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# Resistance Mechanisms to Bt Toxins in Insect Populations

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**Abstract** Insect resistance to *Bacillus thuringiensis* (Bt) toxins has become a significant challenge in biological control and sustainable agriculture. With the widespread use of Bt toxins in genetically modified crops and biopesticides, insect populations have gradually evolved various resistance mechanisms. This study systematically analyzes the genetic, biochemical, and behavioral resistance mechanisms of insects to Bt toxins, revealing multiple pathways such as point mutations, gene amplification, epigenetic modifications, enhanced detoxification enzyme activity, and changes in behavioral patterns. Additionally, the study explores resistance management strategies, such as refuge strategies, Bt gene pyramiding, and integrated pest management (IPM), in delaying the development of resistance. Through case studies, this research summarizes successful resistance management experiences and the challenges faced, and offers directions for future research. This study aims to provide a scientific basis for the continuous improvement of resistance management strategies to ensure the long-term effectiveness of Bt toxins in agriculture.

**Keywords** Bt toxin; Resistance mechanism; Gene mutation; Resistance management; Insect behavior

## 1 Introduction

*Bacillus thuringiensis* (Bt) is a bacterium known for producing insecticidal proteins, commonly referred to as Bt toxins. These toxins have been widely used in sprayable insecticides and transgenic crops as an effective alternative to synthetic pesticides (Jurat-Fuentes et al., 2021). Bt toxins, particularly Cry and Vip proteins, exhibit highly specific insecticidal activity, effectively targeting a wide range of pests while having a relatively minimal impact on non-target organisms and the environment (Cao et al., 2020; Li et al., 2022). The incorporation of Bt genes into crops has revolutionized agricultural pest management, leading to the development of transgenic plants capable of self-protection and continuous pest resistance (Lazarte et al., 2021).

However, with the widespread use of Bt toxins, target insect populations have gradually evolved resistance, posing a significant threat to the long-term efficacy of Bt toxins (Wei et al., 2019; Jurat-Fuentes et al., 2021). Resistance to Bt toxins has been observed in several insect species, particularly those exposed to transgenic Bt crops (Naik et al., 2018; Jurat-Fuentes et al., 2021). Understanding the mechanisms by which resistance develops is crucial for developing effective management strategies and ensuring the sustainability of Bt technology. These resistance mechanisms may include genetic mutations in target receptors, changes in toxin binding sites, and alterations in midgut proteases (Pinos et al., 2021). By studying these mechanisms, we can gain insights into how resistance develops and spreads, enabling the design of more effective pest management strategies and the development of new Bt toxins with novel modes of action (Wang et al., 2019).

This study aims to compile and analyze current research on the mechanisms of resistance to Bt toxins in insect populations, discuss the implications of resistance for the sustainability of Bt technology, and propose potential management strategies to delay the development and spread of resistance. Through a comprehensive analysis of existing studies, this research hopes to provide a scientific basis for improving Bt technology and ensuring its continued application in agricultural pest management.

## 2 Overview of Bt Toxins

### 2.1 Types of Bt toxins

*Bacillus thuringiensis* (Bt) produces a variety of insecticidal proteins, commonly referred to as Bt toxins, which are utilized in both sprayable formulations and transgenic crops to control insect pests. The primary classes of Bt

toxins include Cry and Cyt proteins. Cry proteins, such as Cry1Ac and Cry1F, are the most widely used and are known for their specificity towards lepidopteran and coleopteran pests (Jurat-Fuentes et al., 2021; Kain et al., 2022). These proteins are produced as protoxins that require activation by insect midgut proteases to become toxic (Tabashnik et al., 2015; Guo et al., 2020). Additionally, there are other classes of Bt toxins, such as Vip (vegetative insecticidal proteins), which have different modes of action and target different insect receptors (Wei et al., 2019).

### 2.2 Mode of action

The mode of action of Bt toxins involves multiple steps. First, the toxin binds to receptors on the epithelial cells of the insect midgut, then forms pores that lead to cell lysis and insect death (Figure 1) (Heckel et al., 2021). The active toxin then binds to specific receptors on the midgut epithelial cells, such as cadherin and ATP-binding cassette (ABC) transporters (Zhu et al., 2019). This binding facilitates the formation of pores in the cell membrane, leading to cell lysis and ultimately causing insect death (Chen et al., 2015; Heckel et al., 2021). Recent studies suggest that protoxins and active toxins may have different modes of action, with protoxins potentially being more effective against resistant insect strains (Tabashnik et al., 2015; Guo et al., 2020).

