

Transgenic Bt Corn Hybrids and Pest Management in the USA

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Abstract Corn, *Zea mays* L., grown in many areas of the United States suffers from a variety of insect species that attack virtually all parts of the growing plant. Many conventional pest management programs have been developed to combat these insects with varying degrees of success. In the mid-1990s, the commercial introduction and subsequent widespread adoption of Bt transgenic hybrids has all but transformed conventional corn pest management programs. The initial target of Bt corn, which contains insecticidal protein encoding genes from *Bacillus thuringiensis* (Bt), were stalk boring insects, such as the European and southwestern corn borers. Within a few years of the introduction of Bt hybrids for stalk boring insects, Bt hybrids targeting western and northern corn rootworms were introduced. Since their introduction, however, Bt corn hybrids have come under considerable scrutiny. They have been reported to produce higher yields as well as lower pesticide exposure to humans, non-target organisms, and the environment. Questions, however, have been raised on such issues as contamination of the food chain, resistance development, the overall sustainability of the technology, and more recently, the high costs of Bt hybrids relative to non-Bt hybrids. The present chapter delves into some of the issues and challenges surrounding the continued use of Bt corn hybrids and the strategies employed to address such issues.

Keywords *Bacillus thuringiensis* • Field corn • IPM • Transgenic corn • *Zea mays*

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1 Introduction

Corn, *Zea mays* L., is one of the world's most important crops, with the total production of more than 791 million metric tons in 2007–2008 (USDA 2009a). The United States (U.S.) ranks first among corn growing nations, with a production of over 331 million metric tons in 2007–2008, which is about 42% to the total corn production in the world (USDA 2009).

Considering the economic importance of this crop throughout the world and the U.S., insect pests associated with this crop have received a considerable amount of attention among researchers, growers, policy makers and industry. In North America, about 90 insect pests are found to be associated with this crop (Steffey et al. 1999); however, only a few are considered economically important. Economically important pests of corn can be broadly divided into two groups on the basis of their feeding patterns and plant parts where damage occurs: stalk-tunneling and root-feeding. Among the stalk-tunneling insects, European corn borer, *Ostrinia nubilalis* (Hübner), (Lepidoptera: Crambidae), and southwestern corn borer, *Diatraea grandiosella* Dyar, (Lepidoptera: Crambidae) (Metcalf and Metcalf 1993; Mason et al. 1996; Calvin and Van Duyn 1999; Knutson and Davis 1999; Tiwari et al. 2005a, b) are the most important.

The major root-feeding insects on corn are the northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, western corn rootworm, *D. virgifera virgifera* LeConte, and Mexican corn rootworm, *D. virgifera zea* Krysan and Smith (Coleoptera: Chrysomelidae) (Branson et al. 1982; Levine and Oloumi-Sadeghi 1991). In addition, there are secondary soil insects, which include several species of wireworms (Coleoptera: Elateridae), seedcorn maggot (Diptera: Anthomyiidae), annual white grubs (Coleoptera: Scarabaeidae), and true white grubs (Coleoptera: Scarabaeidae). Depending on the particular species, they can be found feeding on the roots or other belowground parts of the plant (Hunt and Baker 1982; Youngman et al. 1993; Keaster and Riley 1999; Eckenrode and Webb 1999; McLeod et al. 1999; Tiwari et al. 2005a, b).

Historically, management of insect pests in corn has focused on cultural and conventional chemical control programs (Hyde et al. 2000). However, over the past decade, pest management programs for economically important insects have changed dramatically with the commercial availability of *Bacillus thuringiensis* transgenic corn hybrids (Bt hybrids). Under some conditions, pest management programs targeting economically important insects have been reduced to simply planting Bt hybrids. However, some growers choose not to plant Bt hybrids in areas where historically low pest pressures occur. Instead, growers rely on such practices as crop rotation, application of conventional insecticides, and asynchrony between crop susceptibility and pest infestation.

In 2004, adoption of Bt hybrids led to a 10.57 million kilogram reduction in the use of pesticides (Drury et al. 2008), thereby reducing the environmental impact associated with pesticide use and greenhouse gas emissions (Brookes and Barfoot 2008). In addition, Bt hybrids have played a role in increasing net economic benefits

at the farm level (Brookes and Barfoot 2008). Other advantages of planting Bt hybrids include: less need for scouting (Obrycki et al. 2001; Crowder et al. 2006), protection against lepidopteran pests extending to storage (Giles et al. 2000), and lower levels of fungal pathogens and mycotoxins in the absence of stalk borer damage (Munkvold et al. 1999). However, planting Bt hybrids has raised concerns, such as increased cost input (Hyde et al. 1999), resistance development (Obrycki et al. 2001) and effects of Bt toxins on non-target organisms (Hilbeck et al. 1998; Höss et al. 2008; Prihoda and Coats 2008). Efforts have been made to address the aforementioned concerns using scientific research and regulatory approaches. Studies have reported that planting Bt hybrids result in higher returns than non-Bt hybrids under the following conditions: high pest pressure and late plantings (Hyde et al. 1999; Pilcher and Rice 2003; Wolf and Vogeli 2009).

