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Management of Insect Pests with Bt Crops in the United States

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Abstract

Genetically engineered corn and cotton that produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt) have been used to manage insect pests in the United States and elsewhere. In some cases, this has led to regional suppression of pest populations and pest eradication within the United States, and these outcomes were associated with reductions in conventional insecticides and increased profits for farmers. In other instances, pests evolved resistance to multiple Bt traits, compromising the capacity of Bt crops to manage pests and leading to increased feeding injury to crops in the field. Several aspects of pest biology and pest–crop interactions were associated with cases where pests remained susceptible versus instances where pests evolved resistance. The viability of future transgenic traits can be improved by learning from these past outcomes. In particular, efforts should be made to delay resistance by increasing the prevalence of refuges and using integrated pest management.

INTRODUCTION

Since the 1990s, the cultivation of genetically engineered crops that produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt) has played an important role in the management of key insect pests, offering an alternative to the use of conventional insecticides (67, 86). The first Bt crops were corn, *Zea maize* L., and cotton, *Gossypium hirsutum* L., but more recently, farmers in some countries have begun growing Bt soybean, *Glycines max* L., and Bt eggplant, *Solanum melongena* L. (65). Currently, commercially cultivated Bt crops are found in over 20 countries distributed across six continents (65). In the United States, Bt corn and Bt cotton have been grown commercially for more than two decades and have been used to manage both lepidopteran and coleopteran pests (140). Also during this time, scores of studies have been published on Bt crops in the United States that document both the benefits to farmers and society and the shortcomings of this technology, in particular, the loss of efficacy from pests evolving resistance. This review synthesizes the literature on the use of Bt crops in the United States with the goal of providing a better understanding of the factors that have led to successes and failures in pest management and how the use of this technology can be improved in the United States and globally.

The first Bt crops in the United States produced Cry1A and targeted key lepidopteran pests, including tobacco budworm, *Chloridea virescens* Fabricius (Lepidoptera: Noctuidae); bollworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae); and pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) in cotton and European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) and bollworm (also known as corn earworm) in corn (140). These single-toxin crops were replaced over time by Bt crops that produced pyramided toxins (i.e., multiple Bt toxins targeting the same pest), in addition to Bt crops with stacked toxins (i.e., multiple toxins targeting different pest species, including both Lepidoptera and Coleoptera) (**Figure 1**). In the United States, the area planted to Bt corn and cotton has increased substantially over the past quarter century. In 1996, farmers in the United States planted approximately 1 million hectares of

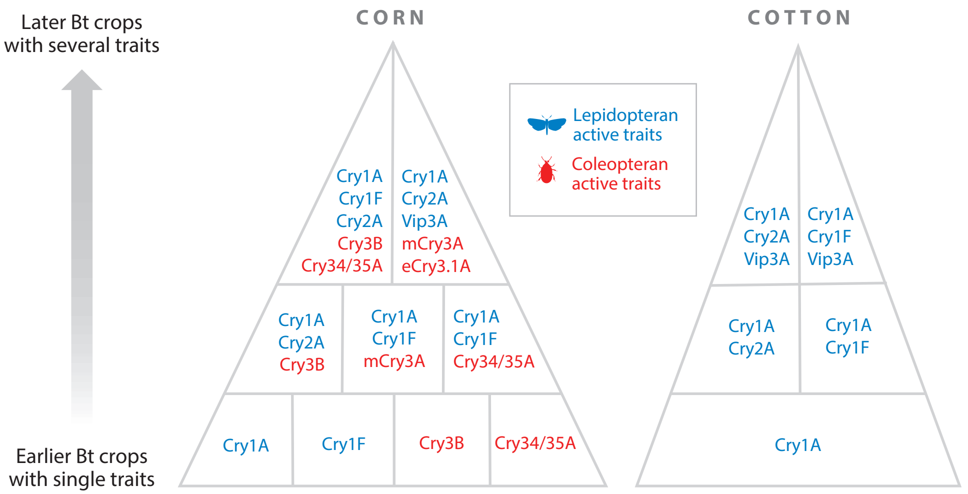

Figure 1

Pattern of pyramiding and stacking of *Bacillus thuringiensis* (Bt) traits over time for Bt corn and Bt cotton in the United States. Subdivisions within larger triangles represent an array of Bt traits found within an individual crop plant. All past and current trait combinations are displayed for cotton, but only a subset of trait combinations are provided for corn. While lepidopteran traits target pests in multiple families, coleopteran traits are only used to manage species in the genus *Diabrotica* (Chrysomelidae) (32, 139–141, 144).

