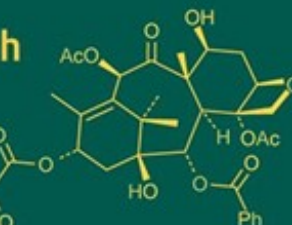
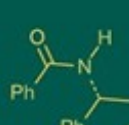
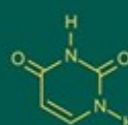
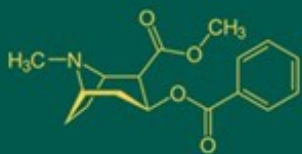


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Current status of insect pest resistance in Bt cotton

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ABSTRACT

The commercialization of transgenic cotton (*Gossypium hirsutum* L.) expressing insecticidal crystalline (Cry) and vegetative insecticidal proteins (Vip) from *Bacillus thuringiensis* (Bt) revolutionized global cotton production by suppressing key lepidopteran pests and reducing synthetic insecticide reliance. However, the long-term sustainability of this technology is severely threatened by field-evolved insect resistance. This review synthesizes the current global status of pest resistance to Bt cotton, contrasting the widespread practical resistance of pink bollworm (*Pectinophora gossypiella* Saunders) in India with its sustained susceptibility in China and ultimate eradication in the United States. We examine the molecular mechanisms driving this resistance, focusing on target-site mutations in cadherin, ABCC2 and ABCA2 midgut receptors, the emerging genetic basis of *Vip3A_a* resistance in *Helicoverpa zea* and evaluate how abiotic stressors such as drought and heat accelerate resistance by altering plant-wide toxin expression. Finally, we analyse next-generation management frameworks, including multi-toxin pyramids, structured versus seed-blend refuges and the integration of emerging RNA interference (RNAi) traits to secure sustainable cotton landscapes.

Keywords: *Bacillus thuringiensis*, *Gossypium hirsutum*, Field-evolved resistance, Pink bollworm, *Vip3A_a*, Pyramids, Refuge strategy, RNA interference

1. Introduction

For nearly three decades, transgenic crops expressing insecticidal proteins from the soil bacterium *B. thuringiensis* (Bt) have stood as a definitive cornerstone of global agricultural biotechnology (Gould, 1998; Tabashnik *et al.*, 2008) [1, 2]. Since its commercial introduction in 1996, Bt cotton (*Gossypium hirsutum* L.) has been adopted by millions of farmers worldwide (James, 2016) [3]. It has provided highly effective, target-specific control of devastating lepidopteran pests, including the pink bollworm (*P. gossypiella*), American bollworm (*Helicoverpa armigera* Hubner) and tobacco budworm (*Heliothis virescens* Fabricius) (Benedict *et al.*, 1996) [4].

The socio-economic and environmental benefits of this adoption have been profound. The technology is characterized by massive reductions in broad-spectrum chemical insecticide applications (Fleming *et al.*, 2018) [5], enhanced populations of beneficial non-target arthropods (Lu *et al.*, 2012) [6] and increased regional yields across both smallholder and large-scale farming systems (Cattaneo *et al.*, 2006 [7]; National Academies of Sciences, Engineering and Medicine [NASEM], 2016; Gassmann and Reisig, 2023) [8]. Despite this historic success, the fundamental biological vulnerability of Bt technology remains an evolutionary inevitability: field-evolved resistance (Comas *et al.*, 2014) [9]. As the spatial and temporal scale of Bt cotton cultivation expanded globally, target insect populations were subjected to relentless, widespread selection pressure (Tabashnik *et al.*, 2013) [10]. Today, the global landscape of Bt cotton efficacy is highly fragmented (Tabashnik *et al.*, 2017) [11]. While some regions continue to enjoy prolonged pest suppression through advanced multi-toxin pyramiding and structural seed-blend mixtures (Quan *et al.*, 2023) [12], others are grappling with severe practical resistance episodes that threaten to return farmers to the chemical pesticide treadmills of the past (Kranthi *et al.*, 2005) [13].

This rapid resistance development is driven by highly complex, multi-layered regulatory and genetic defences within target pests. These defences span target-site mutations such as disruptions in midgut cadherin receptors, ABCC2 and ABCA2 transporters, along with altered midgut protease profiles and accelerated epithelium regeneration driven by internal immune signalling pathways (Fabrick *et al.*, 2023) [14]. Beyond the pink bollworm, an increasingly urgent parallel case is emerging in the polyphagous corn earworm/bollworm, *Helicoverpa zea*, in which practical resistance to Cry toxins and the erosion of susceptibility to *Vip3A_a* now constitute one of the most closely watched resistance fronts in U.S. cotton and maize agroecosystems (Yang *et al.*, 2021; Kerns *et al.*, 2023; Yang *et al.*, 2024) [15, 16, 17].