The issue of insect resistance development to Bt toxins has been addressed by the United States Environmental Protection Agency's (USEPA) mandated Insect Resistance Management (IRM) plan. An IRM plan for Bt transgenic corn requires that a specified percentage of acreage be planted with a regular, non-transgenic corn hybrid. If above threshold levels of target pests are found in the non-Bt hybrid refuge, they can be managed with conventional management programs. Studies on the non-target effects of Bt toxins have yielded inconsistent results among the different taxonomic classes of non-target organisms (Hansen and Obrycki 2000; Höss et al. 2008).

2 Insect Pests of Corn

For the purpose of this chapter, we will focus our discussion on insect pests that are directly or indirectly impacted by currently available Bt hybrids.

2.1 Stalk Tunneling and Leaf/Ear Feeding Insects

Among stalk tunneling insects, European corn borer, *Ostrinia nubilalis* (Hübner), (Lepidoptera: Crambidae) (Mason et al. 1996; Calvin and Van Duyn 1999), and southwestern corn borer, *Diatraea grandiosella* Dyar, (Lepidoptera: Crambidae) (Metcalf and Metcalf 1993; Knutson and Davis 1999) are among the most important pests that occur throughout most of the corn growing areas of the U.S. Crop losses and management costs for European corn borer are reported to exceed \$1 billion annually in the U.S. (Mason et al. 1996). Annual losses from southwestern corn borer are estimated at several million dollars (Morrison et al. 1977). In some corn growing areas, common stalk borer, *Papaipema nebris* Guenée, (Lepidoptera: Noctuidae) is also considered as an occasional pest (Solomon 1988) (Figs. 1 and 2).

The first and second instars of European corn borer feed on leaves in whorl-stage corn causing a shothole-like appearance. The late third instar starts tunneling into the stalks, ears, or ear shanks, with the majority of larvae having bored into the stalks by the fourth instar (Fig. 3). The southwestern corn borer causes injury similar to



Fig. 1 Mature larva of the common stalk borer, *Papaipema nebris*, boring into a corn plant early in the growing season. Feeding within the stalk causes deformed or stunted plants that often lead to the death of the plant



Fig. 2 Damage by common stalk borer larvae resulting in the stunted and abnormal growth of corn plants. Severe damage to the central part of the plant results in the death of central whorl. This condition has been referred as 'dead heart'



Fig. 3 Stalk tunneling by late third and later instars of European corn borer, *Ostrinia nubilalis*, in field corn. Similar tunneling can also be seen in ears or ear shanks

European corn borer, except for one major difference. The mature larva girdles the stalk at the base just above the soil surface late in the season. This late season girdling often results in severe stalk lodging during harvest or from high winds (Knutson and Davis 1999). The southwestern corn borer larva overwinters in a cell, it has made at the base of stalk just below the soil surface (Knutson and Davis 1999).

Other insect pests that feed on corn leaves or the ear include fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and corn earworm, *Helicoverpa zea* (Boddie). Of these, fall armyworm is of greater economic importance (Buntin et al. 2001). Both pests are found during the whorl stage; however, injury also continues to later stages (Buntin et al. 2001). Unlike the corn earworm, which restricts feeding to the ear tips, the fall armyworm is capable of causing severe leaf and kernel damage late in the season (Archer and Bynum 1998).

2.2 Seed and Root Feeding Insects

As mentioned previously, the major root-feeding insects on corn are the northern corn rootworm, western corn rootworm, and Mexican corn rootworm. Crop losses and management costs attributed to corn rootworms have been estimated to cost U.S. growers over \$1 billion annually (Rice 2004). This estimate is now considered to be an underestimate since a soybean variant of the western cornworm has evolved resistance to crop rotation in the central U.S. corn belt (Gray et al. 2009).

Feeding injury on corn roots begins with the first instar. Early instars feed on root hairs and outer root tissue, while older instars burrow and feed in the inner root core.