Figure 1 illustrates the hypothetical model of the mode of action of Bt toxins, including the synergistic mechanisms of transaction and cis-action. It details how toxin monomers bind to receptors to form prepores, which ultimately lead to pore insertion into the insect gut cell membrane, revealing the multi-step action pattern of Bt toxins. This provides an important reference for studying the mode of action of Bt toxins.

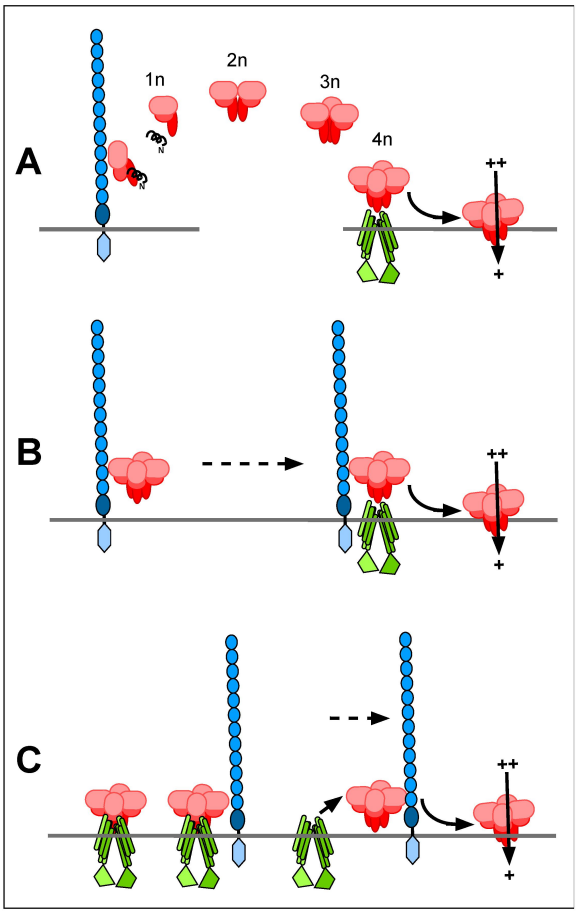



Figure 1 Hypothetical models of the mechanism of synergy between the 12-cadherin domain protein and an ABC transporter (Adopted from Heckel et al., 2021)

Image caption: (A) Trans-acting synergy, due to acceleration of oligomer formation following monomer binding to the cadherin. (B) Cis-acting synergy, due to the cadherin trapping the pre-pore and moving it to the ABC transporter. (C) Cis-acting synergy, due to the cadherin pulling the pre-pore away from the ABC transporter, freeing it to interact with another toxin pre-pore. Dotted lines represent movement of the cadherin within the membrane (Adopted from Heckel et al., 2021)

### 2.3 Target insect populations

Bt toxins are highly effective against a range of insect pests, particularly those in the orders Lepidoptera and Coleoptera. Lepidopteran pests, such as the cotton bollworm (*Helicoverpa armigera*), the diamondback moth (*Plutella xylostella*), and the cabbage looper (*Trichoplusia ni*), are among the primary targets of Cry1Ac and Cry1F toxins (Kain et al., 2022; Xiao et al., 2023). Coleopteran pests, such as the Colorado potato beetle (*Leptinotarsa decemlineat*a), are targeted by other Cry proteins, such as Cry3A (Ren et al., 2021). The effectiveness of Bt toxins against these pests has led to their widespread use in transgenic crops, such as Bt cotton and Bt maize, which express these toxins to provide continuous protection against insect damage (Jurat-Fuentes et al., 2021; Heckel et al., 2021; Kain et al., 2022).

Bt toxins are a diverse group of insecticidal proteins with specific modes of action and target insect populations. Understanding the types, mechanisms, and target pests of Bt toxins is crucial for developing effective pest management strategies and mitigating the risk of resistance development.

## 3 Genetic Mechanisms of Resistance

The genetic resistance mechanisms of insect populations to Bt toxins include point mutations, deletions and insertions, gene silencing, gene amplification, and possible epigenetic modifications. These mechanisms alter the target sites or expression levels of proteins that interact with Bt toxins, thereby reducing the efficacy of these biopesticides and posing a significant challenge to their sustainable use in agriculture. In studies on the mode of action of Bt toxins, mutations in target genes are considered one of the key factors in the development of insect resistance (Figure 2). Research has shown that certain gene mutations in insects can alter the binding capacity of the toxin to its receptor, rendering the toxin inactive (Wang et al., 2019).

