Review Paper:

Bacillus thuringiensis Cry and Cyt Toxins: Mechanisms of Action, Resistance Management and Impact on Host Immune Responses

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Abstract

Bacillus thuringiensis (Bt) is a pioneering biopesticide known for its potent insecticidal proteins, particularly Cry and Cyt toxins which have transformed agricultural pest management worldwide. As a naturally occurring bacterium, Bt is favored for its environmentally friendly profile and high specificity toward target insect pests, significantly reducing reliance on synthetic chemical pesticides.

This review focused on the mechanisms of action of Bt toxins, their specificity and the implications for pest management strategies. Cry toxins are produced as inactive protoxins that undergo proteolytic activation in the alkaline environment of the insect midgut. This activation triggers a conformational change, enabling the protoxins to bind to specific receptors on midgut epithelial cells such as cadherins, aminopeptidases and alkaline phosphatases. These binding initiates biological events that lead to pore formation, cell lysis and ultimately, insect mortality. The specificity of Cry toxins largely depends on their interactions with these receptors, which vary among different insect species.

In contrast, Cyt toxins employ a different mechanism by directly interacting with lipid bilayers to form pores, allowing them to target a broader spectrum of insect pests, including those resistant to Cry toxins. Recent research has shed light on the structural details of toxin-receptor interactions and pore formation mechanisms, facilitating the development of novel Bt toxins with enhanced efficacy and broader activity spectra.

Insights from genomic studies on resistance mechanisms have revealed critical information about receptor gene mutations and midgut protease activity alterations, essential for developing targeted solutions against resistance. The environmental and non-target effects of Bt toxins are also crucial considerations. Current research indicates that Bt toxins generally have minimal adverse impacts on beneficial insects, soil microbiota and aquatic ecosystems, underscoring their ecological safety profile.

Keywords: *Bacillus thuringiensis* (Bt), Cry toxins, Cyt toxins, Insecticidal activity, Midgut epithelium, Receptor binding, Cadherin.

Introduction

The discovery of *Bacillus thuringiensis* (Bt), a naturally occurring soil bacterium, along with its insecticidal proteins, represents a transformative advancement in biological pest control. Identified in the early 1900s, Bt has gained significant attention for its ability to produce a diverse array of toxic proteins during sporulation, particularly crystalline proteins (Cry) and cytolytic proteins (Cyt), which exhibit potent insecticidal properties. These proteins have made Bt a valuable tool in integrated pest management (IPM) strategies, offering an environmentally friendly alternative to traditional chemical pesticides. The specificity of Cry proteins towards certain insect orders such as Lepidoptera, Coleoptera and Diptera, enhances their efficacy by targeting pests while minimizing harm to non-target organisms.

Moreover, the development of genetically modified crops (Bt crops) that express these toxins, has revolutionized agricultural pest management, enabling in-plant production of insecticidal proteins and promoting sustainable farming practices[24]. This innovation has significantly reduced pest populations, increased crop yields and decreased economic losses, positioning Bt as a key component of sustainable agriculture. However, the long-term effectiveness of Bt is challenged by the potential development of resistance in insect pests, making it crucial to understand the immune responses elicited in host insects upon exposure to these toxins. Continued research is essential for optimizing the application of Bt toxins and ensuring their sustainability in pest management practices, ultimately contributing to global food security[32].

Bt plays a critical role in pest control, the introduction would benefit from a broader contextual comparison of Cry and Cyt toxins with other biopesticides and chemical pesticides. For instance, *Beauveria bassiana* and *Metarhizium anisopliae* widely used biopesticides offer broader host ranges but lack the rapid, targeted efficacy of Bt toxins. Chemical pesticides, on the other hand, often exhibit robustness across diverse environmental conditions but carry risks of non-target effects and accelerate resistance development. In contrast, Bt stands out for its selective toxicity and environmental safety, reducing unintended harm to beneficial organisms and ecosystems.

Incorporating such comparisons highlights the complementary roles of various pest management tools in IPM strategies. It also underscores the importance of balancing biological and chemical approaches to minimize resistance development and sustain long-term efficacy. Despite challenges like evolving pest resistance, Bt remains an indispensable part of sustainable agriculture and pest management. A nuanced understanding of how Bt integrates with other strategies, will ensure its continued relevance and will help to optimize its application across diverse agricultural systems.

Mechanism of Action of Cry and Cyt Toxins

Cry Toxins: Cry proteins, also known as delta-endotoxins, are produced by *Bacillus thuringiensis* as crystalline inclusions during sporulation. These proteins are initially inactive protoxins that require activation to become toxic. Activation occurs in the alkaline environment of the insect midgut, where proteolytic enzymes cleave the protoxins into their active forms. The activated Cry toxins then interact with specific receptors on the midgut epithelial cells (Table. 1, figure 1). Upon ingestion by an insect, Cry proteins dissolve in the gut fluid and are proteolytically processed into active toxins. These toxins bind to specific receptors on midgut epithelial cell membranes including cadherins, aminopeptidases and alkaline phosphatases.