Bt corn and cotton (67). By 2019, Bt corn and cotton were grown on 34.9 million hectares, with more than 85% of that area consisting of Bt corn (65). During the same year, 83% of the area in the United States planted to corn was planted to Bt-traited hybrids (142). Benefits associated with the cultivation of Bt crops in the United States include reduced foliar insecticide use, increased profits for farmers, regional suppression of key insect pests, pest eradication within the United States, and increased biodiversity in agricultural fields (23, 34, 63, 134). In several instances, the application of highly effective insect resistance management has been associated with sustained pest susceptibility to Bt crops (115, 130). By contrast, in other cases, the evolution of Bt resistance has compromised the capacity of Bt crops to prevent feeding injury and preserve yield (35, 51, 52, 54, 55, 132, 146).

INSECT RESISTANCE MANAGEMENT IN THE UNITED STATES

In the United States, resistance of insect pests to Bt crops is managed through the refuge strategy (56, 138). In conjunction with the cultivation of a Bt crop, a refuge of non-Bt host plants, which may consist of either non-Bt varieties or naturally occurring vegetation, is present (5, 56, 138). Refuges provide an environment where Bt-susceptible insect genotypes can survive. Mating between Bt-susceptible insects from refuges and Bt-resistant insects from Bt plants produce progeny that are heterozygous for resistance. To the extent that these heterozygous progeny have lower fitness on a Bt crop than do homozygous resistant individuals, resistance is delayed compared to cases in which refuges are absent (19, 56, 132).

Refuges may be used in conjunction with Bt traits that produce a high dose of Bt toxin, an approach referred to as the high-dose/refuge strategy (56). High-dose Bt traits are defined as those that are capable of killing 99.99% of homozygous susceptible individuals or producing 25 times more toxin than is required to kill susceptible individuals (138). With a high-dose Bt crop, resistance is typically functionally recessive, and consequently, heterozygous progeny, produced from mating between refuge individuals and Bt-resistant individuals, are unable to survive on a Bt crop. In this scenario, substantial delays in resistance may be achieved, as illustrated by both computer models and patterns of pest resistance in the field (128, 132).

Another approach that is used to manage Bt resistance is the pyramid/refuge strategy, which has been used with Bt crops in the United States and other countries (56, 127, 138) (**Figure 1**). Pyramided Bt crops produce multiple Bt toxins that target the same insect pest and delay resistance when individuals that possess alleles for resistance to one toxin in a pyramid are killed by a second toxin and vice versa (56, 104). However, refuges are still essential for managing resistance with pyramided Bt crops because the presence of Bt-susceptible individuals from refuges prevents the accumulation of individuals with resistance to both Bt toxins in a pyramid (18, 19, 104).

DIVERSE OUTCOMES FOR MANAGEMENT OF INSECT PESTS WITH BT CROPS IN THE UNITED STATES

There are some cases in which Bt corn and Bt cotton have led to reduced feeding from target pests, have contributed to reduced pest abundance, and have not been associated with pest resistance in the United States (**Table 1**). European corn borer has been managed with Bt corn since 1996, and cultivation of Bt corn has been associated with substantially reduced feeding injury by European corn borer and regional pest suppression (34, 63). Similarly, Bt cotton has served as an important tool for the management of pink bollworm in the southwestern United States, leading to reductions in feeding injury and insecticide applications (2, 23). Furthermore, in conjunction with sterile insect releases, the planting of Bt cotton enabled the eradication of pink bollworm from the United States (2, 134).

Table 1 Summary of management of key pests with Bt crops in the United States

Scientific name and order of pest[a]	Common name of pest	Bt toxins targeting pest	Crops containing Bt toxins[b]	Outcomes for management with Bt crops	Reference(s)
Chloridea virescens (Lepidoptera)	Tobacco budworm	Cry1A Cry2A	Cotton	Sustained susceptibility to Bt traits and negligible feeding injury to Bt cotton	13, 14, 47, 127
Diabrotica virgifera virgifera (Coleoptera)	Western corn rootworm	eCry3.1A Cry3B Cry34/35A mCry3A	Corn	Resistance to all Bt traits and high levels of feeding injury to Bt corn	54, 66, 98, 120
Helicoverpa zea (Lepidoptera)	Bollworm or corn earworm	Cry1A Cry2A Vip3A	Corn and cotton	Pest suppression but resistance to Cry1A and Cry2A; incipient resistance to Vip3A	12, 33–35, 46, 101, 132, 149, 151
Ostrinia nubilalis (Lepidoptera)	European corn borer	Cry1A Cry1F Cry2A	Corn[c]	Regional pest suppression, sustained susceptibility, and reduced injury to Bt and non-Bt crops	34, 63, 115
Pectinophora gossypiella (Lepidoptera)	Pink bollworm	Cry1A Cry2A	Cotton	Pest eradicated through the combined use of Bt cotton and sterile insect releases	134

[a]Order is given in parentheses below the scientific name for each pest species.
[b]Column lists the transgenic crops that are used for management of each pest species.
[c]European corn borer can be a minor pest of cotton, and similar Bt toxins are also found in transgenic cotton (18, 78).
Abbreviation: Bt, *Bacillus thuringiensis*.