Fig. 4 Developmental stages of Japanese beetle, *Popillia japonica*. Larval stage of Japanese beetle referred as white grubs (a) causes injury during early season by chewing off the fine rootlets of corn plants. This causes wilting and purpling of the stem. Grubs continue to feed until late May or early June when they pupate in an earthen cell (b shows prepupal stage) about 1 – 3 inches deep in the soil. Adults (c) feed on corn silks which interfere with pollination resulting into impaired kernel development and grain yield

Heavy infestation by corn rootworms can seriously weaken the root system, impeding the transport of water and nutrients from the roots to aboveground plant parts, as well as lead to stalk lodging (Chiang 1973; Levine and Oloumi-Sadeghi 1991; Tollefson and Levine 1999; Sutter 1999). After feeding for several weeks, the third instar pupates in a small earthen cell. Adults are active from mid- to late-summer, during which time they mate, feed on corn silk, pollen, and kernels of exposed ear tips (Youngman and Tiwari 2004).

In addition, numerous species of secondary soil insects are considered sporadic pests of germinating corn seeds or early stage corn (Hunt and Baker 1982; Youngman et al. 1993; Keaster and Riley 1999; Eckenrode and Webb 1999; McLeod et al. 1999; Tiwari et al. 2005a, b). Important secondary soil insects include several species of ‘annual’ and ‘true’ white grubs (Fig. 4) (Coleoptera: Scarabaeidae), wireworms (Coleoptera: Elateridae), and seedcorn maggot (Diptera: Anthomyiidae). Damage caused from wireworms, annual white grubs, and seedcorn maggot is primarily due to feeding on the germinating corn seed and emerging roots (Youngman et al. 1993). Damage caused by true white grubs is primarily restricted to the developing corn roots (Hunt and Baker 1982; McLeod et al. 1999).

3 *Bacillus Thuringiensis* (Bt)

Bacillus thuringiensis is a rod-shaped, gram positive, spore forming bacterium that is isolated from various habitats worldwide (Schnepf et al. 1998). *B. thuringiensis* produces a proteinaceous parasporal crystalline inclusion body formed within the bacteria during sporulation (Gill et al. 1992). The crystalline inclusion body contains from one to several δ -endotoxins that are responsible for causing death in certain species of insects, yet are harmless to humans and most non-target insects.

Current classification of Bt toxins is based on the nomenclature system developed by Crickmore et al. (1998). This nomenclature assigns a name to each holotype sequence based on the degree of evolutionary divergence as estimated by phylogenetic tree algorithms. Currently 204 holotype sequences for insecticidal proteins have been identified in various strains of *B. thuringiensis*, of which 195 are Cry and nine are Cyt δ -endotoxins (Crickmore et al. 2010). Based on holotype sequences, Cry endotoxins are currently divided into 60 families (from Cry1 to Cry60) and Cyt endotoxins are divided into two families (from Cyt1 to Cyt2) (Crickmore et al. 2010).

3.1 *Mode of Action of B. Thuringiensis*

The insecticidal activity of *B. thuringiensis* occurs after a susceptible insect ingests the crystalline inclusion body. After reaching the midgut, the ingested crystalline inclusion body is solubilized by the alkaline environment and enzymatic proteases,

resulting in the release of one or more δ -endotoxins (Lambert and Peferoen 1992; Gill et al. 1992; Schnepf et al. 1998; Moellenbeck et al. 2001; Ferré and Van Rie 2002; Whalon and Wingerd 2003). Trypsin-like or chymotrypsin-like proteases in the insect gut start acting on the released endotoxins and continue to act until a trypsin resistant core protein is reached (55–75 kDa) (Schnepf et al. 1998; Moellenbeck et al. 2001; Ferré et al. 2008). This is followed by the protease-resistant core protein passing through the peritrophic membrane and binding to specific receptor (membrane protein complex) on the apical brush border of midgut columnar cells. This binding results in pore formation, cell swelling, cell lysis and ultimately insect death. Binding between the protease-resistant core protein and receptors on midgut columnar cells is highly species specific, so insects lacking the specific receptors are not harmed (Dorsch et al. 2002). Failure or reduction of the protease-resistant core protein to bind with a specific receptor on the apical brush border of the midgut columnar cells is one of the mechanisms of resistance development (Ferré and van Rie 2002).

