as chemical defenses. These compounds can act as feeding deterrents, reduce nutritional value, interfere with development, and produce toxic effects [2,3]. Plant defenses include terpenoids, alkaloids, phenolics, glucosinolates, cyanogenic glycosides, furanocoumarins, benzoxazinoids, and protein-based inhibitors, among others [4,5]. The molecular machinery of insects comprises not only host-encoded detoxification enzymes but also symbiotic microorganisms that contribute enzymatic capacity for xenobiotic metabolism [6,7]. The result is a tritrophic coevolutionary system involving plants, herbivores, and plant-associated or herbivore-associated microbes that continuously shapes ecological interactions and agricultural sustainability. The ecological outcome, such as host acceptance, feeding performance, and population persistence, depends on the insect's ability to detect, tolerate, transform, sequester, or avoid these compounds [4].

Detoxification in insect herbivores is often portrayed as a simple metabolic process. However, it is not merely a sequence of enzymatic reactions acting on isolated compounds; rather, it is an emergent phenotype produced by coordinated molecular networks that operate across multiple tissues, developmental stages, and ecological contexts [8,9]. The efficiency and flexibility of this integrated system ultimately determine whether an insect can exploit a chemically defended host plant, expand its host range, or specialize in a narrow set of toxic resources. For example, a specialist herbivore that feeds exclusively on milkweed encounters cardenolides; one that feeds on Brassicaceae encounters glucosinolates; and another that feeds on Nicotiana encounters nicotine [10,11]. Many generalist herbivores encounter an even broader spectrum of allelochemicals, which requires flexible detoxification strategies [5]. The costs of tolerating these chemicals are substantial; therefore, the synthesis and induction of detoxification enzymes divert resources from growth, reproduction, and survival [11,12]. Understanding the molecular basis of herbivore detoxification is therefore essential not only for evolutionary biology but also for applied entomology, food security, and the sustainable management of both agricultural pests and beneficial pollinators.

At the molecular level, insect detoxification relies heavily on large and evolutionarily dynamic gene families that operate

through an integrated, multi-layered system. Phase I biotransformation enzymes, primarily Cytochrome P450 monooxygenases (CYPs), catalyze the oxidative transformation of a broad range of lipophilic allelochemicals, often generating more polar or reactive intermediates [9,13,14]. Phase II conjugation enzymes, such as glutathione S-transferases (GSTs) and UDP-glycosyltransferases (UGTs), further modify these intermediates, reducing their reactivity and facilitating their excretion. Carboxyl/cholinesterases (CCEs), epoxide hydrolases, and other auxiliary enzymes expand the chemical space that insects can tolerate [13,15,16]. Phase III processes, dominated by ATP-binding cassette (ABC) transporters and other solute carriers, remove toxic metabolites from cells and tissues, providing an essential barrier against intracellular accumulation [16,17]. Importantly, these metabolic and transport processes are tightly coupled to antioxidant and redox-control pathways that mitigate oxidative stress generated by both plant toxins and their metabolic activation.

This review synthesizes the current understanding of the molecular machinery by which insect herbivores detoxify plant chemical defenses. We integrate insights from biochemistry, molecular biology, physiology, ecology, and evolutionary genomics to provide a comprehensive mechanistic framework for herbivore detoxification. We begin by outlining the biochemical phases of xenobiotic metabolism and the major enzyme families involved, emphasizing their structural diversity, substrate specificity, and regulation. We then examine the physiological organization of these systems, where detoxification occurs, how inducibility is achieved, and how costs and constraints influence evolutionary optimization. A central focus is the integration of host-encoded detoxification with symbiotic microbial pathways, exploring the ecological conditions that favor symbiont-based versus host-based detoxification strategies, maintained, and transmitted. Throughout the review, we highlight the coevolutionary arms race between plants and herbivores, illustrating how detoxification mechanisms have shaped, and been shaped by, plant defensive chemistry, herbivore host range, and the structure of ecological networks. This integrative perspective provides a foundation for future research seeking to link molecular detoxification processes with ecological outcomes in an increasingly chemically complex world.