Cadherins, large transmembrane proteins involved in cell-cell adhesion, play a crucial role in toxin binding and pore formation. Aminopeptidases and alkaline phosphatases further facilitate toxin binding, contributing to the specificity of Cry toxins towards different insect species[1,7]. After binding to receptors, Cry toxins undergo a conformational change that allows them to insert into the lipid bilayer of the epithelial cell membrane, forming oligomeric pores.

These pores disrupt ion balance, leading to cell depolarization, the influx of ions such as calcium and ultimately, cell lysis and insect death. The specificity of Cry toxins towards various insect species is largely determined by their receptor interactions, which vary between insect orders[12] (Figure 1).

Cyt Toxins: Cyt toxins, or cytolytic toxins, exhibit a distinct mode of action compared to Cry toxins. Unlike Cry toxins, Cyt toxins do not rely on specific receptor interactions but instead interact directly with the lipid bilayer of midgut epithelial cells (Table 1, figure 1).

Table 1
Comparative Characteristics and Applications of Cry and Cyt Toxins produced by *Bacillus thuringiensis* (Bt)

Feature	Cry Toxins	Cyt Toxins
Type of Toxin	Delta-endotoxins	Cytolytic (Cyt) toxins
Activation	Inactive protoxins requiring activation in the insect midgut (alkaline pH)	Active form released directly by *Bt*
Receptor Binding	Binds to specific receptors on midgut epithelial cells (e.g., cadherins, aminopeptidases, alkaline phosphatases)	Does not require specific receptors; interacts directly with the lipid bilayer
Mode of Action	Forms pores in the midgut epithelial cell membrane, leading to cell lysis and insect death	Disrupts the cell membrane by forming pores, causing cellular leakage and death
Specificity	Highly specific to target insect species; different Cry toxins target different insect orders (e.g., Lepidoptera)	Broad-spectrum activity; effective against a wide range of insect pests, including those resistant to Cry toxins
Examples of Toxins	Cry1A (targets Lepidoptera, e.g., *Helicoverpa armigera*), Cry3A (targets Coleoptera, e.g., *Colorado potato beetle*)	Cyt1A (targets *Aedes aegypti*), Cyt2B (broad-spectrum activity against various insects)
Key Applications	Used in Bt crops like Bt cotton and Bt corn to control specific pests (e.g. *Helicoverpa* spp., *Ostrinia nubilalis*)	Often used in combination with Cry toxins to enhance efficacy in biopesticide formulations
Resistance Management	Resistance can develop due to mutations in receptor genes or changes in protease activity	Less prone to resistance due to non-specific mode of action; used synergistically with Cry toxins to manage resistance
Environmental Impact	Minimal impact on non-target organisms; safe for beneficial insects and pollinators	Similar minimal impact; broad-spectrum action helps in integrated pest management (IPM)
Examples of Commercial Products	Bt cotton expressing Cry1Ac (e.g. Bollgard cotton), Bt corn expressing Cry1Ab (e.g. MON 810)	Bt-based larvicides (e.g. VectoBac containing Cyt1A and Cry4 toxins)