Another key insect pest that has been managed successfully with Bt cotton is tobacco budworm. This pest has remained susceptible to Bt cotton since 1996, with Bt varieties experiencing almost no feeding injury compared to those that lack Bt traits (13, 14, 47, 127). The impact of Bt cotton in the southern United States is particularly striking. By 1993, tobacco budworm had evolved resistance to three classes of foliar insecticides, and as a result, in 1994 and 1995, this pest caused extensive damage to cotton fields in Alabama and Mississippi (7, 72, 119). During 1995, cotton farmers in Alabama experienced the highest monetary losses from insect pests since record keeping began in 1979 (119). However, after Bt cotton was introduced in 1996, Alabama cotton growers sprayed the fewest insecticides since synthetic insecticides were introduced in the 1940s (119).

In contrast to pests such as European corn borer, pink bollworm, and tobacco budworm, there are several pest species that evolved resistance to Bt traits and imposed substantial feeding injury to Bt crops. Resistance to Cry1F corn by fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), was first associated with outbreaks of this pest in Bt cornfields in the US territory of Puerto Rico (124). Later, Cry1F resistance was detected in the mainland US states of Florida, North Carolina, and Texas (61, 74, 143). Because populations of fall armyworm are highly panmictic, and because this pest does not overwinter in much of the US mainland, it was initially hypothesized that resistant populations had migrated to the United States from Puerto Rico (61, 107). However, resistance alleles identified from Bt-resistant populations in Puerto Rico were not detected in the United States (9). Consequently, it appears more likely that fall armyworm evolved resistance to Cry1F corn in both the US mainland and Puerto Rico, rather than evolving resistance in Puerto Rico and then spreading to the United States (9, 45).

The first Bt traits in corn were not effective at reducing feeding injury from western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae); however, when Cry1F corn was commercialized in 2003, it reduced feeding injury from this pest by 51–100% (22, 42). Bioassay data from populations sampled in 2003 and 2004 revealed 10-fold interpopulation variation in susceptibility of western bean cutworm to Cry1F (88). This finding suggested that western bean cutworm could rapidly evolve Cry1F resistance due to high levels of genetic variation for survival on Cry1F corn and, by implication, elevated frequencies of resistance alleles in some populations. Following the commercial cultivation of Cry1F corn, western bean cutworm expanded from its historical range in the western United States and became established in the midwestern and northeastern United States and in southeastern Canada (116). This expansion was associated with field-evolved resistance and loss of efficacy by Cry1F corn in both the United States and Canada (25, 88, 116, 118). Currently, Vip3A is the primary Bt toxin used for management of western bean cutworm in corn (43, 82). Importantly, Vip proteins are derived from the vegetative growth stage of *Bacillus thuringiensis*, unlike Cry proteins, which are produced during sporulation; because the structures of these toxins are dissimilar, cross-resistance is typically absent (18, 70).

Planting of Cry1A cotton and corn was associated with reduced feeding injury by bollworm (6, 125). However, because bollworm could still inflict 20–50% feeding injury to these Bt crops, there was initial disagreement within the scientific community concerning the presence of resistance in the field and the effects of resistance on crop injury (81, 131, 132). Nonetheless, as early as 2003, bioassays suggested that field-evolved resistance to Cry1A was present in some bollworm populations (132). These single-toxin Bt crops were replaced by corn and cotton producing a pyramid of Cry1A and Cry2A, which reduced feeding injury and bollworm abundance (34, 46). Over time, injury by bollworm was observed for Bt cotton, Bt field corn, and Bt sweet corn with a pyramid of Cry1A and Cry2A (35, 37, 46). By 2016, resistance was widespread for Bt corn and Bt cotton pyramided with Cry1A and Cry2A (35, 55, 101). Currently, Vip3A plays a key role in reducing feeding injury from bollworm in cotton and corn (100, 102).