2 | PLANT CHEMICAL DEFENSES: A FUNCTIONAL CLASSIFICATION FOR DETOXIFICATION BIOLOGY

Plant chemical defenses can be organized into functional classes that reflect their chemistry, molecular targets, and the detoxification circuits they engage in insect herbivores. Below, we expand a mechanistic framework that links classes of plant defenses to the detoxification priorities they impose on insect herbivores (Figure 1).

2.1 | Electrophilic and reactive toxins

Many plant toxins are electrophilic or generate oxidative species, causing macromolecular damage and stress. Insects, therefore, prioritize neutralizing these threats through two linked systems: GST-mediated conjugation, which detoxifies electrophiles for removal, and integrated antioxidant pathways that maintain redox balance [18,19] (Figure 1). This "Phase II plus redox" strategy centers on preventing irreversible damage, even at high metabolic cost [19].

2.2 | Lipophilic neuroactive compounds

A second major category of defenses includes lipophilic alkaloids, terpenoids, and phenylpropanoids, which interfere with neural signaling and membrane integrity by partitioning into lipid bilayers and modulating ion channels or receptors [4,5] (Figure 1).

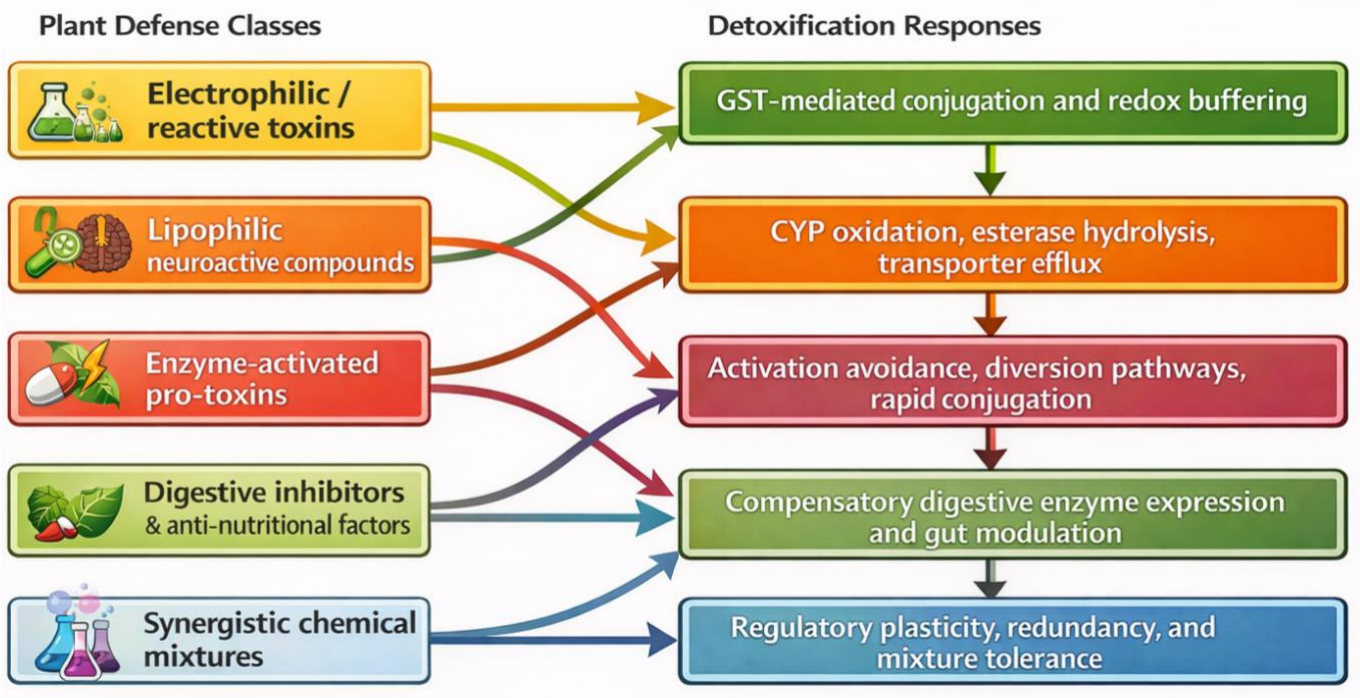

FIGURE 1 | Functional classification of plant chemical defenses and detoxification. Plant chemical defenses are grouped by their mode of action and linked to the dominant detoxification strategies deployed by insect herbivores.