To counter these adaptive mechanisms, current structural shifts in Insect Resistance Management (IRM) emphasize deploying next-generation pyramided multi-toxin cultivars (such as those introducing *Vip3A* along

side *CryIA_c* and *Cry2A_b*) and exploring innovative seed-mixture strategies (or “Refuge-in-a-Bag”) to ensure field-level compliance without requiring active farm layout modifications (Ives *et al.*, 2011; Quan and Wu, 2023) [18, 12]. In parallel, plant-mediated RNA interference is advancing from proof-of-concept toward a genuinely complementary mode of action that can be pyramided with *Bt* toxins to counter resistance that has already fixed in some field populations (Ni *et al.*, 2017; Qi *et al.*, 2024) [19, 20].

2. Global spectrum of resistance: regional case studies

The evolutionary trajectory of insect resistance to *Bt* cotton varies drastically by geography (Bourguet *et al.*, 2005) [21]. These differences are largely unlinked to the biology of the insects themselves; rather, they are dictated by regional agricultural infrastructures, farm sizes and varying levels of compliance with insect resistance management (IRM) frameworks (Onstad, 2014; Sisterson *et al.*, 2005) [22, 23].

2.1 India: the epicentre of practical resistance

India represents the most critical warning sign for transgenic crop durability (Gutierrez *et al.*, 2015) [24]. The country rapidly adopted first-generation single-toxin cotton (Bollgard I, expressing *CryIA_c*) in 2002, followed by second-generation dual-toxin cotton (Bollgard II, expressing *CryIA_c* + *Cry2A_b*) in 2006 (Kranthi, 2014; Fabrick *et al.*, 2023) [25, 14].

Within a few years of intensive cultivation, populations of the pink bollworm (*P. gossypiella*) developed widespread, high-level practical resistance to *CryIA_c*, followed closely by cross-resistance and complete field breakdown of *Cry2A_b* in major cotton growing states such as Gujarat, Maharashtra and Andhra Pradesh (Dhurua *et al.*, 2011; Naik *et al.*, 2018) [26, 27]. Multi-location monitoring across the north, central and south cotton zones between 2007 and 2023 has confirmed that *P. gossypiella* has firmly established itself as a devastating mid- and late-season pest, with green-boll damage on *Bt* genotypes rising sharply after 2014 in central and southern India and after 2018 in the north (Kumar *et al.*, 2025a, 2025b; Fabrick *et al.*, 2023) [28, 29].

This was the first documented field resistance of pink bollworm to *CryIA_c* in Bollgard cotton reported anywhere globally, established through F2 screening and diagnostic-dose bioassays across Gujarat during 2009–2010 (Mohan *et al.*, 2016) [30], and subsequent national surveillance confirmed its spread and rising economic impact across all three Indian cotton zones (Nagrare *et al.*, 2023) [31]. The primary driver behind this rapid breakdown was a systemic failure of the high-dose/refuge strategy (Fabrick *et al.*, 2023) [14]. India’s agricultural landscape is dominated by millions of smallholder farmers operating on fragmented plots of land (Choudhary *et al.*, 2015) [32]. Implementing and monitoring structured, non-*Bt* cotton refuges proved socio-economically and logistically unfeasible (Mohan *et al.*, 2019; Fabrick *et al.*, 2023) [33, 14]. Furthermore, the unauthorized sale of unapproved, unmonitored F2-generation *Bt* seeds and the prolonged survival of cotton stalks in fields past the normal season created a continuous, sub-lethal selection pressure that accelerated the fixation of resistance alleles (Kranthi, 2014; Venugopalan *et al.*, 2009) [25, 34].

As a consequence of total *Bt* failure against pink bollworm, growers have increasingly had to revert to heavy chemical interventions such as modern synthetic insecticides, including chlorantraniliprole, spinosad and indoxacarb, to salvage lint yields (Rakesh *et al.*, 2024) [35]. Meanwhile, the American bollworm (*H. armigera*), though still relatively suppressed compared to the pre-*Bt* era, has shown steady decreases in baseline susceptibility, demanding constant vigilance (Kranthi *et al.*, 2005) [13].

2.2 China: natural refuges and controlled susceptibility

In stark contrast to India, China has successfully maintained the field susceptibility of *P. gossypiella* to *CryIA_c* cotton for nearly three decades (Wu *et al.*, 2008) [36]. This success stems from a unique “natural refuge” system (Wu *et al.*, 2002; Fabrick *et al.*, 2023) [14, 37].