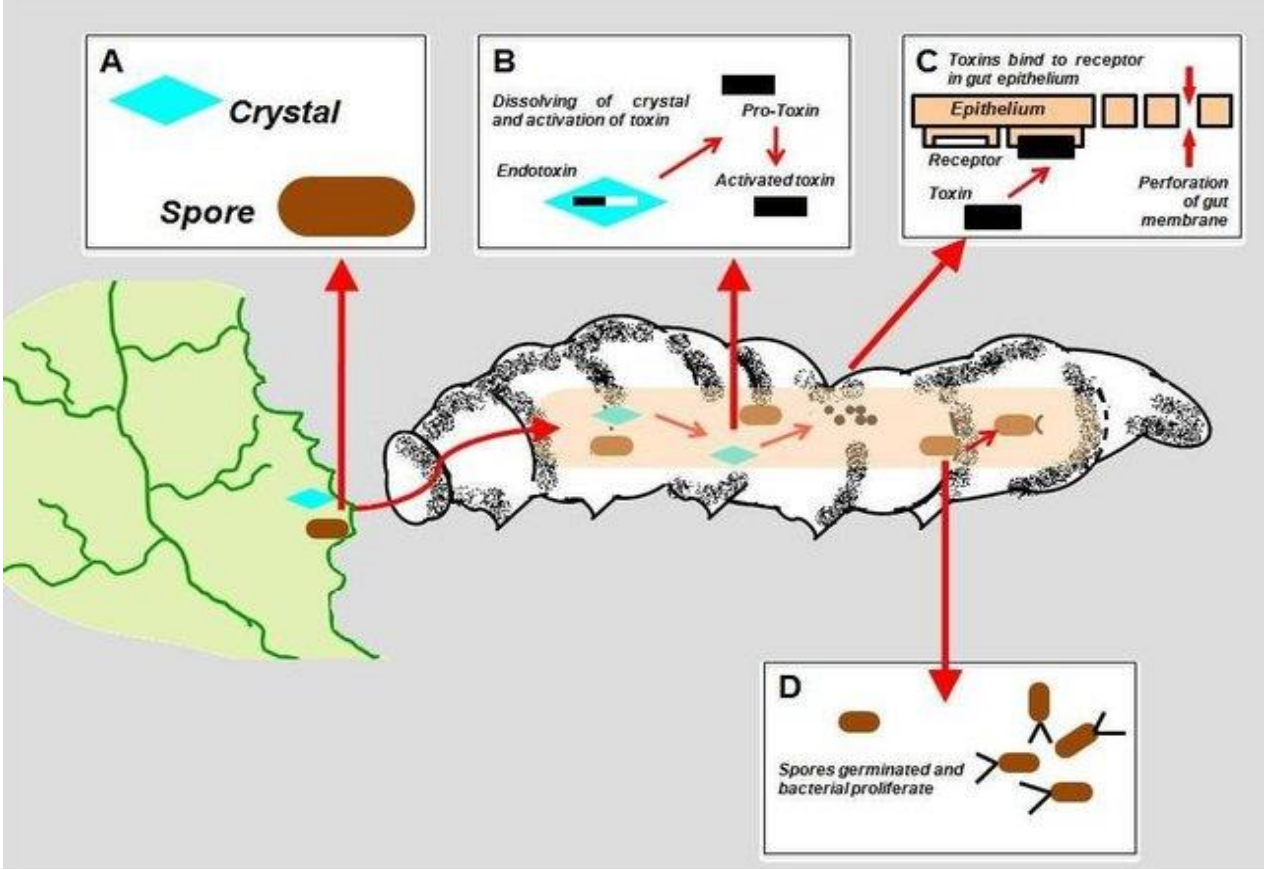

Figure 1: Action Mechanism of *Bacillus thuringiensis* Cry Proteins in Insect Larval Midgut.
A: Formation of protoxin crystals during bacterial sporulation, B: Dissolution and activation of protoxins by insect midgut proteases in an alkaline pH environment, C: Interaction of activated toxins with specific midgut epithelial receptors (e.g. cadherins, aminopeptidases), D: Disruption of epithelial cells via pore formation, leading to cellular damage and leakage, ultimately causing insect death[21]

Cyt toxins bind directly to the lipid components of cell membranes, forming pores that disrupt cellular integrity, leading to ce3lular leakage and death. This mode of action allows Cyt toxins to target a broader range of pests including those resistant to Cry toxins[7]. The lack of specificity in receptor binding allows Cyt toxins to target various insect species, making them effective in integrated pest management strategies where multiple pest species must be controlled. Cyt toxins can act synergistically with Cry toxins, enhancing the overall efficacy of Bt-based pest control measures[13].

The insecticidal proteins produced by *Bacillus thuringiensis* (Bt) can be categorized into two main types: Cry toxins and Cyt toxins, each exhibiting distinct characteristics and mechanisms of action. Table 1 provides a comparative overview of these toxins, highlighting their activation processes, receptor binding affinities, modes of action, specificity towards insect species, applications, resistance management strategies and environmental impacts. Cry toxins are primarily delta-endotoxins that require activation in the insect midgut and display high specificity for certain insect orders, making them valuable for use in genetically modified crops such as Bt cotton and Bt corn. In contrast, Cyt toxins are cytolytic and have broad-spectrum activity, allowing them to act effectively against a wide range of insect pests. Understanding these differences is crucial for optimizing the application of Bt in integrated pest management (IPM) strategies, as well as in addressing challenges such as the development of resistance among pest populations.

Host Immune Response to Bt Toxins: The interaction between *Bacillus thuringiensis* (Bt) toxins and the insect immune system is intricate, encompassing both cellular and humoral immune responses that significantly influence the efficacy of these biopesticides[41].

Insects primarily utilize a cellular immune response mediated by hemocytes, which are key immune cells present in the hemolymph. These hemocytes are critical for recognizing, engulfing and neutralizing pathogens including Bt toxins. Upon entry into the insect body, Bt toxins can either damage midgut cells or can be directly recognized by hemocytes through surface receptors that detect foreign particles and damaged cells. This recognition triggers phagocytosis, a vital process where hemocytes engulf and internalize the toxins or affected cells, thereby facilitating toxin clearance and mitigating further damage[31].

During phagocytosis, hemocytes also release reactive oxygen species (ROS) and other cytotoxic molecules. These highly reactive molecules are instrumental in damaging proteins, lipids and nucleic acids within the toxins or the toxin-damaged cells. This oxidative burst serves as a crucial component of the insect's defense strategy, aimed at neutralizing toxins and limiting their adverse effects. Additionally, hemocytes secrete cytotoxic factors such as proteases and antimicrobial peptides to further inhibit the activity of Bt toxins, enhancing the overall immune response against these biopesticidal agents[34].