Their persistent, dose-dependent toxicity is countered primarily by chemical transformation. Cytochrome P450s introduce polar groups to reduce membrane affinity, while esterases hydrolyze specific compounds, with transporter-mediated excretion being crucial to prevent re-entry into membranes [20,21]. Success also depends on resistance to the enzyme-inhibiting properties of these compounds, a key factor in their potent mixture effects.

2.3 | Enzyme-activated pro-toxins

Glucosinolates and cyanogenic glycosides are stored as inactive precursors, forming potent toxins like isothiocyanates or cyanide only upon enzyme activation. This means herbivore exposure depends critically on feeding patterns and tissue disruption, prompting strategies such as careful feeding to minimize substrate-enzyme mixing [22]. Molecular defenses include diverting activation toward less toxic products (such as nitriles), rapid conjugation of reactive species, and sometimes symbiont-assisted degradation [23]. Thus, detoxification here is defined by controlling the activation cascade itself, integrating behavioral, anatomical, and biochemical adaptations.

2.4 | Digestive inhibitors and anti-nutritional factors

Protease inhibitors, lectins, and tannins function as anti-nutritional defenses, impairing nutrient absorption and gut integrity (Figure 1). Their concentration-dependent, partly reversible effects trigger compensatory physiological responses, not direct detoxification [24]. Insects adjust by upregulating inhibitor-insensitive digestive enzymes, secreting binding proteins, and modifying gut conditions to limit tannin activity [24,25]. These malleable, hormonally-regulated adaptations functionally restore digestion, intertwining detoxification with gut physiology and growth trade-offs.

2.5 | Detoxification as an ecological phenotype

Detoxification is an ecological phenotype shaped by dynamic molecular networks rather than a fixed capacity. An insect's response to an allelochemical is not predetermined but is configured by factors like diet composition, symbionts, life stage, and nutritional state [8,9]. This adaptive network, with its inherent redundancy, hierarchies, and shared resources, determines whether exposure results in tolerance or mortality [8]. Consequently, detoxification capacity is a moving target best understood within realistic ecological contexts. By classifying plant defenses based on their functional challenge, we can map specific chemistries to the enzyme families, regulatory pathways, and symbiotic partnerships they engage, providing a scaffold to predict how plant chemistry translates into herbivore adaptation and ecological outcomes [8,26].

3 | THE CORE DETOXIFICATION ARCHITECTURE: PHASE I, II, III, AND REDOX HOMEOSTASIS

Insect herbivores detoxify plant chemical defenses through a conserved three-phase biochemical architecture, comprising functionalization (Phase I), conjugation (Phase II), and excretion (Phase III), which is critically dependent on a fourth pillar: redox homeostasis [4,15-17,26,27] (Figure 2).

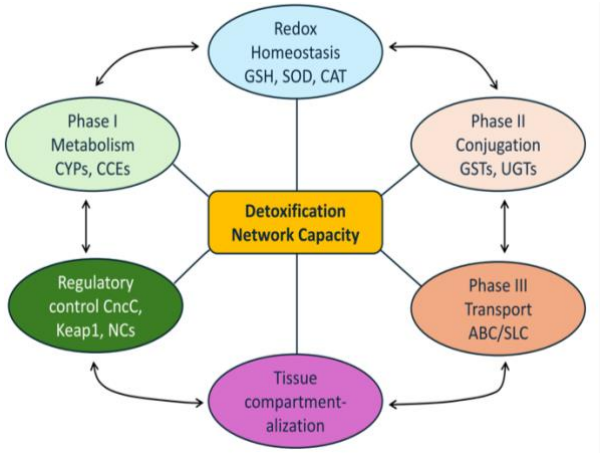

FIGURE 2 | Detoxification of plant chemical defenses as a network phenotype. Detoxification network capacity emerges from the coordinated action of Phase I functionalization (e.g., cytochrome P450s, esterases), Phase II conjugation (GSTs, UGTs), Phase III transport (ABC and SLC transporters), and redox homeostasis.