Unlike the vast monocultures seen in western countries, China’s small-scale agricultural system features high crop diversity (Cui *et al.*, 2019) [38]. Cotton fields are intrinsically interplanted alongside non-*Bt* host crops of *P. gossypiella* and *H. armigera*, such as corn, peanuts, soybeans and vegetables (Lu *et al.*, 2010; Wan *et al.*, 2012) [39, 40]. These diverse, non-*Bt* crops generate an abundance of homozygous susceptible (*S^S*) individuals that emerge naturally and mate with the rare homozygous resistant (*R^R*) individuals emerging from *Bt* fields (Wang *et al.*, 2018) [41].

Long-term multi-decadal panel evaluations confirm that this natural refuge mechanism, paired with dual-strategy resistance management, has prevented field control failures of target lepidopteran pests across key production zones (Quan *et al.*, 2023) [12]. While localized resistance alleles have occasionally risen in areas such as the Yangtze River Valley due to shifting cropping patterns, aggressive management transitions towards multi-gene traits such as stacking GFM *CryIA_{b/c}* with CpTI (cowpea trypsin inhibitor) genes successfully suppressed target populations while significantly slashing historical insecticide footprints (Jin *et al.*, 2015; Wan *et al.*, 2017; Fabrick *et al.*, 2023) [42, 43, 14]. Screening of field-collected pink bollworm from the Yangtze River Valley confirms that, where resistance alleles do arise, recessive cadherin mutations remain the dominant mechanism and their frequency is kept low by the natural refuge, reinforcing the durability of this regionally distinct management model. It is important to note that this durability is not uniform across pest species within China: long-term bioassay monitoring of *Helicoverpa armigera* field populations from 2005 to 2017 detected a significant, sustained increase in *CryIA_c* resistance frequency in northern China, in contrast to the sustained susceptibility documented for pink bollworm over the same period (Zhang *et al.*, 2019) [44]. This divergence has prompted the development of dedicated resistance-monitoring protocols for pyramided (*CryIA_c* + *Cry2A_b*, or *CryIA_c* + Vip3A) *Bt* cotton specifically targeting *H. armigera*, reflecting a recognition that China’s natural-refuge success with pink bollworm cannot be assumed to extend automatically to every lepidopteran pest sharing the same cotton landscape (Ruan *et al.*, 2024) [45].

2.3 The United States and Australia: eradication and advanced pyramiding

The United States and Australia represent highly regulated, industrialized agricultural systems that have successfully delayed resistance through strict legal mandates and rapid technological transitions (Tabashnik *et al.*, 2010) [46].

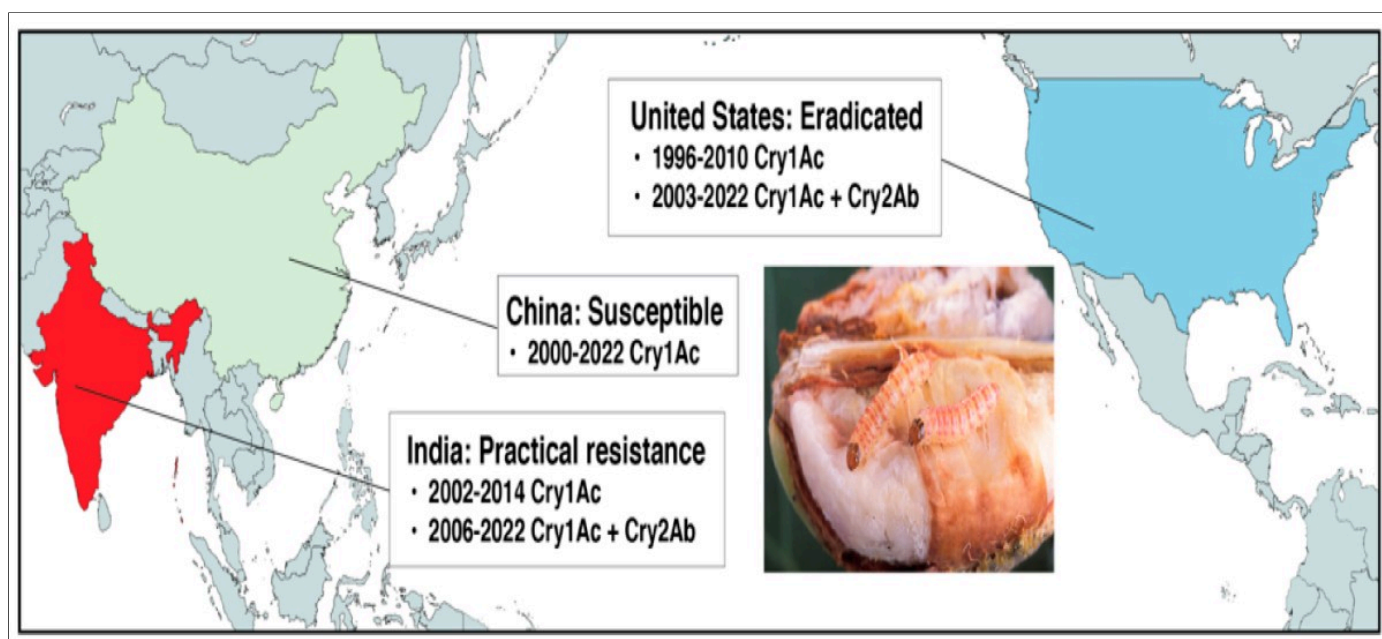
In the southwestern United States and northern Mexico, a historic milestone was achieved with the complete eradication of the pink bollworm from all cotton-producing regions (Carriere *et al.*, 2018; Tabashnik *et al.*, 2023) [47, 48]. This was accomplished through a tightly coordinated, multi-tactic area-wide IPM program that blended near-100 per cent *Bt* cotton adoption with the sterile insect technique (SIT), intensive pheromone mating disruption and strict structured refuge enforcement (Fabrick *et al.*, 2023) [14].