Figure 2 provides a detailed illustration of the receptor screening process for Bt toxins in insect cells. By overexpressing receptor proteins, the binding abilities of the toxin to different receptors were evaluated. These mutations weaken the binding capacity of Bt toxins to the receptors, leading to the development of resistance in insects. The varying binding effects of different toxins and receptors depicted in the figure offer critical experimental evidence for studying resistance mechanisms.

### 3.1 Mutations in target genes

#### 3.1.1 Point mutations

Point mutations in target genes are a common mechanism by which insects develop resistance to Bt toxins. For instance, in the fall armyworm (*Spodoptera frugiperda*), point mutations in the *ABCC2* gene have been linked to resistance to the Cry1F protein (Guan et al., 2020). Similarly, a single point mutation in the cadherin gene of the cotton bollworm (*Helicoverpa armiger*a) results in mislocalization of the cadherin protein, which underpins resistance to Cry1Ac toxin (Xiao et al., 2017). These mutations alter the structure and function of the proteins that Bt toxins target, thereby reducing the efficacy of the toxins.

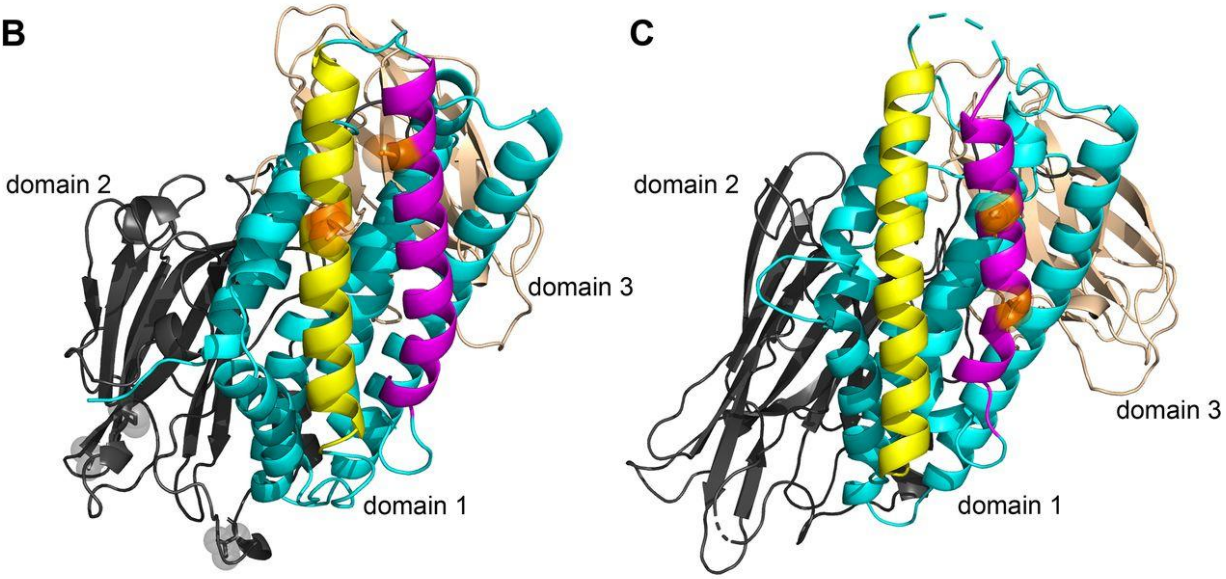
#### 3.1.2 Deletions and insertions

Deletions and insertions in target genes may also confer resistance to Bt toxins in insects. For example, in *Plutella xylostella* (diamondback moth), CRISPR/Cas9-mediated deletion mutations in the *PxABCC2* and *PxABCC3* genes resulted in high resistance to the Cry1Ac toxin (Guo et al., 2019). Studies have found multiple point mutations in target genes such as acetylcholinesterase 1 (ace-1), including A201S, G227A, and F290V, which are associated with resistance to organophosphates and pyrethroids (Figure 3). Similarly, in some populations of Spodoptera frugiperda in Brazil, a 12-base pair insertion mutation was found in the *ABCC2* gene, which is associated with resistance to Bt proteins (Guan et al., 2020). These genetic changes may lead to the production of truncated, non-functional proteins that are unable to effectively bind Bt toxins.