3.1 | Phase I: Functionalization (Oxidation, reduction, hydrolysis)

Phase I reactions modify xenobiotics by introducing or unmasking polar functional groups, enhancing reactivity and enabling subsequent conjugation [14,28]. In insect herbivores, this phase is primarily mediated by cytochrome P450 monooxygenases (CYPs) (Figure 2). CYPs catalyze diverse oxidative transformations (hydroxylation, epoxidation, dealkylation), converting lipophilic plant allelochemicals into more polar metabolites [14,29]. CYP-driven detoxification is marked by lineage-specific expansions, especially in the CYP3 and CYP4 clades, shaped by host plant chemistry [27,30]. Tandem duplications and neofunctionalization yield paralogs with distinct substrate specificities, while coordinated regulatory evolution enables rapid, inducible responses to dietary toxins. Metabolism is spatially organized: the midgut performs first-pass detoxification under high toxin exposure; the fat body integrates xenobiotic processing with energy and redox homeostasis; and Malpighian tubules handle later-stage metabolite processing for excretion [14,27].

A key functional trade-off exists between catalytic breadth and efficiency. Generalists deploy broadly active, highly inducible CYPs to handle diverse diets, whereas specialists evolve a few highly efficient CYPs fine-tuned to specific host defenses, shaping host range and adaptive potential [27,31]. Beyond CYPs, carboxyl/cholinesterases (CCEs) hydrolyze ester-containing allelochemicals and can sequester toxins via non-catalytic binding, buffering exposure during metabolic overload [31,32]. Auxiliary enzymes, including alcohol/aldehyde dehydrogenases, reductases, epoxide hydrolases, and short-chain dehydrogenases, provide complementary pathways. Notably, epoxide hydrolases neutralize reactive epoxides generated by CYPs, preventing macromolecular damage [30,31]. Together, these systems confer metabolic flexibility and redundancy, minimizing the risk of lethal bottlenecks.

3.2 Phase II: Conjugation (increasing polarity and excretability)

Phase II detoxification enhances xenobiotic excretion by covalently attaching polar endogenous groups, primarily via

glutathione GSTs and UDP-glycosyltransferases (UGTs), to Phase I metabolites or parent compounds [16,33] (Figure 2). GSTs conjugate reduced glutathione (GSH) to electrophilic sites, neutralizing reactive intermediates (often generated by CYPs) and linking detoxification to cellular redox balance [34,35]. Some GSTs also act as ligand-binding proteins, sequestering toxins non-catalytically to limit cellular damage, highlighting their dual roles in protection and regulation [34,35]. UGTs transfer sugar moieties (typically glucose) to hydroxylated substrates, dramatically increasing solubility and often inactivating toxins. They are especially vital for neutralizing phenolics, flavonoids, and terpenoid alcohols. In specialist herbivores, UGT-mediated glycosylation can enable toxin sequestration for anti-predator defense, turning plant allelochemicals into ecological assets [16,33,36]. Additional conjugation systems, such as sulfotransferases and N-acetyltransferases, exist in select insect lineages and may fine-tune responses to specific plant chemicals, though they remain underexplored due to taxonomic restriction and methodological challenges [9,36]. Broadening research into these pathways could uncover further dimensions of insect–plant coevolution.

3.3 Phase III: Transport and Excretion

Phase III detoxification involves the active efflux of xenobiotics and their conjugated metabolites from cells, preventing intracellular accumulation that could otherwise cause toxicity, even when metabolites are chemically inert [17] (Figure 2). In insects, this phase is primarily mediated by ATP-binding cassette (ABC) transporters, especially members of the ABCB, ABCC, and ABCG subfamilies, alongside various solute carrier (SLC) transporters [17,37]. ABC transporters are strategically positioned at key physiological barriers, such as the gut epithelium and Malpighian tubules, where they pump substrates into the gut lumen or excretory system [27,37]. By regulating intracellular concentrations, they critically influence tissue-specific toxicity and overall physiological fitness. Consequently, even modest changes in transporter expression or activity can profoundly affect detoxification capacity, independent of metabolic enzyme function [17,38]. Beyond excretion, these transporters reinforce barrier integrity and enable chemical compartmentalization, shielding sensitive tissues while confining toxins to designated elimination routes [39,40]. This spatial control, however, can create physiological trade-offs, such as interference with nutrient uptake or ion homeostasis, potentially constraining the evolution of resistance mechanisms [39,40].