Currently, the primary target pests in the US are *H. zea* Boddie (bollworm/corn earworm) and *H. virescens* (tobacco budworm) (Welch *et al.*, 2021) [49]. Because *H. zea* has inherently lower baseline susceptibility to Cry proteins and rapidly developed field resistance to dual-toxin cottons (Bollgard II and WideStrike), the US and Australia have aggressively transitioned to third-generation multi-toxin pyramids (Fleming *et al.*, 2018) [5].

Technologies such as Bollgard 3, WideStrike 3 and TwinLink Plus layer a third, structurally distinct vegetative insecticidal protein (Vip3A) alongside standard Cry proteins to eliminate individuals resistant to older Cry toxins (Santiago *et al.*, 2023) [50]. However, resistance monitoring continues to be critical. Multi-year diet-bioassay surveillance across Arkansas, Louisiana, Mississippi, Tennessee and Texas documented a significant decline in *H. zea* susceptibility to Vip3A_a between 2016 and 2020, including field-derived strains with up to 13-fold resistance relative to susceptible laboratory reference strains an early warning that field-evolved resistance to this last-effec

tive Cry-independent toxin is already underway (Yang *et al.*, 2021) [17]. Extended sentinel monitoring using *Bt* sweet corn across twenty-five US states and five Canadian provinces between 2020 and 2022 confirmed widespread resistance to *Cry1A_b*, *Cry2Ab₂* and *Cry1A₁₀₅*, together with measurable shifts in *Vip3A_a* allele frequencies, underscoring that resistance evolution in *H. zea* is a moving, multi-toxin problem rather than a single-gene event (Dively *et al.*, 2023) [51]. In addition, localized populations of secondary or sporadic pests such as

the beet armyworm (*Spodoptera exigua* Hubner) have demonstrated the capacity to evolve highly potent, autosomal recessive resistance to *Vip3A_a* proteins under intense selection pressures (Reisig *et al.*, 2026) [52], reinforcing that even the newest single mode of action is not immune to the same evolutionary dynamics that eroded *Cry1A_c* and *Cry2A_b*.



Source: Fabrick *et al.*, 2023

Figure 1. Pink bollworm responses to *Bt* cotton in the U.S., China and India

3. Molecular and genetic mechanisms of resistance

The biochemical arms race between target lepidopterans and *Bt* proteins occurs primarily at the midgut membrane interface (Jurat-Fuentes *et al.*, 2021; Fabrick *et al.*, 2023) [14, 53]. For a *Bt* toxin to kill a larva, it must undergo proteolytic activation in the midgut, bind to highly specific epithelial receptors and oligomerize to form lethal, membrane-permeable pores (Heckel *et al.*, 2007; Soberón *et al.*, 2007; Fabrick *et al.*, 2023) [54, 55, 14].

Mode of action of *Bt* Cry toxins in insects

- 1) **Ingestion and Activation:** Insect larvae ingest Cry protoxins, which are processed by midgut proteases into activated toxins (Fabrick *et al.*, 2023) [14].
- 2) **Receptor Binding:** The toxins bind to midgut receptor proteins such as cadherins and ABC transporters; reduced binding is a major mechanism of resistance (Fabrick *et al.*, 2023) [14].
- 3) **Pore Formation:** In the leading pore formation model, binding causes toxins to aggregate into oligomers that insert into cell membranes to form pores (Fabrick *et al.*, 2023) [14].
- 4) **Lysis and Death:** Uncontrolled influx of cations and water causes midgut cell swelling and lysis, killing the larva via acute tissue damage, starvation or septicemia (Fabrick *et al.*, 2023) [14].

3.1 Receptor alteration in *Cry1A_c* resistance

The most common and intensely studied mechanism of resistance to *Cry1A_c* involves mutations that disrupt target-site receptor binding (Zhao *et al.*, 2003; Fabrick *et al.*, 2023) [56, 14].