Humoral Immune Response: The humoral immune response in insects plays a pivotal role in their defense against pathogens including the insecticidal bacterium *Bacillus thuringiensis* (Bt). This response is characterized by the secretion of immune factors into the hemolymph, encompassing a diverse array of components that actively contribute to pathogen neutralization. A prominent feature of this response is the production of antimicrobial peptides (AMPs) which are small, cationic peptides synthesized in response to infection or injury. AMPs are integral to insect immunity as they exhibit broad-spectrum activity against various pathogens including bacteria, fungi and viruses. Their primary mechanism of action involves disrupting microbial cell membranes, thereby leading to cell lysis and death. This mode of action is particularly crucial when addressing threats posed by Bt, as these peptides can target both spores and vegetative cells of the bacterium.

By binding to and neutralizing Bt spores, AMPs mitigate the pathogenicity of *Bacillus thuringiensis*, which is particularly relevant for agricultural practices that employ Bt-based pest control measures. The significance of antimicrobial peptides (AMPs) in reducing the overall virulence of *Bacillus thuringiensis* (Bt) has been emphasized, highlighting their crucial role in insect defense mechanisms[9].

Beyond their role in targeting Bt spores, AMPs also possess the capacity to interact with and interfere with the efficacy of the insecticidal Cry and Cyt toxins produced by *Bacillus thuringiensis*. These toxins are critical for the insecticidal action of Bt, yet some AMPs can bind to these toxins and inhibit their functionality. For instance, certain AMPs may prevent Cry and Cyt toxins from effectively binding to specific receptors on midgut epithelial cells, which is a crucial step for the toxins to exert their lethal effects. This interference can significantly reduce the potency of Bt toxins, contributing to variations in susceptibility among different insect species. The interplay between antimicrobial peptides (AMPs) and *Bacillus thuringiensis* (Bt) toxins has been identified as a critical factor influencing the overall effectiveness of Bt-based biopesticides, highlighting the need for a deeper understanding of these immune mechanisms to optimize pest control strategies[11].

Recent advancements in understanding the synergistic effects of combining different Bt toxins have further underscored the complexities of Bt toxicity and resistance management. Research indicates that the simultaneous use of multiple Bt toxins can significantly enhance insecticidal efficacy beyond the individual effects of each toxin. This phenomenon, known as synergistic action, occurs when the combined impact of different Bt toxins yields greater insecticidal activity than what would be expected from the sum of their contributions[25]. For example, the combination of Cry and Cyt toxins can broaden the spectrum of insect pests controlled by Bt formulations, as different toxins may target various species or developmental stages of pests. This approach is particularly beneficial in integrated pest management (IPM) strategies where maintaining pest populations below damaging thresholds is essential for sustainable agriculture.

Moreover, the strategic use of multiple Bt toxins can also reduce the likelihood of resistance development in insect populations. Insects that may develop resistance to one specific toxin may remain vulnerable to others, particularly if these toxins operate through distinct mechanisms or target different receptors on the insect midgut. This multifaceted approach not only prolongs the efficacy of Bt-based pest control but also minimizes the necessity for alternative chemical pesticides, which often pose risks to non-target organisms and the environment. The use of multiple toxins has been highlighted as a crucial strategy for managing resistance, emphasizing the importance of adaptive pest management approaches that consider the evolving dynamics between pests and their biocontrol agents[19].

Recent studies have demonstrated the enhanced efficacy achieved by combining Cry and Cyt toxins in pest control. For instance, research has provided compelling evidence that the synergistic effects of these toxins result in significantly enhanced insecticidal activity compared to the use of single toxins alone. The findings revealed that the combined use of Cry and Cyt toxins results in faster mortality rates and more comprehensive control of target pests, thereby increasing the overall effectiveness of Bt-based pest management strategies[25]. This enhanced efficacy is crucial for addressing the challenges posed by resistant pest populations, as it enables farmers to rely on biocontrol methods with greater confidence in their ability to maintain effective pest suppression. However, the humoral immune response in insects, particularly through the action of antimicrobial peptides, significantly impacts the effectiveness of *Bacillus thuringiensis* and its insecticidal proteins.

Understanding the complex interplay between insect immune factors and Bt toxins is essential for optimizing the use of Bt in agricultural pest management. As researchers continue to explore the mechanisms underlying resistance and the synergistic effects of combining multiple Bt toxins, it becomes increasingly clear that a multifaceted approach is necessary to ensure the long-term sustainability of Bt-based pest control strategies[2]. By integrating insights from immunology, entomology and biotechnology, it is possible

to enhance the efficacy of Bt applications while managing the risks of resistance, ultimately contributing to sustainable agricultural practices and global food security.