3.4 Redox homeostasis as a fourth pillar of detoxification

Often regarded as secondary, redox homeostasis is in fact foundational to every phase of detoxification. Many plant allelochemicals either directly induce oxidative stress or generate reactive oxygen species (ROS) as byproducts of metabolic activation [13,15,16,41]. To counteract this, insects rely on a coordinated antioxidant network, including superoxide dismutase (SOD), catalase (CAT), peroxidases, and enzymes involved in glutathione GSH synthesis and recycling [41-43]. Thioredoxin systems and mitochondrial stress responses further bolster cellular redox stability during chemical challenge [41]. Importantly, redox regulation is not merely defensive; it is deeply regulatory. Oxidative and electrophilic stress act as signaling cues that activate transcriptional programs, upregulating genes across Phases I, II, and III [44,45]. At the same time, redox resources are finite: excessive demand for reducing equivalents to fuel detoxification can impair growth, reproduction, and immunity [4,44,45]. Thus, detoxification efficiency reflects a dynamic trade-off among chemical defense, metabolic investment, and physiological resilience.

4 | REGULATORY NETWORKS: HOW INSECTS SENSE AND REPROGRAM DETOXIFICATION

Detoxification in insect herbivores is not a fixed trait but a dynamic, inducible phenotype. Enzyme and transporter expression are finely tuned in response to chemical exposure, development, nutrition, and stress. Insects deploy integrated regulatory networks that sense xenobiotic threats and reprogram metabolism accordingly (Figure 2 and 3).

4.1 The CncC/Keap1 (Nrf2-like) Pathway

The CncC/Keap1 pathway is a highly conserved master regulator of detoxification and oxidative stress responses in insects. CncC-a cap 'n' collar bZIP transcription factor homologous to mammalian Nrf2-activates genes essential for xenobiotic metabolism, antioxidant defense, and redox balance [46,47]. Under basal conditions, CncC is sequestered in the cytoplasm by Keap1, which promotes its ubiquitination and proteasomal degradation. Keap1 acts as a redox- and electrophile-sensitive sensor: electrophilic plant allelochemicals (or their metabolites) modify critical cysteine residues on Keap1, disrupting its repressive function [47,48]. This stabilizes CncC, enabling its nuclear translocation and binding to antioxidant response elements (AREs) in target gene promoters. The resulting transcriptional program coordinately upregulates GSTs, UGTs, antioxidant enzymes, transporters, and, in some species, select cytochrome P450s [14,16,30,37]. Rather than modulating individual genes, CncC/Keap1 orchestrates a network-wide response that integrates conjugation, redox buffering, and efflux, critical for managing electrophilic or oxidative challenges without metabolic bottlenecks or cellular damage [47-49]. However, chronic pathway activation incurs physiological costs, linking detoxification to trade-offs in growth and life-history traits.

4.2 Xenobiotic-sensing nuclear receptors and bHLH-PAS factors

Beyond redox-sensitive systems, insects deploy specialized transcription factors that directly sense the chemical nature of dietary xenobiotics. Key among these are nuclear receptors like HR96 and bHLH-PAS proteins homologous to the vertebrate aryl hydrocarbon receptor (AhR) and its dimerization partner ARNT [50,51]. These factors act as ligand-responsive sensors, often activated by lipophilic or planar plant secondary metabolites. Upon binding, they induce select detoxification genes, especially cytochrome P450s and transporters specialized for hydrophobic compounds. Unlike the broadly stress-responsive CncC/Keap1 pathway, these systems offer greater chemical specificity, allowing insects to fine-tune defenses to particular allelochemical classes [47-49]. Critically, these xenobiotic-sensing pathways are embedded within broader developmental and metabolic networks. Their activity shifts across larval stages, mirroring changes in feeding behavior, tissue maturation, and hormonal cues, and is modulated by nutritional status, which governs the energetic resources available for detoxification [9,52].