Cadherin (Cad): Deletions or point mutations in genes encoding midgut cadherin proteins prevent *Cry1A_c* from binding, halting the toxin activation process (Gahan *et al.*, 2001; Fabrick *et al.*, 2023) [57, 14]. Whole-genome and transcript-level work on field-derived pink bollworm continues to refine this picture: an in-frame 234-bp inser-

tion in exon 12 of the cadherin gene *PgCad₁* (the *r₁₄* allele) was shown to delete 36 amino acids from cadherin repeat 5 without altering overall transcript abundance, conferring 237-fold resistance to *Cry1A_c* and modest cross-resistance to *Cry2A_b* in a recessively inherited pattern direct molecular confirmation that structural loss of a single extracellular repeat, rather than loss of the gene itself, is sufficient to defeat toxin binding (Wang *et al.*, 2020) [58].

ABCC2 Transporters: Functional genomic studies have proven that mutations in the ATP-binding cassette transporter family C2 (ABCC2) are primary drivers of field-evolved *Cry1A_c* resistance across multiple genera, including *Pectinophora*, *Helicoverpa* and *Heliothis* (Wang *et al.*, 2020) [58]. These mutations typically introduce premature stop codons or structural misfoldings that eliminate the extracellular loops where the toxin anchors (Fabrick *et al.*, 2023) [14].

3.2 Distinct pathways in *Cry2A_b* and *Vip3A* resistance

Crucially, Cry2 and Vip3 proteins do not share the same midgut binding sites as Cry1 proteins, which forms the logical foundation of gene pyramiding (Chakraborty *et al.*, 2016) [59].

ABCA2 Transporters: Resistance to *Cry2A_b* is strongly linked to mutations in the ABCA2 transporter rather than ABCC2 (Fabrick *et al.*, 2015) [60]. Field-selected strains of *Cry2A_b*-resistant pink bollworm in India share nearly identical molecular defects in the ABCA2 gene as laboratory-selected strains in the US, highlighting a globally conserved evolutionary path to resistance (Fabrick *et al.*, 2023) [14].

Vip3A Binding: While widespread practical resistance to Vip3A in the field remains comparatively low (Welch *et al.*, 2021; Fabrick *et al.*, 2023) [49, 14], the genetic and biochemical basis of emerging *Vip3A_a* resistance is now being resolved in unusually fine detail in *H. zea*, the pest for which this trait carries the greatest present-day stakes. Reduced binding of *Vip3A_a* to midgut receptors, together with altered proteolytic processing of the toxin, was identified as a resistance mechanism distinct from the cadherin- and ABC-transporter-mediated pathways that dominate Cry resistance, confirming that Vip3A truly acts through an independent route at the receptor level

(Kerns *et al.*, 2023) [15]. Screening of five independent field-derived *H. zea* strains further showed that *Vip3A_a* resistance is controlled by a single, recessive, autosomal gene in each strain, but that at least three distinct genetic loci can underlie this phenotype across populations evidence that resistance is arising through parallel, independent mutational routes rather than spreading from one source, which complicates diagnostic monitoring and management (Yang *et al.*, 2024) [16]. Consistent with this, a polygenic-resolution analysis of wild *H. zea* populations found signatures of ongoing selection at multiple genomic regions associated with Cry resistance, suggesting that resistance evolution in this pest is more genetically complex than the sin-

gle-locus recessive model that historically described pink bollworm (Taylor *et al.*, 2024) [61]. A complementary genetic mapping study likewise identified a resistance locus for *Cry1A_c* in *H. zea* that does not correspond to the classical cadherin or ABC-transporter genes implicated in other lepidopterans, reinforcing that this species may be assembling resistance through a broader and less predictable genetic toolkit (Benowitz *et al.*, 2022) [62]. Together, these findings indicate that early-warning resistance already documented in *H. zea* (Yang *et al.*, 2021) [17] is mechanistically heterogeneous, and that pyramids relying on *Vip3A* as a fail-safe against Cry-resistant genotypes should not be assumed to retain a single, stable point of vulnerability.