The study by Guo et al. (2019) illustrated four mutant alleles (r1-r4) of the *ABCC2* gene associated with resistance to Cry1F toxin, with r4 being a newly discovered mutation in this study. The figure details the positions of these mutations in both the genomic and protein structures, highlighting how these mutations may result in loss of function. This provides an important reference for understanding how deletions and insertions in target genes can confer resistance to Bt toxins in insects.

**A**

| Protein | Structural class | Domain 1 | Domain 2 | Domain 3 | Domain 4-7 | Commercial Trait |
|---|---|---|---|---|---|---|
| Cry1Da_7 | three-domain | 1Da | 1Da* | 1Da | 1Da/1Ab** | |
| Cry1B.868 | three-domain | 1Be | 1Be | 1Ca | 1Ab | |
| Cry1A.105 | three-domain | 1Ab | 1Ab | 1Fa | 1Ac | corn |
| Cry1F.842 | three-domain | 1Fa | 1Fa | 1Fa | 1Ac | corn |
| Cry2Ab | three-domain | 2Ab | 2Ab | 2Ab | N/A | corn/cotton |
| Vip3A | Vip3 | N/A | N/A | N/A | N/A | corn |




**D**

**Domain 2**

| | Cry1Da_7 | Cry1B.868 | Cry1A.105 | Cry1F.842 | Cry2Ab |
|---|---|---|---|---|---|
| Cry1Da_7 | 100 | 32 | 39 | 42 | 14 |
| Cry1B.868 | 29 | 100 | 28 | 29 | 10 |
| Cry1A.105 | 37 | 29 | 100 | 38 | 12 |
| Cry1F.842 | 40 | 31 | 39 | 100 | 16 |
| Cry2Ab | 13 | 10 | 12 | 15 | 100 |

**E**

**Domain 3**

| | Cry1Da_7 | Cry1B.868 | Cry1A.105 | Cry1F.842 | Cry2Ab |
|---|---|---|---|---|---|
| Cry1Da_7 | 100 | 39 | 52 | 52 | 14 |
| Cry1B.868 | 38 | 100 | 42 | 42 | 21 |
| Cry1A.105 | 50 | 42 | 100 | 100 | 21 |
| Cry1F.842 | 50 | 42 | 100 | 100 | 21 |
| Cry2Ab | 13 | 22 | 22 | 22 | 100 |

Figure 2 Sequence and structure relationship between Bt insecticidal proteins in current and next-generation above-ground traits (Adopted from Wang et al., 2019)

Image caption: (A) Protein sequence information on the different NIPs, indicated by Bt toxin holotype nomenclature. The asterisk indicates the Cry1Da domain, in which substitutions were made to enhance CEW activity. Domains 4 to 7 of the three-domain Cry1 proteins are protoxin domains that are digested in vivo and thus are not part of the active ingredient; the Cry1Da_7 active core was appended to both Cry1Da and Cry1Ab protoxin domains and tested separately (double asterisk). Cry2Ab does not have these protoxin domains. Vip3A is of a different structural class whose sequence is different and structurally distinct from those of three-domain Cry proteins. N/A, not applicable. (B) Crystal structure of Cry1Da_7-DIP showing the three-domain architecture of domain 1 (cyan), domain 2 (gray), and domain 3 (light pink) in cartoon representation as well as helix 3 (yellow) and helix 4 (magenta) in domain 1. The key domain 1-disabling cysteine substitutions V108C and E128C are highlighted with orange sticks and semitransparent spheres corresponding to their side chain. The gray sticks and semitransparent spheres in domain 2 indicate the side chains of substitutions (S282V, Y316S, and I368P) that confer increased CEW specific activity. (C) Model of the three-dimensional architecture of Cry1B.868-DIP protein in cartoon representation with the above-described color scheme. The key domain 1-disabling substitutions A160N and N167D are highlighted with orange sticks and semitransparent spheres corresponding to their side chain. (D) Percent sequence identity between domains 2 of FAW-active insecticidal proteins based on comparative sequence analysis by multiple-sequence alignment (74). (E) Percent sequence identity between these proteins in domain 3 (Adopted from Wang et al., 2019)