3.2 *Bt Hybrids*

The first transgenic corn hybrid containing a modified short sequence of genes from *B. thuringiensis* against an insect pest was registered by the USEPA in 1995 (Shelton et al. 2002) under the names of “KnockOut®” (Syngenta Seeds [formerly Novartis Seeds]) and “NatureGard®” (Mycogen Seeds). Both hybrids contain event 176, Cry1Ab endotoxin, for European corn borer and other Lepidoptera pests. However, Bt hybrids with event 176 are no longer registered (Glaser and Matten 2003). In 1996, Bt hybrids containing event Bt11 under the name of “Agrisure™ CB” by Northrup King, and event Mon810 under the name of “YieldGard®” by Monsanto were commercially released; both events encoded the Cry1Ab endotoxin. In the years following, the USEPA registered two more Bt events for use in corn (Youngman and Tiwari 2004): event TC 1507 in 2001 developed jointly by Pioneer/Dupont and Dow AgroSciences under the name “Herculex™ I *Insect Protection*” and event Mon863 in 2003 developed by Monsanto under the name “YieldGard® Rootworm”. Event TC 1507, Cry1F endotoxin, targeted black cutworm, fall armyworm, and European corn borer, and event Mon863, Cry3Bb, targeted corn rootworms.

The USEPA has since registered stacked Bt hybrids designed to control two different types of insects, such as “YieldGard® Plus” (Monsanto) in October 2003, and “Herculex® XTRA *Insect Protection*” (Dow AgroSciences and Pioneer Hi-Bred International) in October 2005. YieldGard® Plus contain events Mon 810 and Mon 863, encoding for Cry1Ab1 and Cry3Bb1 endotoxins, respectively (USEPA 2005a). Herculex™ XTRA *Insect Protection* contains event DAS-59122-7 encoding for Cry34Ab1 and Cry35Ab1 endotoxins, and event TC1507 encoding for Cry1F endotoxin (USEPA 2005b). Cry34Ab1 (14 kDa) and Cry35Ab1 (44 kDa) endotoxins are a relatively new class of insecticidal proteins identified from a *B. thuringiensis* strain PS149B1 that acts against corn

rootworms (Herman et al. 2002; Gao et al. 2004). In October 2006, the USEPA registered Agrisure™ RW Rootworm-Protected Corn (Syngenta Seeds). Agrisure™ RW Rootworm-Protected Corn contains event MIR604, which produces a modified Cry3A (mCry3A) endotoxin (USEPA 2006). The modified Cry3A gene, recreated from *B. thuringiensis* subsp. *tenebrionis*, with its optimized expression in corn claims to have enhanced activity against larvae of the western corn rootworm and northern corn rootworm (USEPA 2006). In 2007, the USEPA registered Agrisure™ CB/RW (Syngenta Seeds) as another stacked hybrid containing events Bt11 and MIR604 expressing Cry1Ab and mCry3A endotoxins, respectively (USEPA 2008). The most recent addition to the list is SmartStax™ (Monsanto and Dow AgroSciences) as another stacked hybrid containing events MON 89034, TC1507, MON 88017 and DAS-59122-7 expressing Cry1A.105 and Cry2Ab2; Cry1F; Cry3Bb1; and Cry34Ab1 and Cry35Ab1 endotoxins, respectively (USEPA 2009). According to Ostlie et al. (1997), Bt hybrids exhibit different levels of protection, depending on the type of genetic event and promoter used in developing a hybrid. The genetic event, in addition to a promoter, affects the amount, type, and location of the production of the endotoxin in the plant. For example, Bt hybrids with events Bt11 and Mon810 provide protection against first and second generation European corn borer larvae. Bt hybrids containing event 176 provide less acceptable protection against second generation larvae. As Ostlie et al. (1997) pointed out; events Mon810 and Bt11 express the Cry1Ab endotoxin in all plant tissues with the exception of root tissues, whereas event 176 expresses endotoxin only in green tissue and pollen. In addition, Ostlie et al. (1997) noted that Bt hybrids with events Bt11 and Mon810 provided 93% control of southwestern corn borer, whereas Bt hybrids with event 176 provided only 19% control of this pest.

Bt hybrids containing event Mon863 continue to produce endotoxins throughout the plant (Vaughn et al. 2005). In a laboratory study conducted by Vaughn et al. (2005), Bt hybrids containing event Mon863 (encoding for Cry3Bb1 endotoxin) exhibited a declining trend in root expression of Cry3Bb1 endotoxin from the V4 to V9 growth stage; however, this declining trend had no negative effect on corn roots despite rootworm pressure. In a study cited by Rice (2004), YieldGard® Rootworm (event Mon863) and YieldGard® Plus (stacked hybrid) (events Mon 810 and Mon 863) were tested against the soil insecticide terbufos (Counter 20CR) and several non-Bt hybrids in protecting corn roots from damage caused by corn rootworms. The study showed that the Bt hybrids were 100% consistent in protecting corn roots from economic damage, whereas Counter 20CR was only 63% consistent. Moreover, little or no protection from corn rootworm feeding was detected in the non-Bt hybrids.