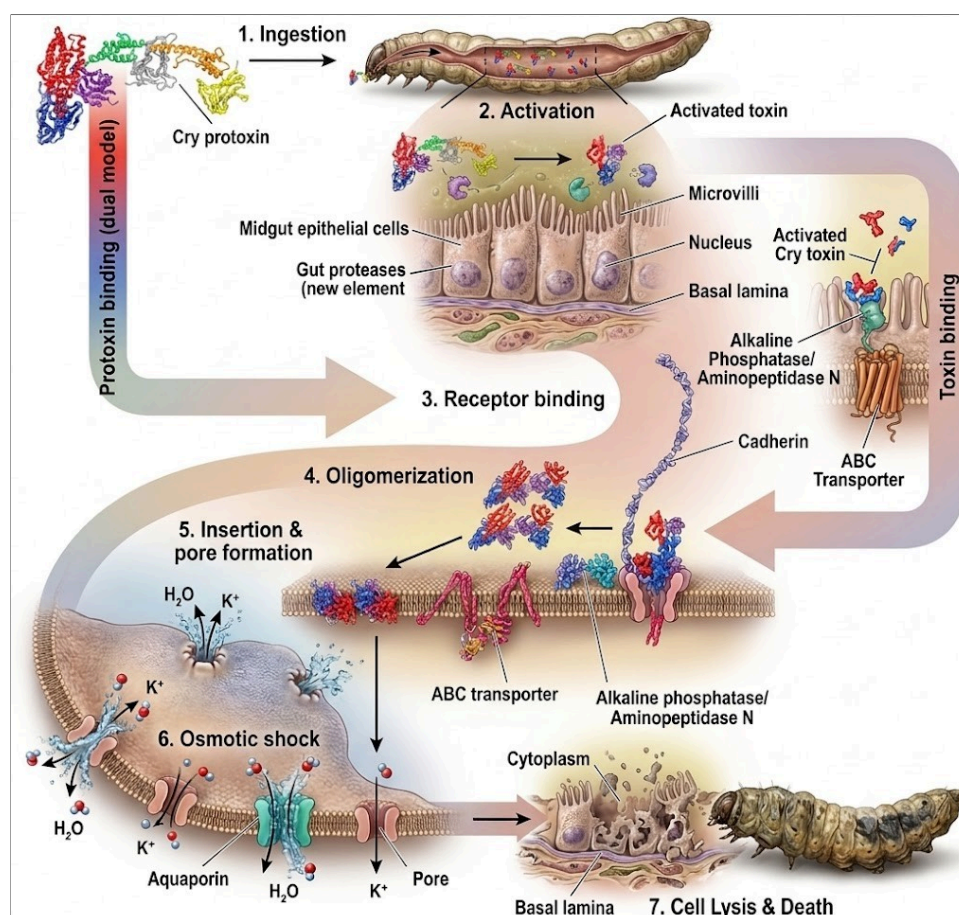


Figure 2. Modified dual-model mechanism of Cry toxin action in lepidopteran larvae

4. Environmental and agronomic drivers of resistance

While insect genetics dictate how resistance occurs, environmental and plant-physiochemical factors dictate how fast it spreads (Tabashnik and Carriere, 2020). A core assumption of the high-dose/refuge strategy is that the transgenic plant must express a consistently high concentration of the toxin throughout its lifecycle to guarantee the death of heterozygous (R^S) individuals (Onstad, 2014) [22]. Field observations demonstrate that this assumption is frequently violated by abiotic stress (Razzaq *et al.*, 2023) [63].

4.1 Temporal fluctuations and phenological declines

Bt toxin expression is highly dynamic and correlates strongly with the physiological age of the cotton plant (Kranthi *et al.*, 2005) [13]. Toxin concentrations are highest during the early vegetative stages but experience a profound, natural decline during late-stage flowering and boll development (Holman *et al.*, 2026) [64]. This drop in expression creates a dangerous “sub-lethal dose window” late in the season. During this phase, the toxin concentration falls to a level where it fails to kill heterozygous (R^S) larvae, allowing individuals with single copies of a resistance allele to survive, reproduce and rapidly increase the frequency of resistance alleles within the local gene pool (Tabashnik and Carriere, 2020).

4.2 Abiotic stress-induced gene silencing

Extreme climatic events severely compromise the insecticidal efficacy of *Bt* cotton (Razzaq *et al.*, 2023) [63]. Under prolonged environmental stressors such as high-temperature spikes (greater than 40 °C), severe drought or waterlogging, the transgenic plant’s metabolic activity shifts toward survival, frequently leading to a down-regulation of the CaMV-35S promoter driving the *Bt* gene (Razzaq *et al.*, 2023) [63]. This reduction in protein synthesis results in sharp drops in *Cry1A_c* and *Cry2A_b* concentrations within key reproductive structures such as squares and bolls, leaving them vulnerable to late-season pest complexes.

4.3 Tissue-specific heterogeneity

Bt proteins are not distributed evenly throughout the plant (Holman *et al.*, 2026) [64]. While young terminal leaves express robust levels of toxins, reproductive tissues particularly the boll endocarp, petals and square bracts often express substantially lower baseline concentrations (Holman *et al.*, 2026) [64]. This spatial heterogeneity allows opportunistic pests such as *S. litura* or *H. armigera* to feed on microhabitats within a single *Bt* plant where the toxin dose is sub-lethal, effectively providing them with a “built-in” spatial refuge that inadvertently screens for tolerant phenotypes (Kranthi *et al.*, 2005) [13].