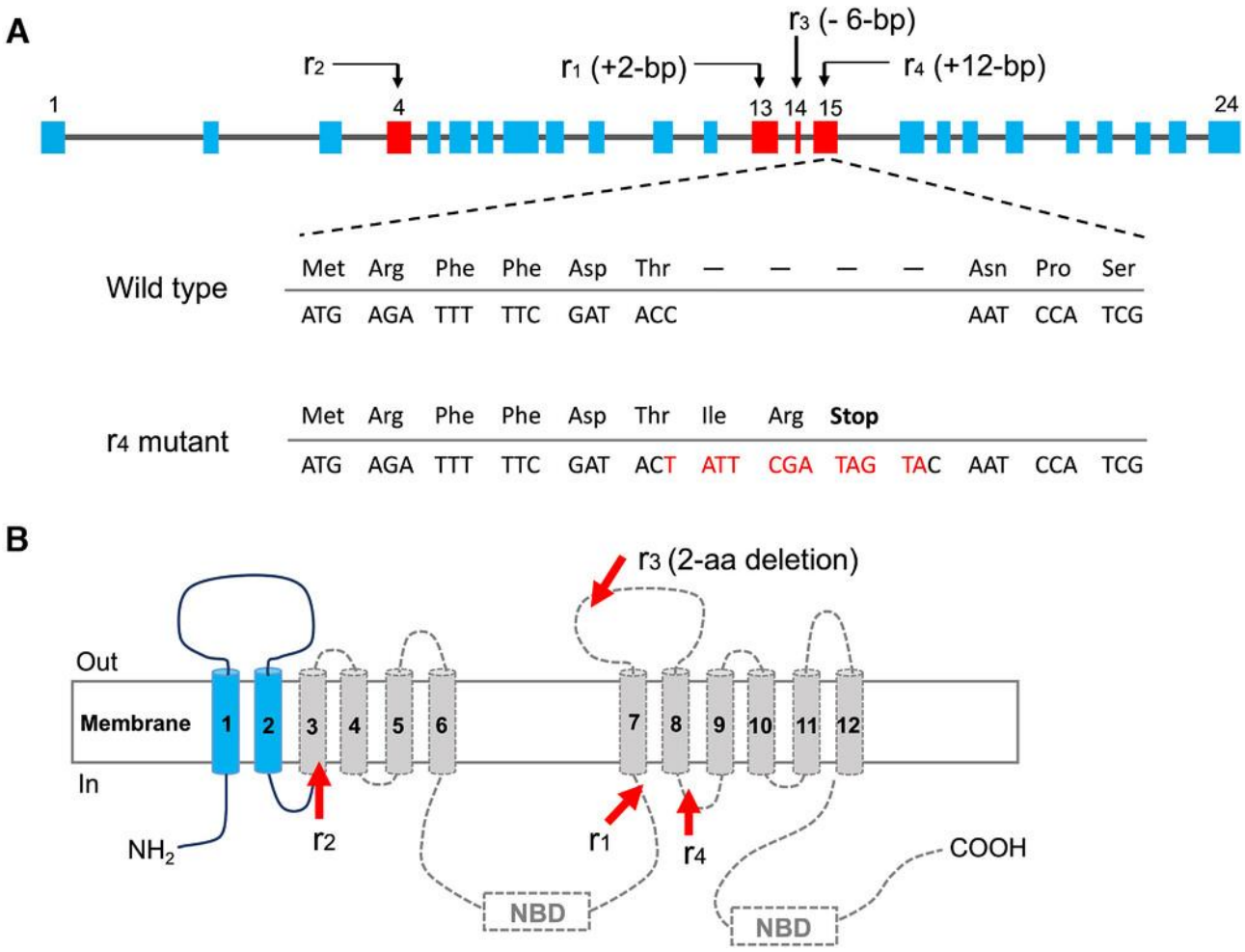



Figure 3 Four mutant alleles (r1–r4) of SfABCC2 associated with Cry1F resistance in *Spodoptera frugiperda*. (A) Genomic structure of SfABCC2. (B) Protein structure of SfABCC2. The r1, r2 and r3 alleles were reported respectively by Banerjee et al. (2017), Flagel et al. (2018) and Boaventura et al. (2019). The r4 allele was detected in the present study (Adopted from Guo et al., 2019)

#### 3.1.3 Gene silencing

Gene silencing mechanisms, such as RNA interference (RNAi), can also play a role in resistance. Although specific examples of RNAi-mediated resistance to Bt toxins were not detailed in the provided papers, the down-regulation of key genes involved in Bt toxin binding and activation could theoretically contribute to resistance. For instance, the down-regulation of ABCC2 and ABCC3 genes has been linked to resistance in several lepidopteran species (Guo et al., 2019).

### 3.2 Gene amplification

Gene amplification, where multiple copies of a resistance gene are produced, can enhance resistance levels. While the provided papers did not specifically mention gene amplification as a resistance mechanism to Bt toxins, it is a well-documented phenomenon in other contexts of insecticide resistance. The increased expression of resistance genes can lead to higher levels of the corresponding proteins, which can sequester or degrade Bt toxins more effectively.