Bt corn for management of western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), was grown commercially beginning in 2003 and produced Cry3B; three additional Bt toxins, mCry3A, eCry3.1A, and Cry34/35A (since renamed Gpp34/Tpp35A), were subsequently used in management (48, 49). Beginning in 2009, high levels of feeding injury to Cry3B corn and field-evolved resistance were detected in several midwestern states (17, 51–53, 108, 146, 152). Resistance to Cry3B was widespread in some parts of the Midwest by 2013, while other regions showed a more heterogeneous distribution of resistance (97, 111, 121, 122). Western corn rootworm populations with resistance to Cry3B were cross-resistant to mCry3A and eCry3.1A, an effect that likely arose because of the structural similarity among these toxins (18, 66). This resistance was mitigated by planting Bt corn that contained a pyramid of Cry3 and Cry34/35A (41). Rootworm populations, in turn, evolved resistance to these pyramided hybrids; consequently, there are now some western corn rootworm populations with resistance to all commercially available Bt toxins (54, 98). Additionally, the closely related northern corn rootworm, *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), has evolved resistance to Cry3B corn in some field populations (17). Given the lack of a high dose for Bt traits targeting northern corn rootworm, the evolution of resistance in additional areas and to additional Bt traits seems likely (92, 93).

CASE STUDIES OF KEY PESTS MANAGED WITH BT CROPS IN THE UNITED STATES

Management of European Corn Borer with Bt Corn

Management of European corn borer with Bt corn represents an exemplar for successful, long-term pest management with Bt crops. Because European corn borer feeds within corn stalks and ears, management with foliar insecticides is challenging and needs to be carefully timed to coincide with larval eclosion (78). The systemic production of Bt toxins by genetically modified corn simplified and improved management of this pest. Beginning in the late 1990s, Bt corn was readily adopted by farmers in the northern corn-growing states (34, 63). Alleles for resistance to Cry1F corn and Cry1A corn in European corn borer were rare, and populations remained susceptible to these Bt toxins following the commercial cultivation of Bt corn (3, 115). These toxins were augmented with a third Bt toxin, Cry2A, beginning in 2009 (114).

Long-term data from several midwestern states showed that the planting of Bt corn was associated with regional suppression of European corn borer populations (63). Moreover, farmers who grew Bt corn enjoyed increased profits because of reduced feeding injury from European corn borer (63). Interestingly, profits also increased for farmers who grew non-Bt corn because regional suppression of this pest resulted in lower levels of feeding injury to non-Bt corn (63). Similarly, in the eastern United States, where European corn borer is also a pest of vegetables, the widespread planting of Bt corn led to regional suppression of European corn borer and decreased feeding injury by this pest to various vegetable crops (34).

There are several factors that likely contributed to the successful management of European corn borer with Bt corn. Importantly, Bt toxins targeting European corn borer typically met the criterion of high dose, and survival of heterozygous resistant insects on Bt corn closely resembled survival of susceptible insects (26, 91, 114). Additionally, adult European corn borer show a high degree of movement, with adults emerging from cornfields and dispersing to grassy field edges to mate, after which females disperse back into cornfields for oviposition (95). This pattern of movement is important because it facilitates mating between insects that developed on Bt corn and Bt-susceptible insects from refuges. Furthermore, high rates of dispersal prevent the accumulation of resistance alleles within individual fields (30, 105). Additionally, fitness costs appear to accompany Bt resistance by European corn borer, which should act to reduce the frequency of resistance alleles within refuge populations (27, 94).

Two final features in the successful management of European corn borer with Bt corn were the cultivation of corn with a pyramid of Bt toxins and the introduction of these pyramided hybrids before populations developed resistance to Bt hybrids with single toxins (114). Resistance to Cry1F corn by European corn borer in Canada, where farmers continued to grow this single-traited Bt crop rather than using hybrids with a pyramid of toxins, illustrates that even high-dose Bt traits can be compromised by pest resistance and indicates the benefit of pyramiding Bt toxins, in conjunction with the use of non-Bt refuges, to bolster resistance management (117).

Management of Pink Bollworm with Bt Cotton

In the southwestern United States, pink bollworm was managed successfully with Bt cotton for more than two decades, beginning in 1996 (134). This pest is challenging to manage with foliar insecticides because it is multivoltine, enabling populations to build rapidly within a growing season, and because larvae feed within cotton bolls and flower buds, which provide shelter from foliar insecticides (85). However, the systemic production of Bt toxins by transgenic cotton plants killed pink bollworm larvae in these structures that were hard to reach with foliar insecticides

(76). Although Bt cotton was planted widely throughout the Southwest for management of pink bollworm, resistance monitoring data from Arizona suggest that resistance alleles remained at a low frequency, despite an initial resistance allele frequency of approximately 16% (130, 135). Furthermore, the widespread planting of Bt cotton, coupled with sustained pest susceptibility, led to regional suppression of pink bollworm and fewer applications of foliar insecticides to cotton fields (20, 21, 23). Populations of pink bollworm in the Southwest were further reduced, and eventually eradicated, through the combined use of Bt cotton and sterile insect releases (134).