5. Next-generation insect resistance management (IRM)

To safeguard the future of transgenic cotton and prevent a widespread resurgence of synthetic chemical pesticide use, IRM frameworks must evolve beyond simple, single-toxin paradigms (Tabashnik *et al.*, 2013) [10].

5.1 Evolution of the refuge strategy: from structured to seed-blends

The traditional structured refuge model, which requires farmers to plant a clean, distinct block of non-*Bt* cotton (usually 5% to 20% of the total area), has failed in smallholder-dominated regions due to socio-economic constraints and low compliance (Sisterson *et al.*, 2005) [23]. To overcome this human variable, the seed-blend strategy also known as Refuge-in-a-Bag (RIB) has been widely deployed in industrialized nations and is under serious consideration for developing markets (Ives *et al.*, 2011; Onstad, 2014) [18, 22].

By mechanically mixing a designated percentage (e.g., 5% to 10%) of non-*Bt* cotton seed directly into the commercial bag, compliance becomes 100 per cent mandatory and automated (Ives *et al.*, 2011) [18]. Every field automatically contains a random, scattered distribution of susceptible host plants, ensuring the survival of susceptible alleles without requiring conscious field modifications by the grower (Tabashnik *et al.*, 2010) [46]. India mandated a full transition from structured refuges to RIB from the 2020 season, with commercial *Bt* seed packets required to contain 5–10% non-*Bt* refuge seed blended directly into each 475 g bag (Mohan, 2020) [65]. However, the durability of this fix depends entirely on seed-industry stewardship: an ICAR-Central Institute for Cotton Research assessment of commercial RIB packets found irregularities including admixture of *Bt* seed into the refuge fraction, poor germination of the non-*Bt* component, and asynchronous flowering between the refuge and the main *Bt* crop problems that, if uncorrected, would functionally recreate the same non-compliance that undermined the structured refuge it replaced (Mohan, 2020) [65].

5.2 Multi-toxin pyramiding and novel proteins

The industry must continue transitioning away from dual-toxin models toward multi-toxin pyramids that integrate Vip3A alongside distinct Cry proteins (Santiago *et al.*, 2023) [50]. Because Vip3A shares no structural homology or midgut binding sites with Cry toxins, the probability of an insect simultaneously mutating two or three independent receptor pathways is mathematically very low, provided that resistance to the companion Cry toxins is not already advanced in the target population (Chakraborty *et al.*, 2016) [59]. However, as the recent identification of multiple independent genetic routes to Vip3A_a resistance in *H. zea* (Yang *et al.*, 2024) [16] means pyramid durability can no longer be treated as guaranteed by biochemical independence alone; sustained baseline monitoring of Vip3A susceptibility is now essential wherever this trait is deployed.

5.3 Emergence of RNA interference (RNAi) and next-gen biotech

As resistance to standard crystalline proteins evolves, the integration of RNA interference (RNAi) traits represents the next major technological leap (Razzaq *et al.*, 2023) [63]. Pyramiding a traditional *Bt* gene with a plant-mediated dsRNA construct targeting vital insect genes (such as v-ATPase or ribosomal proteins) introduces a completely unique, non-protein mode of action. Larvae feeding on these plants ingest the dsRNA, triggering gene silencing within their cells, bypassing midgut membrane receptor mutations entirely (Razzaq *et al.*, 2023) [63].

Proof-of-concept work in cotton itself supports this approach: transgenic cotton engineered to express dsRNA against juvenile-hormone-related genes (HaJHMT and HaJHBP) in *H. armigera* was effective against *Bt*-resistant larvae and computer simulations parameterized on northern Chinese cropping conditions where millions of farmers grow *Bt* cotton alongside abundant non-transgenic host plants showed that

pyramiding a *Bt* toxin with an independent RNAi trait substantially delayed the spread of resistance relative to *Bt* cotton deployed alone (Ni *et al.*, 2017) [19]. More broadly, dsRNA-based biopesticide formulations are progressing rapidly toward field and commercial use across insect pest systems, though recent resistance-management analyses caution that dsRNA resistance can itself evolve relatively quickly under intense, sustained selection, primarily through reduced cellular uptake of the dsRNA molecule rather than target-gene mutation underscoring that RNAi should be deployed as one pillar of a pyramided strategy rather than as a stand-alone replacement for *Bt* (Qi *et al.*, 2024) [20].