4 Insect Resistance Management

Adoption of Bt corn hybrids worldwide has increased tremendously since the first commercial release in 1995. In 2009, 85% of the corn acreage in the U.S. was under Bt hybrids; this includes all the available transgenic Bt hybrids (USDA 2009b)

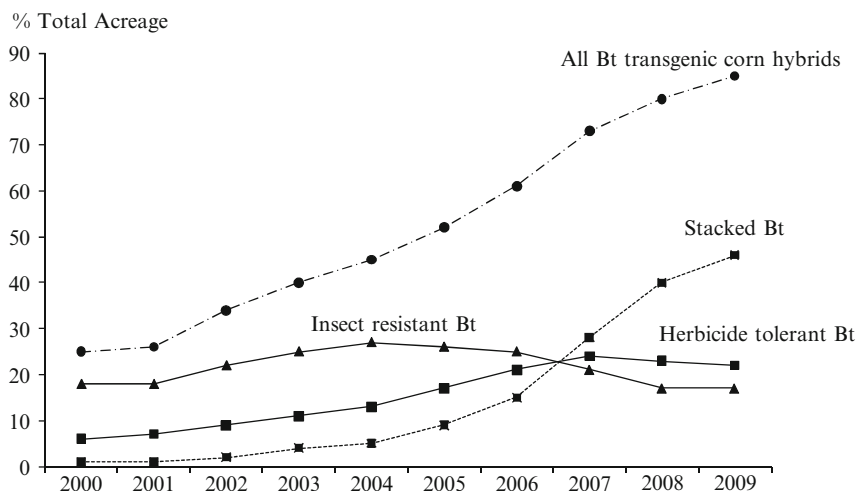


Fig. 5 Increase in the acreage under Bt corn hybrids from 2000 to 2009 in the United States. The acreage under Bt corn hybrids has been continuously increasing since the first commercial release of Bt hybrids in 1995. Bt corn hybrids are genetically modified corn hybrids containing a modified short sequence of genes from a bacterium, *Bacillus thuringiensis*. These genetically modified hybrids produce toxins in the host plant that are targeted against specific insect pests.

Source: <http://www.ers.usda.gov/Data/BiotechCrops/alltables.xls>, accessed April 12, 2010

(Fig. 5). The increased acreage under Bt hybrids has benefited growers in several ways, such as fewer applications of insecticides, higher yields and less exposure to humans and environment from insecticides. However, it has presented some new challenges. One of the most important challenges presented by the increasing acreage under Bt hybrids is the risk of developing resistance to Bt expression in the target insects (Gould 1998; Zhao et al. 2003). Scientists, pest management practitioners, and environmental regulators have responded to this challenge by developing insect resistance management strategies (IRM) for the purpose of delaying development of resistance to Bt events in the target pests (Hyde et al. 2000; Glaser and Matten 2003; Zhao et al. 2003; Bates et al. 2005; Bourguet et al. 2005). Considering the importance of this issue, the USEPA mandated that all companies registering Bt hybrids develop and deploy IRM strategies to delay the development of resistance in target pests.

During the early stages of developing IRM strategies, several tactics were designed and developed (Roush 1997). One strategy is the planting of Bt hybrids, which express moderate levels of toxin to delay the development of resistance in the target pest (Bates et al. 2005). The idea behind using a moderate level of toxin was to maintain survival of a susceptible proportion of the population. Another tactic to mitigate resistance development is planting Bt hybrids expressing a high level of toxin (high-dose strategy) (Zhao et al. 2003). The idea being that the expression of endotoxin is high enough to kill any heterozygous resistant larvae, which would otherwise survive and reproduce. Planting Bt hybrids expressing a high level of toxin in addition to planting a non-Bt refuge has become the primary

element of IRM strategies (Dalecky et al. 2006; Tabashnik 2008; USEPA 2005a, b). A Scientific Advisory Panel Subpanel to FIFRA defined high dose as “25 times the toxin concentration needed to kill susceptible larvae” (USEPA 1998). The Subpanel also defined structured refuges to “include all suitable non-Bt host plants for a targeted insect that are planted and managed by people”. However, Bt hybrids containing the event Mon863, which produces Cry3Bb endotoxin that targets corn rootworms, as well as other Bt hybrids targeting corn rootworms are reported to express low-moderate levels of toxin (Siegfried et al. 2005; Vaughn et al. 2005; Meihls et al. 2008).