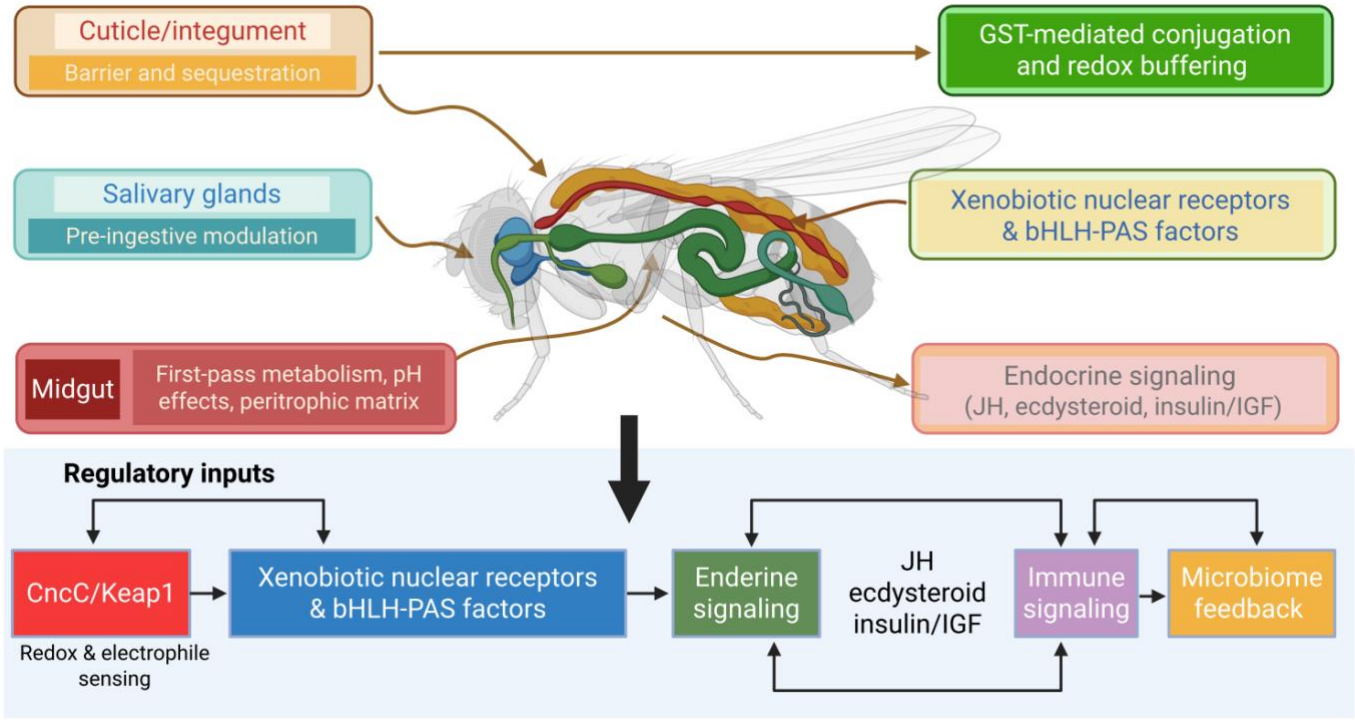

FIGURE 3 | Spatial and regulatory control of detoxification in insect herbivores. Detoxification is spatially partitioned across specialized tissues, with first-pass metabolism in the midgut, systemic processing in the fat body, excretion via Malpighian tubules, and pre-ingestive modulation by salivary glands, while the cuticle limits exposure and enables sequestration. These tissue-specific processes are coordinated by regulatory networks that integrate chemical sensing, redox status, endocrine signaling, and immune inputs.