5.4 Ecologically based IPM and sucking pest vacuum management

Because *Bt* toxins are strictly lepidopteran-specific, their deployment has inadvertently created an ecological vacuum in cotton ecosystems (Lu *et al.*, 2012) [6]. Secondary, non-target sucking pests such as the cotton jassid (*Amrasca biguttula biguttula* Ishida) and whiteflies (*Bemisia tabaci* Gennadius) have emerged as primary threats, necessitating chemical intervention (Nyesse *et al.*, 2026) [66]. To prevent these secondary sprays from destroying the natural biological control agents that suppress target lepidopterans, modern IPM must incorporate highly selective, narrow-spectrum insecticides (e.g., flonicamid, afidopyropen) alongside behavioural disruptions such as pheromone trapping (Nyesse *et al.*, 2026) [66]. Maintaining robust populations of natural predators is a critical, overlooked line of defence that eliminates rare *Bt*-resistant larvae surviving on transgenic plants (Lu *et al.*, 2012) [6]. The scale of this secondary-pest pressure is illustrated by whitefly dynamics in north India, where a severe *Bemisia tabaci* outbreak in 2015 was followed by an even more prolonged infestation in 2022 that exceeded the economic threshold level for more than ten consecutive weeks across surveyed districts of Punjab, Haryana and Rajasthan; the analysis attributed the resurgence to a combination of susceptible cotton genotypes, delayed sowing, sub-optimal insecticide use and favourable weather rather than to *Bt* trait failure per se, but the episode underscores that whitefly and the cotton leaf curl virus it transmits now rival lepidopteran resistance as a threat to *Bt* cotton profitability in this region (Kumar *et al.*, 2024) [67].

6. Gaps in current knowledge and future directions

Despite extensive research into transgenic crop durability, several critical blind spots remain that require urgent attention from academic and industrial researchers:

- 1. Vip3A Baseline Susceptibility Mapping and Mechanistic Diversity:** With the rapid adoption of Bollgard 3 and equivalent traits, global baseline susceptibility monitoring for Vip3A remains uneven (Welch *et al.*, 2021) [49]. Because Vip3A_a resistance in *Helicoverpa zea* already appears to arise from at least three distinct genetic loci across independent field populations (Yang *et al.*, 2024) [16].
- 2. Climate Change Interactions:** Quantitative models mapping the precise impact of rising ambient temperatures and carbon dioxide levels on the tissue-specific expression of pyramided *Bt* traits remain scarce (Razzaq *et al.*, 2023) [63]. Understanding these interactions is vital for predicting future pest outbreaks (Holman *et al.*, 2026) [64].
- 3. Smallholder-Compatible RIB Models:** Research must focus on the agronomic viability of seed-blend strategies in smallholder settings, particularly regarding the risk of larval movement between adjacent *Bt* and non-*Bt* plants, which can unintentionally accelerate resistance development (Ives *et al.*, 2011) [18].
- 4. RNAi Resistance Risk Assessment:** As plant-mediated RNAi traits move toward commercial pyramiding with *Bt* in cotton, field-realistic resistance-risk data are still limited. Laboratory selection experiments in other cropping systems show that dsRNA resistance, driven mainly by reduced cellular uptake, can evolve within relatively few generations under intense selection (Qi *et al.*, 2024) [20]; equivalent data for lepidopteran cotton pests under realistic field exposure regimes are needed before RNAi can be relied upon as a durable independent mode of action.

5. Cross-Species Refuge Adequacy: China's natural-refuge model is well validated for pink bollworm but appears insufficient on its own for *Helicoverpa armigera* (Zhang *et al.*, 2019) [44]; multi-species resistance-monitoring frameworks that do not assume uniform refuge adequacy across the pest complex are needed.

6. RIB Seed-Quality Assurance: Where refuge-in-a-bag has replaced structured refuges, resistance-management outcomes now depend on seed-industry quality control (blend accuracy, non-Bt seed germination, and flowering synchrony) as much as on the underlying genetics; routine, independent auditing of commercial RIB packets should be considered a core component of IRM rather than a one-off compliance check (Mohan, 2020) [65].

7. Conclusions

The current status of insect pest resistance in *Bt* cotton serves as a powerful reminder of evolutionary resilience. The divergence between India's practical resistance disasters and the sustained management successes in China and the United States demonstrates that the durability of biotechnology is fundamentally dependent on human management. Single-toxin models are thoroughly obsolete and dual-toxin systems are under severe pressure. The emerging, mechanistically diverse resistance to *Vip3A_a* now documented in *H. zea* shows that even the newest single-protein technologies are not exempt from this trajectory. Sustaining the immense socio-economic benefits of *Bt* cotton requires a rapid, global transition toward three-protein pyramids, automated refuge mechanisms such as seed-blends, and the proactive, carefully risk-assessed incorporation of alternative modes of action such as RNAi. Ultimately, biotechnology must not be viewed as a stand-alone solution, but rather as a highly dynamic, evolving component embedded within a broader, ecologically intensive Integrated Pest Management framework.

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