4.1 High-Dose Toxin and Refuge Strategy for Single Event Bt Hybrids against Corn Borers

This strategy involves two components: planting Bt corn hybrids, which express a high dose of toxin; and refuge planting of non-Bt corn hybrids. This strategy has shown to be an effective way of delaying the development of resistance to Bt toxins (Alstad and Andow 1995; Gould 1998; Zhao et al. 2003; Bates et al. 2005; Dalecky et al. 2006; Eizaguirre et al. 2006). Under this strategy, the Cry1Ab toxin produced by Bt hybrids is high enough to kill all susceptible homozygous, and most of the resistant heterozygous target pests. The few resistant heterozygous individuals remaining will most likely breed with susceptible homozygous individuals from refuge areas. The effect of which being a greatly diminished production of resistant heterozygous individuals in subsequent populations (Gould 1998; Vacher et al. 2003; Bourguet et al. 2005). Another advantage of the high-dose toxin approach is maintaining host plant damage below the economic threshold (Bates et al. 2005). The USEPA has mandated various plans on the size and layout of refuge planting based on agronomic conditions and the target pest (USEPA 2001; USEPA 2005a, b) (Fig. 6).

According to the USEPA requirements, refuge area requirements for Bt hybrids targeting European corn borer, southwestern corn borer, and other lepidopteran pests has been divided into two categories: non-cotton growing areas and cotton growing areas (Youngman and Tiwari 2004). The USEPA requirements state that growers in non-cotton growing areas may plant up to 80% of their corn hectares using a Bt hybrid, with the remaining 20% serving as the refuge (USEPA 2000). In cotton growing areas, growers may plant up to 50% of their corn hectares using a Bt hybrid, with the remaining 50% serving as the refuge (USEPA 2000). The large percentage of refuge in cotton areas was recommended to prevent resistance development in corn earworm populations (Gould et al. 2002) given that corn is a major host source for corn earworm development in the mid-Atlantic.

A refuge may be located within, adjacent, or up to 0.8 km (0.5 mile) from the Bt hybrid field. Distance of refuge from the Bt hybrid field is based on information on insect flight and oviposition behavior (Glaser and Matten 2003). The purpose of which is to promote random mating between susceptible moths from refuge areas

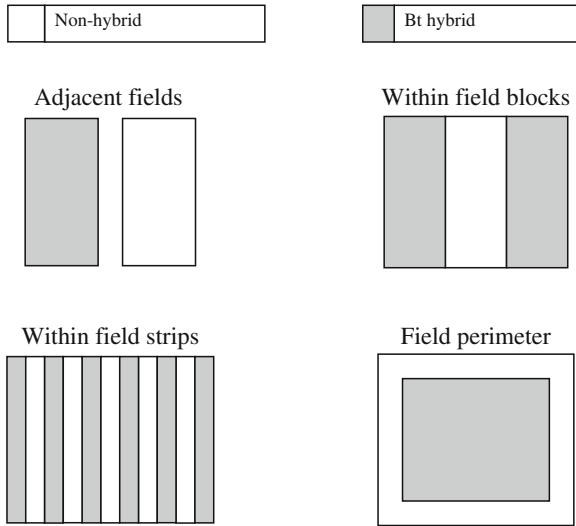


Fig. 6 Different layouts of non-Bt refuge and Bt corn hybrid plantings for insect resistance management. The United States Environmental Protection Agency has mandated various plans on the size and layout of refuge planting by non-Bt corn hybrids to delay the development of resistance against Bt toxins

with resistant survivors from Bt hybrid fields. A refuge up to 0.8 km (0.5 mile) away from the Bt hybrid field must not be treated with any insecticides for corn borers. If the refuge is to be treated for corn borers, it must be within 0.4 km (0.25 mile) of the Bt hybrid field. Also, sprayable formulations of Bt insecticides cannot be applied to the refuge. A refuge can be planted as strips (i.e., strips that are at least 6 and preferably 12 adjacent rows wide) within the Bt hybrid field, or as blocks within, adjacent, or away from the Bt hybrid field. A minimum of six rows was based on a simulation modeling of insect movement and mating (Onstad and Guse 1999). In addition, a refuge can be planted as a field perimeter or as end rows (Fig. 4). Mixing seeds of Bt hybrids and non-Bt hybrids is not recommended for managing corn borers (Youngman and Tiwari 2004).

4.2 Moderate-Dose Toxin and Refuge Strategy for Single Event Bt Hybrids against Corn Rootworms

The IRM strategy for Bt hybrids targeting corn rootworms is similar to the IRM strategy for Bt hybrids targeting European corn borer and other lepidopterans (Youngman and Tiwari 2004). According to Gray (2001), an IRM strategy involving numerous within-field refuge strips would be more effective than separate block refuges in the case of Bt hybrids targeting corn rootworms. Gray (2001) noted that pre-mating dispersal of adult corn rootworms away from their field of emergence

is very limited compared with European corn borer where mating occurs in tall grass outside of cornfields, with subsequent egg laying occurring randomly across the landscape.