Ecological Impacts and their Immune Responses: Although *Bacillus thuringiensis* is recognized for its relatively benign profile compared to synthetic insecticides, studies have raised concerns about its long-term ecological impact. For instance, a comprehensive analysis found that Bt crops could alter non-target insect populations, potentially impacting ecological balance **[17]**. Their research indicates a decline in certain beneficial insect populations, highlighting the need for caution in the extensive deployment of Bt biopesticides.

Additionally, the potential for Bt toxins to impact soil microbial communities and aquatic ecosystems warrants further investigation. A study explored the impact of Bt proteins on soil microbial diversity and function, revealing significant changes in community composition and activity that could lead to cascading effects on soil health and nutrient cycling[28]. Furthermore, the implications of Bt toxin persistence in the environment, particularly regarding its degradation rates and effects on microbial resistance, remain underexplored. Understanding these dynamics is crucial for assessing the sustainability of Bt-based pest management strategies[27].

Recent studies on the ecological impacts and immune responses of *Bacillus thuringiensis* (Bt) would significantly strengthen a review focused on its biopesticide applications. The interaction between Bt toxins and non-target species, along with the complexities of insect resistance mechanisms, has been highlighted in multiple studies, providing a nuanced understanding of its environmental and biological effects. Abbas[1] provides a comprehensive overview of the controversies surrounding Bt crops, particularly emphasizing environmental safety and the ecological consequences of Bt toxin exposure. For instance, while Bt toxins, such as Cry and Cyt proteins, are highly effective against target pests, their persistence in the environment and potential effects on non-target organisms still require thorough evaluation.

The widespread adoption of Bt crops has raised concerns about unintended effects on beneficial insect populations, pollinators and soil health. Studies have shown that pollen and plant debris from Bt crops can persist in the soil, potentially altering soil microbial communities and impacting soil health. This was further supported by the findings in the 2022 study by Celi et al[13] who reported that the introduction of Bt can significantly impact hemocyte profiles and immune responses in *Rhynchophorus ferrugineus*[13].

In terms of immune responses, recent research has delved into the specific interactions between Bt toxins and insect immune systems. Exposure to Bt toxins in red palm weevil larvae was found to alter hemocyte counts and stress responses, with variations depending on the insect's developmental stage and sex. For instance, males exhibited different patterns of immune modulation compared to females, with variations in the expression of heat shock proteins (Hsp70) within the brain and hemolymph cells. This study highlights the potential for sex-specific differences in Bt susceptibility, adding a new dimension to our understanding of resistance development[13].

Moreover, the ecological impacts of Bt crops extend beyond insect interactions, as shown by recent studies examining non-target organisms. For example, research has pointed out that Bt toxins can impact aquatic ecosystems and can alter the dynamics of microbial populations in soils. The importance of long-term field studies has been emphasized to accurately evaluate the potential ecological risks associated with the widespread deployment of Bt crops. These findings are particularly crucial, given that Bt has been promoted as an environmentally friendly alternative to chemical insecticides.

Genomic Insights into Resistance Mechanisms: The evolution of resistance in insect pests to *Bacillus thuringiensis* (Bt) toxins is a complex process influenced by various genomic factors. Recent advancements in genomics, transcriptomics and proteomics have revealed critical insights into the genetic mechanisms underpinning this resistance. Notable studies have elucidated how genomic alterations in different insect species contribute to varying levels of susceptibility to Bt toxins. One of the primary mechanisms of resistance involves mutations in the genes encoding the receptors that bind to Bt toxins[35,45]. These receptors, primarily cadherin proteins, are essential for the initial interaction between Cry toxins and the midgut epithelial cells of insects. For example, in several Lepidopteran species, mutations in cadherin genes have been shown to reduce the binding affinity of Cry1A toxins, preventing them from exerting their toxic effects[19,44].

The specific nature of these mutations can vary between species, leading to differential susceptibility. For instance, *Spodoptera frugiperda* has developed mutations that confer higher resistance to Cry toxins compared to other Lepidopterans, reflecting the adaptive nature of these receptor alterations. Midgut proteases are critical for the activation of Cry toxins, as they process the toxins into their active forms. Changes in the expression or activity of these proteases can significantly influence an insect's susceptibility to Bt. For instance, upregulation of specific protease inhibitors in resistant populations can prevent the necessary processing of Cry toxins, resulting in reduced susceptibility[44].

Different insect species exhibit varying protease profiles; thus, the ability to activate Bt toxins can differ widely. For example, the expression levels of serine proteases can vary significantly between Spodoptera species, impacting their

overall sensitivity to Bt toxins. The immune response of insects is another layer of complexity that influences resistance to Bt toxins. Insects possess an innate immune system that includes cellular and humoral responses, which can vary significantly among species. Enhanced immune responses can mitigate the effectiveness of Bt toxins by promoting their clearance before they interact with target receptors. For instance, immune pathways, such as those mediated by Toll and Imd signalling, can be activated in response to the presence of Bt toxins, leading to the production of antimicrobial peptides and other immune factors[36].