This integration underpins the pronounced ontogenetic and condition-dependent variation in detoxification capacity throughout the insect life cycle.

4.3 Endocrine and immune cross-talk

Detoxification is tightly integrated with endocrine and immune systems, reflecting trade-offs in resource allocation and stress response. Key hormones, including juvenile hormone (JH), ecdysteroids, and insulin/IGF signaling, modulate detoxification gene expression by shifting metabolic priorities across development and nutritional states [53,54]. For instance, JH and ecdysteroids can up- or downregulate detox capacity during life-stage transitions, while insulin signaling links nutrient availability to investment in detoxification machinery [54,55]. Immune activation, triggered by pathogens or gut dysbiosis, often competes for the same energetic and redox resources, potentially suppressing detoxification [56,57]. Conversely, chronic xenobiotic exposure can impair gut barrier function, alter immune tone, and disrupt microbiota interactions [57-59]. These bidirectional influences mean detoxification phenotypes arise not from toxin exposure alone, but from dynamic trade-offs among chemical defense, immunity, and growth. Recognizing and accounting for these interactions is essential for mechanistic interpretation and ecological relevance.

5 | TISSUE COMPARTMENTALIZATION: WHERE DETOXIFICATION HAPPENS

Detoxification in insect herbivores is spatially organized across specialized tissues, each performing distinct yet complementary roles. Understanding the tissue-specific architecture of detoxification is essential for interpreting molecular data, elucidating adaptations to plant defenses, and designing mechanistically rigorous experiments (Figure 3).

5.1 Midgut: First-pass metabolism and chemical partitioning

The midgut is the primary site of contact with ingested plant allelochemicals and thus the frontline of detoxification. It is richly endowed with Phase I and II enzymes, especially cytochrome P450s, GSTs, UGTs, and carboxyl/cholinesterases, and fortified with ABC transporters that efflux metabolites back into the gut lumen, minimizing intracellular accumulation [16,30,37] (Figure 3). Key physiological features shape its detox capacity: the peritrophic matrix acts as a selective barrier, limiting access of large or reactive compounds to epithelial cells, while midgut pH, which is highly variable across taxa and gut regions, modulates toxin ionization, stability, and enzyme activity [60,61]. For instance, the alkaline midgut of many Lepidoptera can attenuate or amplify the toxicity of specific plant metabolites, steering which detox pathways are engaged [61]. By determining whether toxins are neutralized locally or enter systemic circulation, the midgut exerts an outsized influence on whole-organism tolerance [23,61]. Consequently, even subtle shifts in its enzyme profiles or barrier properties can drive host-plant adaptation, making it a critical hub of evolutionary innovation.

5.2 Fat body: Systemic detoxification and metabolic integration

The fat body, functionally analogous to the vertebrate liver and adipose tissue, serves as a central hub that integrates

detoxification with energy metabolism, nutrient allocation, and redox homeostasis [62]. It plays a key role in systemic detoxification, processing allelochemicals and metabolites that bypass midgut first-pass metabolism [62,63]. While it expresses many of the same detoxification enzymes as the midgut (CYPs, GSTs, UGTs), their regulation reflects whole-organism demands rather than local exposure (Figure 3). The fat body is crucial for sustaining redox balance during prolonged detoxification, supplying reducing equivalents, synthesizing glutathione, and coordinating antioxidant responses [62,63]. This metabolic integration creates trade-offs: resources diverted to detoxification can constrain growth, reproduction, and immunity, tying chemical tolerance to life-history strategies [62,64]. Because fat body activity reflects cumulative rather than acute exposure, it reveals the physiological costs of feeding on chemically defended plants, but may obscure the primary sites of toxin action. Thus, tissue-resolved analyses remain essential for a complete understanding of detoxification dynamics [62,65].