### 3.3 Epigenetic modifications

Epigenetic modifications, such as DNA methylation and histone modification, can alter gene expression without changing the DNA sequence. These modifications can potentially contribute to Bt resistance by regulating the expression of genes involved in toxin binding and activation. Although the provided papers did not explicitly discuss epigenetic modifications in the context of Bt resistance, this area remains an important avenue for future research to fully understand the complexity of resistance mechanisms.

## 4 Biochemical Mechanisms of Resistance

### 4.1 Altered toxin binding

Altered toxin binding is a common biochemical mechanism by which insects develop resistance to Bt toxins. This mechanism involves changes in the receptors on the insect midgut cells, where Bt toxins typically bind. Mutations or modifications in these receptors can reduce the binding affinity of the toxins, thereby diminishing their efficacy. This resistance mechanism has been observed in various insect species and poses a significant challenge in managing Bt resistance. Due to receptor alterations, the toxins cannot effectively bind to their target sites, reducing their toxic effect and allowing insects to survive exposure to Bt toxins. The widespread occurrence of this mechanism makes it a crucial area of study (Jurat-Fuentes et al., 2021).

### 4.2 Enhanced detoxification enzymes

Insects also resist Bt toxins through enhanced detoxification enzyme activity, which is another critical biochemical resistance mechanism. These enzymes can metabolize and neutralize Bt toxins before they reach their target sites, thereby reducing the toxins' effectiveness. Enhanced detoxification capacity is often associated with the overexpression or increased activity of these enzymes.

#### 4.2.1 Cytochrome P450 monooxygenases

Cytochrome P450 monooxygenases (P450s) play a crucial role in detoxifying xenobiotics, including Bt toxins. These enzymes are involved in the metabolic detoxification of a wide range of substances, such as phytochemicals, insecticides, and environmental pollutants. The overexpression of P450s is closely linked to the enhanced detoxification capabilities observed in resistant insects. P450s can work through multiple pathways to detoxify xenobiotics, and their regulation involves complex networks of transcription factors and signaling pathways. Studies have shown that P450s can be induced through specific signaling pathways in resistant insects, significantly enhancing their detoxification capabilities (Lu et al., 2020; Nauen et al., 2021).

#### 4.2.2 Glutathione S-transferases

Glutathione S-transferases (GSTs) are another group of critical detoxification enzymes involved in the resistance to Bt toxins. GSTs catalyze the conjugation of glutathione to toxic substances, making these substances more water-soluble and easier to excrete. Although the role of GSTs in Bt toxin resistance is less documented compared to P450s, studies indicate that they play a significant role in detoxifying other insecticides and xenobiotics. Therefore, GSTs' potential role in Bt resistance is an important area for further investigation (Jurat-Fuentes et al., 2021).

#### 4.2.3 Esterases

Esterases are enzymes that hydrolyze ester bonds in various substrates, including insecticides. These enzymes can detoxify Bt toxins by breaking down their ester linkages, thereby reducing the toxins' potency. In resistant insect populations, the expression or activity of esterases is often significantly increased, further accelerating the detoxification process of Bt toxins. Esterases, like P450s and GSTs, contribute to the enhanced detoxification capabilities observed in resistant insects, and their role in Bt resistance is increasingly recognized (Nauen et al., 2021).

### 4.3 Changes in gut microbiota

Changes in the gut microbiota of insects can also play a critical role in Bt toxin resistance. The gut microbiota not only affects the overall health and metabolic capacity of the host insect but can also directly or indirectly participate in the detoxification of xenobiotics. Research has shown that changes in the composition and function of the gut microbiota can enhance insect resistance to Bt toxins in several ways. Some microbes can directly degrade Bt toxins, reducing their toxicity, while others may enhance the host's immune response and detoxification enzyme activity, further increasing resistance. Although this area of research is still developing, preliminary studies suggest that gut microbiota plays an essential role in Bt resistance, and future research will provide more scientific insights into this mechanism (Pinos et al., 2021).

By understanding these biochemical mechanisms in depth, researchers can develop more effective resistance management strategies to mitigate and delay the development of insect resistance to Bt toxins, ensuring the continued efficacy of Bt-based pest control methods.