In different insect species, the intensity and type of immune response can vary, affecting how effectively Bt toxins can function. For example, while some Lepidopterans exhibit robust immune responses to Bt exposure, others may show minimal activation of immune pathways, allowing for greater susceptibility to the toxins. Additionally, insects like *Plutella xylostella* (diamondback moth) have been documented to exhibit a higher degree of immune tolerance to Cry toxins, suggesting that specific adaptations in immune responses can facilitate resistance[41].

Host Immune Modulation: Modulating the host immune response offers a potential strategy to enhance the effectiveness of Bt toxins and overcome resistance. By manipulating the insect immune system, researchers aim to increase the susceptibility of pests to Bt toxins[37]. Certain compounds and treatments can suppress the insect immune response, thereby increasing the effectiveness of Bt toxins. Compounds that inhibit the production or activity of immune factors, such as antimicrobial peptides, can enhance susceptibility to Bt toxins[11]. Incorporating immune modulation strategies into IPM programs can improve the effectiveness of Bt-based control measures. Combining Bt toxins with immune-modulating agents can enhance pest control by both increasing toxin efficacy and reducing the likelihood of resistance development[36,37].

The environmental and non-target effects of Bt toxins are crucial factors in evaluating the safety and sustainability of Bt-based pest control strategies. Recent studies have focused on assessing the impact of Bt toxins on non-target organisms and ecosystems[4]. Bt toxins generally have minimal adverse effects on beneficial insects, such as pollinators and natural enemies of pests. For instance, research indicates that Bt toxins have low toxicity to honeybees and other pollinators, making them a relatively safe option for crops that rely on insect pollination.

The impact of Bt toxins on natural enemies of pests, such as predatory beetles and parasitoid wasps, has also been found to be minimal, which is important for maintaining ecological balance and ensuring effective pest control[16,33]. Studies have assessed the effects of Bt crops and biopesticides on soil microbiota and aquatic ecosystems. While there is some evidence of Bt toxin persistence in soil, the overall impact on soil microbial communities appears to be low. Similarly, the impact on aquatic ecosystems, particularly non-target aquatic invertebrates, has been found to be minimal, further supporting the environmental safety of Bt-based pest control[23].

Empirical Data Analysis: Empirical data analysis reveals the effectiveness of various *Bacillus thuringiensis* (Bt) toxins against specific mosquito populations and other agricultural pests. For instance, Boonserm[5] demonstrated that the Cry4Aa and Cry4Ba proteins exhibit significant toxicity against *Anopheles* mosquitoes, resulting in mortality rates of 80-85%. The study emphasized that these toxins induce cell lysis in target larvae through pore formation in gut cells. Further investigation showed that two proteins utilize different binding domains on a specific *Culex* alkaline phosphatase isoform, suggesting differential actions on target larvae, highlighting the specificity of Cry toxins against various mosquito species[15].

Additionally, it was detailed that Cry toxins bind to specific receptors in the midgut of susceptible insects, initiating a cascade of events leading to gut cell lysis and death, underscoring the importance of receptor interactions in determining biopesticide effectiveness[6].

Table 2
Empirical Data on Bt Toxins and Their Impact

Toxin Type	Target Pest	Mortality Rate (%)	Resistance Observations	Environmental Impact
Cry4Aa and Cry4Ba	*Aedes aegypti*	85-90%	None reported in study	Minimal non-target effects[15]
Cry4Ba	*Anopheles spp.*	80-85%	Reduced binding in resistant strains	Pollen impact on soil microbes[5]
Cry1Ac	*Helicoverpa armigera*	65-70%	Resistance through receptor mutations	Limited effects on beneficial insects[6]
Cyt1A	*Aedes spp.*	75%	Synergistic with Cry toxins, low resistance observed	Broader toxicity to non-targets[10]
Vip3Aa	*Spodoptera frugiperda*	60-80%	Resistance due to altered protease activity	Altered soil enzyme activities[12]

Cyt1A has been shown to enhance the insecticidal effects of Cry toxins through a synergistic mechanism, achieving a mortality rate of approximately 75% against various *Aedes* species. Resistance mechanisms in target pests have also been observed, as resistance to Cry toxins in *Spodoptera frugiperda* is often linked to alterations in protease activity, with a mortality range of 60-80% for Vip3Aa against this pest [10, 12]. Collectively, this empirical evidence underscores the crucial interactions between Bt toxins and their target pests, emphasizing the need for continued research to optimize the sustainable application of Bt-based biopesticides while addressing potential resistance issues (Table 2).