In certain areas of the Midwest Corn Belt, where crop rotation as a cultural control option is no longer effective against larvae of western and northern corn rootworms, it has been recommended to use a refuge for first-year corn if a Bt corn rootworm hybrid targeting corn rootworms is planted. Western corn rootworm has adapted to crop rotation by switching from corn to soybean to lay eggs (Gray et al. 1998; Rondon and Gray 2004). Northern corn rootworm has adapted to crop rotation by extending egg diapause from one to two years (Krysan et al. 1984; Levine et al. 1992; Gray et al. 1998). In both cases, planting a Bt corn rootworm hybrid is recommended as one of the best ways to manage the rotation resistant problem associated with corn rootworms (Onstad et al. 2003).

4.3 Refuge Planting for Stacked Bt Hybrid against Corn Borers and Corn Rootworms

According to the USEPA, refuge area requirements for stacked Bt hybrids targeting corn borers and corn rootworms have been divided into two categories: non-cotton growing areas and cotton growing areas (USEPA 2005a, b). USEPA requirements state that growers in non-cotton growing areas may plant up to 80% of their corn hectares using a Bt hybrid targeting corn borers, with the remaining 20% serving as the refuge (USEPA 2005a, b). However, in cotton growing areas, growers may only plant up to 50% of their corn hectares using a Bt hybrid targeting corn borers, with the remaining 50% serving as the refuge (USEPA 2005a, b). The refuge may be planted in two ways: either as a common refuge for both corn borers and corn rootworms or as separate refuges for corn borers and corn rootworms (USEPA 2005a, b).

The common refuge involves planting corn hybrids that do not contain Bt events for either corn borers or corn rootworms. The refuge area must represent at least 20% (in non-cotton growing areas) and 50% (in cotton growing areas) of a grower's corn hectares (i.e., sum of stacked Bt hybrid hectares and refuge hectares). The refuge can be planted as a block, perimeter strips, or in-field strips. If perimeter or in-field strips are planted, the strips must be at least six, and preferably 12 adjacent rows wide. The common refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae and other soil pests. In addition, the refuge may be treated with a non-Bt foliar insecticide for control of late season pests if pest pressure reaches an economic threshold; however, if corn rootworm adults are present at the time when foliar applications are made then the stacked Bt hybrid acres must be treated in a similar manner.

The second option is planting separate refuge areas for corn borers and corn rootworms. A corn borer refuge involves planting corn hybrids that do not contain Bt events for corn borers on at least 20% of the hectares in non-cotton growing areas, and on at least 50% of the hectares in cotton growing areas. These refuge

areas are based on the total corn hectares a grower plants each season (i.e., the sum of stacked Bt corn hybrid hectares and corn borer refuge hectares), and must be planted within 0.8 km (0.5 mile) of the stacked Bt hybrid field. The corn borer refuge can be treated with a soil-applied or seed-applied insecticide for corn rootworm larval control, or a non-Bt foliar-applied insecticide for corn borer control if pest pressure reaches an economic threshold.

A corn rootworm refuge involves planting corn hybrids that do not contain Bt events for corn rootworm, but can be planted with Bt corn borer hybrids. The corn rootworm refuge must represent at least 20% in non-cotton growing areas, and 50% in cotton growing areas. These refuge areas are based on the total corn hectares a grower plants each season, i.e., the sum of stacked Bt corn hybrid hectares and corn rootworm refuge hectares. The refuge can be planted as an adjacent block, perimeter strips, or in-field strips. The corn rootworm refuge can be treated with soil-applied or seed-applied insecticides to control rootworm larvae and other soil pests. The refuge can also be treated with a non-Bt foliar insecticide for control of late season pests; however, if rootworm adults are present at the time when foliar applications are made then the stacked Bt hybrid field must be treated in a similar manner.