## 5 Behavioral Mechanisms of Resistance

### 5.1 Avoidance behavior

Avoidance behavior is a critical mechanism by which insects can resist the effects of Bt toxins. Insects may develop the ability to detect and avoid Bt-treated plants or areas, thereby reducing their exposure to the toxins. This behavior can be particularly effective in heterogeneous environments where non-Bt plants are available as refuges. For instance, the presence of abundant refuges of non-Bt host plants has been shown to favor sustained susceptibility to Bt crops by providing alternative feeding sites for pests, thereby reducing the selection pressure for resistance (Tabashnik et al., 2023).

### 5.2 Changes in feeding patterns

Changes in feeding patterns represent another behavioral adaptation that insects can employ to mitigate the impact of Bt toxins. Insects may alter their feeding habits, such as feeding at different times of the day or targeting different parts of the plant that have lower concentrations of Bt toxins. For example, studies have shown that the western corn rootworm, a significant pest of maize, exhibits changes in its feeding behavior when exposed to Bt-expressing maize. Resistant insects were found to maintain a more stable microbiome compared to susceptible insects, suggesting that their altered feeding patterns might help them avoid the detrimental effects of Bt toxins (Paddock et al., 2021).

### 5.3 Altered life cycle

Insects may also develop resistance to Bt toxins through alterations in their life cycle. This can include changes in developmental timing, such as faster or slower growth rates, which can help them avoid peak periods of Bt toxin expression in plants. For example, the larvae of *Anoplophora glabripennis*, when fed on transgenic poplar lines expressing dual Bt toxins, showed significant changes in the expression of genes related to their growth and development. These changes suggest that the larvae are adapting their life cycle to mitigate the effects of Bt toxins (Ren et al., 2021). Additionally, the nutritional environment can influence the susceptibility of insects to Bt toxins. Helicoverpa zea, a polyphagous pest, showed a 100-fold increase in LC50 when reared on protein-biased diets compared to carbohydrate-biased diets, indicating that diet-mediated plasticity can alter the life cycle and resistance levels of insects (Deans et al., 2017).

## 6 Management Strategies to Combat Resistance

### 6.1 Refuge strategies

Refuge strategies involve planting non-Bt crops near Bt crops to maintain a population of pests that remain susceptible to Bt toxins. This approach helps to delay the evolution of resistance by ensuring that susceptible pests can mate with potentially resistant individuals, thereby diluting resistance genes in the pest population. The high-dose/refuge strategy has been particularly successful in North America, where it has helped maintain susceptibility in major pests like the European corn borer and the pink bollworm (Huang et al., 2011). However, the effectiveness of this strategy can be compromised by non-compliance with refuge requirements and the presence of pests with non-recessive resistance (Brewerand Bonsall, 2020). Natural refuges, such as non-Bt host plants, have also been shown to delay resistance, although they may not be as effective as structured non-Bt cotton refuges (Jin et al., 2014).

### 6.2 Pyramiding Bt genes

Pyramiding involves stacking multiple Bt genes that produce different toxins within the same crop. This strategy aims to provide multiple modes of action against pests, making it more difficult for them to develop resistance. Pyramided Bt crops, such as those expressing both Cry and Vip3Aa toxins, have shown promise in delaying resistance. The synergistic action of multiple toxins can effectively manage pest populations even when some pests have developed resistance to one of the toxins. However, the design of these pyramids must consider

potential cross-resistance and antagonism between toxins, which can reduce their effectiveness (Carrière et al., 2015). Products like SmartStax and PowerCore have demonstrated improved resistance management benefits compared to single-toxin products, allowing for smaller refuges and less dependence on high mortality rates among susceptible pests (Storer et al., 2012).

### 6.3 Integrated pest management (IPM)

Integrated Pest Management (IPM) combines multiple control tactics to manage pest populations sustainably. This approach includes the use of Bt crops, refuges, biological control agents, and cultural practices. IPM aims to reduce the reliance on any single control method, thereby delaying the evolution of resistance. For instance, combining Bt crops with the release of sterile insects or transgenic insects has been proposed as a method to manage pest populations and resistance simultaneously (Brewerand Bonsall, 2020; Zafar et al., 2020). Additionally, IPM strategies can be tailored to regional pest pressures, ensuring that Bt crops are used where they are most beneficial and reducing their use in areas with low pest pressure. By integrating various control methods, IPM can enhance the sustainability of Bt crops and delay the development of resistance in pest populations (Gassmann and Reisig, 2022).