Recent Insights into Bt Toxin Action and Insect Resistance Mechanisms: Recent research has provided deeper insights into the molecular mechanisms underlying the action of Cry and Cyt toxins. Advanced techniques such as cryo-electron microscopy and X-ray crystallography have revealed crucial structural details of toxin-receptor interactions and the process of pore formation. These studies have significantly enhanced our understanding of the diverse Bt toxins and their modes of action. The classification of Bt toxins into three-domain α-PFTs, Cyt-type β-PFTs and aerolysin-type β-PFTs offers a valuable framework for exploring their distinct functions and potential applications. Ongoing research focused on the structural features of these toxins as essential for addressing challenges like resistance development in pests. This knowledge will support the design of more effective biopesticides and transgenic crops, ensuring sustainable pest management. Furthermore, these findings have paved the way for the development of novel Bt toxins with improved activity and specificity, expanding their utility in agriculture[42].

Additionally, ongoing research into the mechanisms of resistance development in insect populations is crucial for maintaining the effectiveness of Bt toxins. Identifying genetic mutations and molecular pathways associated with resistance can inform the design of new toxins and resistance management strategies[39]. One area ripe for exploration is the genetic and molecular basis of insect resistance to Bt toxins. Recent advancements in genomic technologies including next-generation sequencing and CRISPR/Cas9 gene editing, have opened avenues to dissect the genetic factors that contribute to resistance. For example, studies have identified specific mutations in receptor genes such as cadherins and aminopeptidases, that confer resistance to Cry toxins in various insect populations[43].

A detailed examination of these genetic adaptations, particularly in relation to varying environmental pressures and pest management practices, could provide critical insights into how resistance develops and propagates within insect communities. Additionally, there is a growing body of research focused on the role of the gut microbiota in influencing insect susceptibility to Bt toxins. Recent findings suggest that the composition of gut microbial communities can significantly affect how insects respond to Cry and Cyt toxins. For instance, certain microbial species may enhance or diminish the efficacy of these biopesticides by modulating the insect's immune responses or aiding in the metabolism of the toxins[14]. Exploring the interactions between Bt toxins and the gut microbiome could yield valuable insights into potential strategies for mitigating resistance and enhancing the efficacy of Bt biopesticides through microbial interventions.

Furthermore, the ecological implications of Bt toxins are an area that warrants deeper investigation. While the review touches upon the environmental impacts of Bt crop cultivation, it often reiterates well-known findings without delving into emerging research that assesses long-term ecological consequences. For example, studies have indicated that the widespread use of Bt crops can lead to shifts in non-target insect populations, potentially disrupting ecological balance and affecting biodiversity[18]. Incorporating data on these ecological dynamics, along with the implications for integrated pest management (IPM) strategies, would provide a more nuanced understanding of how Bt applications can be optimized while minimizing negative environmental effects. Moreover, the review could benefit from a discussion of the latest innovations in biotechnological approaches for enhancing the specificity and efficacy of Bt toxins.

Recent developments in protein engineering, such as the design of synthetic Cry toxins with tailored receptor binding properties, could present novel strategies for overcoming existing resistance challenges[22]. Highlighting such innovations would not only enrich the review but will also inspire future research directions aimed at developing next-generation Bt biopesticides. The 63-fold increase in resistance to Cry1Ac toxin observed in the *ABCB1KO* strain confirms the crucial role of *PxABCB1* as a functional receptor for Cry1Ac in the diamondback moth (DBM). This study validates earlier findings that linked the downregulation of *PxABCB1* expression to Cry1Ac resistance.

The Cry toxins act by binding to specific midgut receptors, disrupting gut epithelial cells through pore formation, ultimately leading to insect death. However, the absence of functional *PxABCB1* reduces Cry1Ac's binding efficiency, diminishing its toxic effect. Interestingly, the knockout strain did not exhibit resistance to other Bt Cry proteins, indicating that the role of *PxABCB1* is highly specific to Cry1Ac. This suggests that different Bt toxins may rely on distinct receptors or receptor complexes for their activity. Such specificity provides insight into the variability in receptor-ligand interactions which may contribute to the development of selective resistance in insect populations.

The CRISPR/Cas9-based functional characterization of *PxABCB1* provides valuable insights into its role in *Plutella xylostella* resistance to Cry1Ac and avermectin insecticides.

The study highlights the complex interplay between Bt toxin receptors and detoxification pathways, offering practical implications for pest management. By leveraging the specificity of *PxABCB1*'s dual role, pest control strategies can be optimized to minimize resistance development while ensuring sustainable pest suppression[29]. These findings underscore the need for continued research into receptor-based resistance mechanisms and the strategic use of Bt and chemical insecticides in integrated pest management programs. An important recent study provides insights into the role of three aminopeptidase N (APN) genes—*HaAPN1*, *HaAPN2* and *HaAPN5*—in mediating the toxicity of *Bacillus thuringiensis* (Bt) toxins in *Helicoverpa armigera*. Utilizing CRISPR-Cas9 gene-editing technology, Wang et al[38] generated homozygous knockout strains for each APN gene in a susceptible strain (SCD) of *H. armigera* to assess their involvement in the mode of action of Cry1A and Cry2A toxins.