As with European corn borer, there are several aspects related to the biology of pink bollworm, and the interaction between this pest and Bt cotton, that likely facilitated successful management. In particular, the first Bt cotton varieties produced Cry1A and achieved a high dose against pink bollworm, leading to recessive inheritance of resistance, which, in conjunction with non-Bt refuges and fitness costs of resistance, likely acted to maintain resistance alleles at low frequencies, even though elevated resistance allele frequencies were observed immediately following the commercialization of Cry1A cotton (50, 76, 126, 130). The cultivation of Cry1A cotton was followed by the inclusion of cotton with a second Bt toxin, Cry2A, in a pyramid with Cry1A, and the Cry2A trait also achieved a high dose against pink bollworm (140). Importantly, there was no evidence of resistance to Cry1A cotton when the pyramid of Cry1A and Cry2A was commercially cultivated (127, 130). Finally, during the eradication program, releases of sterile insects served as a source of refuge individuals to prevent mating between Bt-resistant insects (134).

The successful management of pink bollworm with Bt cotton in the United States stands in stark contrast to the use of Bt cotton in India, where pink bollworm populations evolved resistance first to single-toxin Cry1A cotton and then to the pyramid of Cry1A with Cry2A (31, 83). This outcome, which has substantially diminished the utility of Bt cotton for pest management, appears to have resulted primarily from inadequate planting of non-Bt refuges, although other factors, such as a longer temporal window over which cotton was grown each year and the use of cotton hybrids that produced a mixture of Bt and non-Bt cotton seeds within bolls, may have also contributed to this outcome (83, 129).

Management of Western Corn Rootworm with Bt Corn

Western corn rootworm is a univoltine pest, with adults ovipositing in cornfields during the summer, eggs overwintering in the soil, and larvae hatching the following spring (59). Thus, fields where corn is grown for at least two consecutive years provide a suitable habitat for this pest (73). Most yield losses caused by western corn rootworm result from larval feeding on corn roots (73, 137). Prior to the commercialization of Bt corn, management of this pest focused on soil insecticides applied at planting to kill larvae, rotation of fields out of corn production for one or more years (i.e., crop rotation), and the use of foliar insecticides to kill adults (73). It is noteworthy that western corn rootworm in the US Corn Belt evolved resistance to all three of these management practices (59, 80). Because Bt corn targeted corn rootworm larvae, it fit readily with existing management practices, taking the place of soil-applied insecticides. Initial efficacy data highlighted the capacity of Bt corn to substantially reduce root feeding injury and rootworm survival, and Bt corn was quickly adopted by US farmers for rootworm management (68, 92, 93). However, the rapid adoption of this technology was matched by a rapid counter-response by this pest. The first Bt corn targeting corn rootworm was commercialized in 2003 and produced Cry3B; by 2009, field-evolved resistance to Cry3B corn was documented for some populations of western corn rootworm in Iowa (52). Furthermore, resistance was associated with high levels of feeding injury to Bt corn plants in the field (51, 52, 66). Over time, the magnitude of Cry3B resistance increased among western corn rootworm populations and was documented throughout the Midwest (17, 51, 108, 146, 152).

Both pest biology and the pest–crop interaction likely facilitated the rapid evolution of Bt resistance and the high levels of injury observed in the field (49). In particular, adult western corn rootworm display limited dispersal, and many individuals travel only approximately 40 m per day (62). Limited adult movement may have contributed to the evolution of resistance in multiple ways. First, initial cultivation of Bt corn used only spatially segregated refuges; consequently, limited adult movement allowed resistant genotypes to accumulate within populations. Second, cases of Bt resistance were often associated with continuous cultivation of Cry3B corn, which, in combination with limited adult dispersal, allowed resistance to increase within these fields (51–53, 120, 146). Because of this pest's limited adult movement, adult corn rootworm that emerged from a field of Cry3B corn would oviposit into the same field, in which Cry3B corn would be planted again the next season (49). Laboratory data indicated that continuous selection could result in resistance to Cry3B corn in three generations (79). An additional factor facilitating resistance evolution was the lack of a high dose for Cry3B corn and other Bt traits targeting western corn rootworm, which had the concomitant effect of conferring nonrecessive inheritance of resistance (64, 90, 112, 113). Furthermore, there appear to be minimal fitness costs associated with Cry3B resistance (64, 90, 112). Given these risk factors, the availability of non-Bt refuge corn was likely inadequate to enable meaningful delays in resistance (4, 133).