## 7 Case Studies

### 7.1 Successful management of resistance

The successful management of resistance to Bt toxins in insect populations has been achieved through various strategies. One notable example is the implementation of proactive resistance management strategies such as the refuge strategy and the pyramid strategy. These strategies have been effective in sustaining the control of target pests for nearly two decades (Wu, 2014). Additionally, the use of multi-toxin Bt crops has been shown to delay resistance evolution by providing redundant killing mechanisms, which is supported by field outcomes and theoretical predictions (Tabashnik et al., 2013). In the United States, regional suppression of pest populations and even pest eradication have been achieved in some cases, leading to reduced reliance on conventional insecticides and increased profits for farmers (Gassmann and Reisig, 2022).

### 7.2 Challenges faced in different regions

Despite the successes, several challenges have been encountered in different regions. For instance, practical resistance to Bt crops has been documented in some populations of 11 pest species across seven countries, affecting nine widely used Bt toxins (Tabashnik et al., 2023). In the United States, pests have evolved resistance to multiple Bt traits, compromising the effectiveness of Bt crops and leading to increased crop damage (Gassmann and Reisig, 2022). The spatial heterogeneity of Bt crop deployment in small-holder farm systems has also been identified as a factor that can accelerate the regional evolution of resistance, highlighting the need for spatially explicit resistance management strategies (Huang et al., 2017). Furthermore, the genetic diversity of resistance mechanisms, including mutations in ABC transporters and enhanced immune responses in insects, poses additional challenges for understanding and managing resistance (Gahan et al., 2010; Heckel et al., 2012; Tay et al., 2015; Xiao et al., 2023).

### 7.3 Lessons learned

Several lessons have been learned from the management of Bt resistance in insect populations. The importance of maintaining abundant refuges of non-Bt host plants has been underscored as a key factor in delaying resistance evolution (Tabashnik et al., 2013; Tabashnik et al., 2023). Understanding the molecular genetic basis of resistance, such as the role of ABC transporters in toxin mode of action, is crucial for developing effective resistance detection methods and management strategies (Gahan et al., 2010; Heckel et al., 2012; Tay et al., 2015). The integration of multiple strategies, including the use of multi-toxin Bt crops and spatially explicit deployment patterns, can enhance the sustainability of Bt technology (Tabashnik et al., 2013; Huang et al., 2017). Finally, continuous monitoring and proactive adaptation of resistance management strategies are essential to address the evolving threat of resistance and ensure the long-term success of Bt crops (Wu, 2014; Gassmann and Reisig, 2022).

## 8 Concluding Remarks

Research on the resistance mechanisms of insect populations to *Bacillus thuringiensis* (Bt) toxins has revealed several key findings. The evolution of resistance to Bt toxins has become a major challenge to Bt-based pest control strategies, particularly in transgenic crops where lepidopteran and coleopteran insects have shown significant resistance. Key resistance mechanisms include mutations in ABC transporters, which play a crucial role in the mode of action of Bt toxins. Additionally, the dynamics of cross-resistance between Bt and other insecticides indicate that resistance development involves complex genetic and biochemical pathways. The continuous evolution of Bt toxins to overcome resistance underscores the need for ongoing innovation in Bt technology.

Continuous monitoring and research are essential for managing and mitigating the evolution of Bt resistance. The changes in global resistance patterns emphasize the importance of early detection and timely countermeasures. A deep understanding of the genetic and biochemical bases of resistance, particularly the role of ABC transporters, is crucial for developing effective management strategies. Moreover, research shows that spatial heterogeneity in the deployment of Bt crops can significantly influence the speed of resistance evolution, necessitating targeted management practices based on specific environmental conditions.

Future research needs to further explore how ABC transporters and other genetic factors contribute to Bt resistance, which will deepen the understanding of resistance mechanisms. Investigating the cross-resistance dynamics between Bt and other insecticides is also crucial, as it can lead to the development of pest management strategies that address multiple resistance pathways. Continuous development and testing of new Bt toxin variants will enhance their effectiveness against resistant insect populations. Expanding field studies and monitoring will provide more data to optimize resistance management strategies and ensure their effectiveness in different agricultural environments. Promoting Integrated Pest Management (IPM) practices that combine Bt crops with other control methods can effectively reduce the selection pressure for resistance.

By advancing these key research areas, future studies will not only enhance the sustainability of Bt technology but also provide innovative solutions for broader pest control. As the understanding of resistance mechanisms deepens and new Bt toxins are developed, scientists will be able to design more precise management strategies, delaying the onset of resistance and mitigating its impact on agricultural production. These efforts will ultimately ensure that Bt technology continues to serve as an effective and sustainable tool, contributing to global agricultural security and environmental protection.

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### Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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