Surprisingly, qualitative binding assays demonstrated no significant impact on the interaction between the toxins and midgut brush border membrane vesicles in the knockout strains. Moreover, bioassays revealed no substantial change in susceptibility to Cry1A or Cry2A toxins compared to the wild-type strain, suggesting that these APN genes may not play a critical or exclusive role in Bt toxin binding or toxicity, as previously hypothesized. This finding challenges the conventional understanding of APNs as key receptors and highlights the need for further research to identify alternative receptors or redundant pathways involved in Bt toxin activity.

The emergence of insect resistance to *Bacillus thuringiensis* (Bt) Cry1 toxins poses significant challenges for sustainable agricultural practices relying on biopesticides and transgenic crops. This study utilized the CRISPR/Cas9 genome engineering system to elucidate the role of the ABC transporter subfamily C genes, *PxABCC2* and *PxABCC3*, in mediating resistance to Cry1Ac in *Plutella xylostella*. The successful construction of homozygous knockout strains ABCC2KO and ABCC3KO—allowed for a direct investigation into the contributions of these genes to the resistance phenotype. The significant resistance levels observed in the knockout strains (724-fold for ABCC2KO and 413-fold for ABCC3KO) compared to the susceptible DBM1Ac-S strain strongly implicate both *PxABCC2* and *PxABCC3* in the Cry1Ac resistance mechanism.

The incompletely recessive nature of the resistance alleles further suggests a complex interaction between the two genes, which could involve multiple mechanisms including altered toxin binding and downstream signaling pathways in response to Cry1 toxins. Qualitative binding assays demonstrated a marked reduction in Cry1Ac binding to midgut brush border membrane vesicles (BBMVs) in both knockout strains. This finding is critical as it indicates that both *ABCC2* and *ABCC3* function as midgut receptors for Bt toxins, reinforcing the hypothesis that ABC transporters play a pivotal role in the susceptibility of *P. xylostella* to Cry1 toxins[20]. The decreased binding in the knockout strains also highlights the importance of these proteins in the initial step of the toxin's mode of action which is binding to specific receptors on the midgut epithelial cells.

Furthermore, the identification of the classic BtR-1 resistance locus as the location for Cry1Ac resistance alleles, further supports the notion that resistance mechanisms in *P. xylostella* are genetically linked and possibly influenced by various evolutionary pressures. This aligns with previous studies indicating that resistance to Cry toxins can arise from genetic mutations that impact the structure and function of toxin receptors, leading to reduced efficacy of Bt-based biopesticides.

Conclusion

A comprehensive understanding of the mechanisms of action of Cry and Cyt toxins, alongside insights into host immune responses and recent advances in resistance management, highlights the critical need for ongoing research in Bt-based pest control. While resistance to Bt toxins presents a significant challenge, innovative strategies such as toxin synergy, immune modulation and the application of genomic tools offer promising avenues for enhancing the efficacy and sustainability of Bt technologies in agriculture. However, to ensure the long-term success of these biopesticides, it is vital to balance their benefits with considerations of environmental impact and non-target effects. This conclusion not only summarizes the key findings of this review but also underscores the need for further research in specific areas such as the development of novel Bt toxins, the exploration of alternative pest control strategies and the investigation of ecological interactions.

Future studies should focus on understanding the genetic basis of resistance, optimizing toxin formulations and assessing the long-term effects of Bt applications on agroecosystems. By addressing these areas, researchers can contribute to the sustainable integration of Bt toxins into pest management practices, ensuring their continued role in promoting agricultural productivity and environmental health.

To advance the understanding and application of *Bacillus thuringiensis* (Bt) toxins in pest management, several avenues for future research should be explored. First, there is a need for the continued exploration and characterization of novel Bt toxins with enhanced specificity and efficacy to improve pest control outcomes. Investigating the genetic and biochemical mechanisms of resistance in target insect populations is crucial for developing effective management strategies and ensuring the long-term utility of these toxins. Additionally, research should focus on integrating Bt toxins with other pest management approaches including biocontrol agents and cultural practices, to optimize pest control effectiveness while minimizing environmental impacts.

Comprehensive ecological risk assessments are also necessary to evaluate the long-term effects of Bt toxins on non-target organisms and overall ecosystem health. While the review acknowledges the advantages of Bt toxins, it is essential to adopt a balanced viewpoint by addressing the challenges and limitations associated with this technology. Resistance development poses a significant threat to the effectiveness of Bt toxins, necessitating the exploration of management practices that can mitigate this risk.

Furthermore, the environmental impacts of Bt toxin applications including non-target effects on beneficial insects and soil microorganisms, require thorough investigation. Lastly, the commercial landscape surrounding Bt products presents challenges such as market concentration and access for smallholder farmers, which must be addressed to ensure the equitable deployment of Bt technologies on a global scale. By considering these research directions and critical perspectives, we can enhance the sustainability and effectiveness of Bt-based pest management strategies.

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