Management of western corn rootworm using Bt corn was further complicated by the presence of cross-resistance among some Bt toxins and field-evolved resistance to single toxins prior to combining traits into two-toxin pyramids. Cross-resistance in western corn rootworm was found among the structurally similar three-domain toxins: Cry3B, mCry3A, and eCry3.1A, but not to the binary toxin Cry34/35A (18, 66). Using a pyramid of Cry3 and Cry34/35A offered one avenue to delay resistance, despite the lack of a high dose for these toxins (92, 93, 113). However, the presence of Cry3 resistance in the field prior to the use of this pyramid compromised the pyramid's capacity to delay resistance (17, 51–53, 108, 146, 152). In some parts of the Midwest, farmers relied heavily on pyramids of Cry34/35A with Cry3 to mitigate Cry3 resistance (41). This, in turn, facilitated the evolution of resistance to Cry34/35A and the presence of rootworm populations with resistance to all commercially available Bt toxins (54, 98). Farmers responded to the loss of efficacy of Bt traits to manage corn rootworm by increasing their use of soil-applied insecticides (41).

Somewhat contrasting outcomes have arisen from the use of Bt corn to manage western corn rootworm versus northern corn rootworm. Both species co-occur within the agricultural landscape, and patterns of mortality from Bt corn appear to be similar between these species, suggesting similar selection intensity for resistance (40, 92, 93). However, northern corn rootworm was relatively rare in fields that suffered high levels of feeding injury from large populations of Bt-resistant western corn rootworm (41, 51). Furthermore, evidence of resistance to Bt corn in northern corn rootworm began to emerge seven years after resistance in western corn rootworm (17, 52). One difference between these species is that adult northern corn rootworm disperse more readily than adult western corn rootworm, leaving cornfields to feed on other sources of pollen before returning to cornfields to oviposit (24, 84). These higher rates of dispersal may slow the accumulation of resistance alleles within populations by enabling an influx of Bt-susceptible individuals into a population and facilitating the movement of resistant individuals out of a population, thereby extending the time until large populations of Bt-resistant individuals impose substantial feeding injury to Bt corn. However, it is also possible that other interspecific biological differences, such as the presence of extended diapause in northern corn rootworm, may have contributed to differences in the rate of resistance evolution (73).

Management of Bollworm with Bt Corn and Bt Cotton

The case of bollworm represents an example of pest management with Bt crops where there is evidence for both regional suppression and Bt resistance. Bollworm is a pest of corn and cotton in the United States and encounters Cry1A, Cry2A, and Vip3A when feeding on these crops (18, 102) (**Figure 1**). While bollworm can substantially reduce yield for cotton, injury to field corn tends to be subeconomic and typically does not warrant management with either Bt toxins or insecticides (11, 29, 96). Bollworm abundance in the eastern United States began declining after Cry1A corn was initially planted and continued to decline with the planting of corn that produced a pyramid of Cry1A and Cry2A (34, 46). As the cultivation of Bt corn increased, and bollworm abundance declined, injury by bollworm to both Bt and non-Bt varieties of corn and cotton declined (34, 37). However, bollworm populations were evolving resistance to Cry1A and Cry2A at the same time that abundance was declining; subsequently, both bollworm abundance and crop injury increased, leading to greater use of foliar insecticides (12, 33, 35, 37, 46, 101, 103, 132). Currently, Vip3A plays a central role in reducing feeding injury to Bt cotton (102, 149, 151). However, evidence of incipient field-evolved resistance to Vip3A has been found in some bollworm populations, as indicated by increases in resistance allele frequencies, pest survival, and crop injury (33, 149, 151). One factor that likely has contributed to resistance evolution is the lack of a high dose for either the Cry1A or Cry2A traits targeting bollworm in corn and cotton (100, 128). While Vip3A traits may not meet the criterion of high dose against bollworm, available data suggest that inheritance of resistance to Vip3A in bollworm is recessive, unlike resistance to both Cry1A and Cry2A, which is nonrecessive (16, 102, 150). Consequently, bollworm may not evolve resistance to Vip3A as quickly as it did to Cry1A and Cry2A.

Additionally, larval diet breadth and adult dispersal behavior may have affected patterns of Bt resistance evolution. Adult bollworm display high rates of both short-range and long-range dispersal (109). High rates of dispersal should facilitate mating between Bt-selected individuals and individuals from non-Bt refuges, in addition to diluting resistance alleles within populations (44, 57). However, despite high rates of dispersal, available data demonstrate the effect of local selection (within $\leq$1 km) on Bt resistance in bollworm (5, 37). Consequently, local refuges appear important for delaying Bt resistance in bollworm (5).