5.3 Malpighian tubules: Excretion and final clearance

The Malpighian tubules serve as the insect's primary excretory organ and a key site of Phase III detoxification [27,37]. They are enriched in ABC transporters and solute carriers that actively shuttle conjugated xenobiotics from the hemolymph into the excretory lumen for elimination [14,27]. Some tubules also express Phase II enzymes, allowing final metabolic modifications before excretion [27,66]. Excretory efficiency directly shapes systemic toxin exposure and toxicity duration. Thus, variation in transporter expression or activity in the Malpighian tubules can significantly influence detoxification capacity, even when metabolic enzyme levels elsewhere remain unchanged [27,66]. However, this clearance process is energetically costly, linking excretory performance to broader fitness trade-offs.

5.4 Salivary glands: Pre-ingestive modulation of plant defenses

Though often overlooked, salivary glands contribute critically to detoxification by shaping plant-insect chemical interactions before ingestion [67]. Saliva can contain enzymes, binding proteins, or effector molecules that suppress plant defense signaling or alter the activation of pro-toxins, such as inhibiting the hydrolysis of glucosinolates or phenolic glycosides [67,68] (Figure 3). By chemically manipulating the feeding site, salivary secretions reduce the toxin load entering the gut. This pre-ingestive strategy is especially refined in specialist herbivores, which exploit specific biochemical weaknesses in their host plants, making salivary function an integral, though understudied, component of detoxification [67,68].

5.5 Cuticle and integument: Barriers and potential sequestration compartments

The cuticle and integument act as the first line of defense against environmental toxins, limiting dermal uptake of plant-derived chemicals encountered during feeding or oviposition [69]. Beyond passive barrier function, the integument in some insects serves as a sequestration site, storing conjugated or even unmetabolized allelochemicals away from sensitive tissues [69,70]. This not only mitigates toxicity but, in certain species, repurposes plant compounds for anti-predator defense. Variation in cuticular composition and permeability thus influences exposure routes and detoxification demands, adding a critical layer of spatial and functional complexity to insect chemical tolerance [69,70].

6 | CONCLUSION

Insect herbivores overcome plant chemical defenses through a dynamic, multi-scale detoxification system that integrates Phase I–III metabolism, redox homeostasis, regulatory networks, and tissue compartmentalization, not as a linear pathway, but as a coordinated network phenotype. This architecture enables insects to neutralize, tolerate, or even co-opt plant secondary metabolites, shaping host range, feeding ecology, and evolutionary trajectories. Core components, including cytochrome P450s, GSTs, UGTs, esterases, transporters, and antioxidants, are widely conserved, but their deployment varies with host chemistry, life history, and evolutionary history. Gene family expansions, regulatory plasticity, and tissue-specific expression support both generalist flexibility and specialist precision. Crucially, detoxification capacity depends not just on enzyme levels, but on redox balance, energy status, endocrine and immune signals, rendering it highly condition-dependent and often decoupled from simple gene expression to tolerance correlations.

7 | FUTURE DIRECTIONS AND PERSPECTIVES

Advancing insect detoxification research requires shifting from gene catalogs to functional flux and quantifying how enzyme kinetics, cofactor availability, and transporter activity jointly determine metabolic outcomes through integrated metabolomics, activity assays, and genetic tools. Studies must also embrace chemical realism by testing complex plant metabolite mixtures rather than single compounds to capture ecological relevance. Spatial resolution is key: single-cell and tissue-specific omics, combined with imaging, will clarify compartmentalized detox dynamics across gut, fat body, and Malpighian tubules. Unraveling the regulatory logic of detox networks, especially their integration with endocrine and immune signals, will enable predictive models of phenotype across environments. Rigorous causal tests, including gnotobiotic experiments, are needed to define the microbiome's context-dependent role. Evolutionary genomics can reveal how gene family expansions and regulatory rewiring drive rapid adaptation to plant and synthetic toxins. Ultimately, these insights should inform sustainable pest management by exploiting metabolic trade-offs and minimizing cross-resistance between plant defenses and insecticides.

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AUTHOR CONTRIBUTIONS

Conceptualization: A.M. | Visualization: J.I., M.U, and S.B. | Writing – original draft: A.M. | Writing – review and editing: J.I., M.U, and S.B. The authors confirm their contributions to the paper as follows.

DATA AVAILABILITY STATEMENT

The authors have nothing to report.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the author(s) used ChatGPT 3.5 in order to improve readability and language. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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