The larval diet breadth of bollworm likely has a complex effect on resistance evolution, acting to either delay or accelerate the evolution of Bt resistance depending on the host plants available for oviposition and subsequently consumed by larvae. Bollworm is polyphagous, feeding on weedy species and non-Bt crops, such as peanut, sorghum, soybean, and tobacco, in addition to Bt corn and Bt cotton (69). Both weedy species and non-Bt crops provide a source of refuge individuals and should act to delay resistance evolution (5, 36, 60). However, polyphagy also appears to be a key factor in facilitating resistance evolution in this multivoltine pest, since sequential generations of bollworm use corn, then cotton, as a larval host in the southern United States. Therefore, in a single year, successive generations of bollworm are exposed to structurally similar Bt toxins shared between Bt corn and Bt cotton, and cross-resistance has been found for these structurally similar Bt toxins (18, 70, 102, 147).

Bollworm prefer to oviposit in flowering hosts, and sequential generations of bollworm follow a series of host plants as they initiate flowering at different times over the growing season (69). Typically, corn serves as a larval host for one or more generations before oviposition begins in cotton (57, 60). Corn is a preferred host for bollworm and contributes more adults to bollworm populations than any other crop (57, 60, 69). Consequently, bollworm populations experience selection for resistance on Bt corn before using Bt cotton as a host. Further complicating the issue, most farmers in the Cotton Belt do not plant required refuges when growing Bt corn, and refuges are not required for Bt cotton because this crop contributes very few bollworm to the overall population (99, 101, 102). Because Vip3A is present in both corn and cotton, the same pattern of selection imposed by sequential generations of bollworm feeding on Bt corn followed by Bt cotton, which accelerated resistance to Cry1A and Cry2A, also will impose selection for resistance to Vip3A (102). Given the importance of Bt corn in driving the evolution of Bt resistance by bollworm to both Bt corn and Bt cotton, increasing refuges of non-Bt corn in the landscape should aid in delaying resistance to Vip3A (102).

The cases of tobacco budworm in the United States and Old World bollworm, *Helicoverpa armigera* Hübner, in Australia provide interesting contrasts to the challenges encountered with managing bollworm. Both Cry1A and Cry2A traits in cotton produce a high dose against tobacco budworm, a factor that should contribute to the sustained susceptibility of this pest to Bt cotton (128). By contrast, Cry1A and Cry2A traits do not produce a high dose against Old World bollworm, and resistance alleles to both Bt toxins are present within Australian populations of this pest (77). However, Australia required a 70% non-Bt cotton refuge for single-traited Cry1A cotton and, later, a 10% non-Bt cotton refuge (or 5% refuge of pigeon pea, *Cajanus cajan*) for cotton pyramided with Cry1A and Cry2A (38). Additionally, the transition from Cry1A cotton to pyramided cotton took place in a single growing season, which reduced the risk of Old World bollworm evolving resistance to either type of Bt cotton (127). Use of large refuges with single-traited cotton and the consistent application of resistance management across the landscape appear to be important factors that enabled the successful management of Old World bollworm with Bt cotton in Australia (38, 148).

LESSONS LEARNED AND FUTURE PROSPECTS

After more than two decades of commercial cultivation of Bt crops in the United States, it is possible to examine general patterns among cases where pests have evolved resistance, and presented management challenges, versus instances where pests have been successfully managed without meaningful resistance evolution; these findings are relevant for regions around the world where Bt crops are grown. Among the key factors affecting the risk of resistance development is whether a Bt trait produces a high dose of Bt toxin against a target pest, as well as the subsequent inheritance of resistance (56, 128). However, even when a high dose is present, refuges are still essential to delay resistance (56, 128, 129). High-dose Bt events were present in all instances where pests have been managed successfully in the United States without meaningful resistance evolution (i.e., European corn borer, pink bollworm, and tobacco budworm) (13, 114, 134). However, it is noteworthy that many of the Bt traits that target insect pests in the United States do not achieve a high dose, and that, while these traits have provided benefits to farmers by reducing feeding injury and associated yield losses, these benefits often have been compromised to a greater or lesser extent by the evolution of resistance (25, 34, 51, 54, 88, 101, 116). Thus, one key question is how pest management can be improved for Bt traits that do not meet the criterion of high dose. In addition to the dose of Bt provided by a transgenic crop, several other factors may affect the risk of resistance evolution, and appear to be especially important when a high dose is lacking. These factors include the availability of non-Bt refuges; the frequency of resistance alleles; the presence of fitness costs; patterns of pest dispersal and mating; and, for polyphagous pests, exposure to Bt crops versus non-Bt hosts within the agricultural landscape (49, 50, 100, 127, 128).

Many of the current resistance issues are associated with insufficient planting of refuges, and larger refuges may have helped to delay resistance (4, 52, 100, 132, 133). Furthermore, computer simulation models and laboratory experiments demonstrate the capacity of refuges to delay resistance (28, 132, 136). The current approach for managing resistance has focused on spatial refuges and the extent to which larger spatial refuges should accompany the planting of Bt crops. In general, ensuring that non-Bt refuges are present when Bt crops are grown appears to be essential for sustainable pest management with Bt crops (56, 133). However, refuges may also be temporal, with Bt crops not grown in certain years, and this could occur at spatial scales ranging anywhere from an individual field to a larger geographical region. For example, farmers could rotate between Bt and non-Bt varieties over time, an approach that is highly compatible with the tenets of integrated pest management (49, 100, 123). Ideally, a Bt crop would not be grown during a period of low pest abundance, although this would require the ability to accurately predict pest abundance prior to planting. Additionally, alternative pest management approaches could be used when Bt crops were not grown. Such an approach would increase the extent to which Bt crops are used as a component of integrated pest management and aid in delaying the evolution of Bt resistance. However, extrinsic factors, such as periods of high commodity prices, can cause farmers to increase their reliance on Bt traits, which in turn can drive resistance evolution, as was observed with Bt corn targeting western corn rootworm (120).

Pest biology and interactions between insect pests and Bt crops are important factors to consider when assessing the likelihood of Bt resistance developing and, consequently, how to most effectively manage pests with Bt crops over the long term. Resistance evolution and field failures for Bt corn targeting western corn rootworm provide an example of how limited adult movement, coupled with Bt traits that do not produce a high dose, can lead to rapid development of resistance (49). This observation is concordant with past research on resistance to conventional insecticides, with resistance developing more rapidly for less mobile pests due to the accumulation of resistance alleles within a population and the reduced movement of susceptible insects into populations experiencing selection (105). Additionally, an elevated presence of resistance alleles within populations at the time that a Bt trait was introduced likely contributed to resistance evolution for pests such as western bean cutworm and western corn rootworm (87, 88). For polyphagous pests, the number of generations per year that are exposed to Bt toxins is an important factor to consider, as illustrated by instances of Bt resistance in bollworm, where many of the problems posed by resistance to Bt cotton are driven by selection for resistance on Bt corn (102). Considering these risk factors, in addition to the dose of Bt toxin produced by a Bt crop, can improve risk assessments for resistance management. In general, when a high dose is absent, additional care should be taken to ensure that a robust resistance management approach is in place (4, 49, 100, 128).

To date, Bt traits have been used to manage lepidopteran and coleopteran pests. Recently, Bt cotton producing Cry51A was developed for management of Hemiptera and Thysanoptera, including *Lygus* spp. and thrips (Thripidae) (1, 58). Additionally, it appears likely that future management of lepidopteran and coleopteran pests will include transgenic crops with novel Bt traits (8, 89, 145). For management of corn rootworm, future transgenic traits may include Bt toxins and insecticidal proteins from *Brevibacillus laterosporus* and *Pseudomonas chlororaphis*, in addition to double-stranded RNA molecules, which kill pests though RNA interference (10, 15, 106). The expanded array of transgenic traits beyond Bt toxins that is being developed and deployed for management of corn rootworm may be a model that is followed for management of other pests (8, 75). One of the challenges in developing future transgenic traits will be extending the accessibility and benefits of these technologies to farmers that grow crops other than major commodities such as corn, cotton, and soybean. The recent commercialization of Bt eggplant provides one model for how this might be accomplished (110).

It will be important to apply insights from management of pests with current Bt traits to improve pest management with future transgenic traits based on both Bt toxins and other insecticidal molecules. It is likely that many of the future transgenic traits will not meet the criterion of high dose. For example, Cry51A does not produce a high dose against its target pests, and past research has found that western corn rootworm can adapt rapidly to transgenic corn that employs RNA interference (1, 71). When a high dose is absent, the most effective approaches for insect resistance management include pyramiding of multiple transgenic traits and increasing refuges within the agricultural landscape (104). One of the key challenges to date for pyramiding of Bt toxins has been the deployment of pyramids after pests have been exposed to toxins singly and resistance issues have already emerged; management of both bollworm and western corn rootworm provides examples of this phenomenon (12, 35, 49, 55, 101, 102, 132). Computer simulation models demonstrate that the resistance management benefit of using pyramids is substantially compromised when resistance to one of the toxins in a pyramid is already present (104). The use of novel pyramids, coupled with adequate use of refuges, will help to improve the sustainability of future insecticidal traits in transgenic crops by delaying the evolution of resistance. Additionally, timely resistance monitoring will make it possible to assess the success of these resistance management approaches and, if necessary, refine them. In general, the sustainability of future transgenic crops can be enhanced by developing approaches for their use within broader strategies for multifaceted integrated pest management, rather than by focusing on using transgenic traits as a stand-alone approach.

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