4.4 Limitations with the High-Dose Toxin and Refuge Strategy

The high-dose toxin and refuge planting strategy for preventing resistance development in the target insect is based on three strict assumptions: inheritance of resistance is recessive in the target insect population (Liu et al. 2001), low presence of resistance alleles ($<10^{-3}$) in the target insect population (Roush and Miller 1986), and random or preferential mating between susceptible individuals from the refuge and resistant individuals from the Bt hybrid field (Vacher et al. 2003, Bates et al. 2005). However, there are examples where inheritance of resistance to Bt toxins is found to be incomplete or non-recessive, such as in a strain of *Helicoverpa armigera* to Cry1Ac toxin (Akhurst et al. 2003), *H. zea* to Cry1Ac toxin (Burd et al. 2003), and *O. nubilalis* to the Bt toxins in Dipel ES (Dipel ES contains Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, and Cry2B endotoxins of *Bt*. Cry1Ab and Cry1Ac) (Huang et al. 1999). The idea behind the high-dose toxin refuge strategy is that it targets individuals with incompletely dominant resistance or heterozygous resistance (Bourguet et al. 2000). In a study conducted in 1997, strains of pink bollworm, *Pectinophora gossypiella*, collected from 10 Arizona cotton fields revealed that the estimated frequency of a major resistance allele to Cry1Ac toxin has increased to 0.16 (Tabashnik et al. 2000). In cotton, the variable developmental period found between resistant larvae of *P. gossypiella* on Bt transgenic cotton hybrids expressing Cry1Ac toxin and susceptible larvae on non-Bt transgenic cotton hybrids could lead to non-random mating between resistant and susceptible individuals (Liu et al. 2001). In a study on pre-copulatory dispersal and mating in *O. nubilalis*, it was found that females prefer mating near the emergence site before

dispersal (Dalecky et al. 2006). In a similar study conducted by Bailey et al. (2007), they found that the mean (\pm SEM) distance flown by *O. nubilalis* adults was 5.05 ± 7.3 m in 12 h from the release site. This could be a limiting factor for random mating to take place between resistant and susceptible individuals. However, no significant violations have been reported to date with respect to target pests subjected to the high-dose refuge strategy (Bates et al. 2005; Tabashnik et al. 2008).

There are new issues facing the high-dose refuge strategy in terms of adhering to the physical limitations. Contamination of Bt hybrid seeds with non-Bt hybrid seeds as a result of off-types may promote more rapid development of resistance (Gould 1998; Bates et al. 2005). The movement of the target insect between Bt hybrids and other non-Bt host plants or weedy plants can lead to ingestion of intermediate doses of toxin by the target insect, which may eventually expedite the development of resistance (Gould 1998). In addition, pollen mediated gene flow from Bt hybrids to non-Bt hybrids (refuge) has been found to result in low to moderate levels of Bt toxin in refuge plants (Chilcutt and Tabashnik 2004).

5 Resistance Monitoring

Resistance monitoring has been an integral part of the IRM strategy to detect the development of resistance in target insects to Bt hybrid toxins. Several methods have been suggested for monitoring the development of resistance: annual damage reports by growers, direct monitoring of insect population susceptibility, dose–response bioassays, diagnostic/discriminating dose bioassays, F_2 screen, feeding disruption assays, and feral assays (Venette et al. 2000; Bourguet et al. 2005). However, the dose–response and diagnostic/discriminating dose bioassays are currently the most widely used methods (Bates et al. 2005; Bourguet et al. 2005; Huang 2006; Huang et al. 2007).

The dose–response bioassay as described by Bourguet et al. (2005) measures the change in EC_{50} and LC_{50} values in a natural population of the target pest over a period of time. This is done by exposing insects to a series of Bt toxin concentrations, and then using probit analysis to determine EC_{50} and LC_{50} values. The dose–response bioassay is more efficient in detecting high levels of resistance or resistance conferred by a dominant allele than in detecting early development of resistance conferred by a recessive allele (Bates et al. 2005; Huang et al. 2007). In addition, the dose–response bioassay can test large number of insects in a relatively efficient manner (Ferré et al. 2008).

The diagnostic/discriminating dose bioassay is based on the use of a single dose of a Bt toxin (i.e., the diagnostic/discriminating dose) (Huang 2006). The most commonly used diagnostic/discriminating dose is the LC_{99} value for susceptible strains, which is developed from a dose–response bioassay (Huang 2006). This single dose method is more efficient in detecting dominant resistance alleles or recessive resistance alleles at high levels (Huang et al. 2007). However, some of the limitations with the diagnostic/discriminating dose method are the need for a large

sample size (Roush and Miller 1986) and decreased efficiency at detecting recessive alleles resistant to Bt toxins (Venette et al. (2000). Bourguet et al. (2005)) noted that this method is unlikely to detect early stages of resistance development.

Given that both the dose–response and diagnostic/discriminating dose bioassays are not suitable for detecting low levels of recessive alleles resistant to Bt toxins, suggestions have been made to integrate other resistance monitoring tools into the program. The F_2 screen and DNA marker methods are reported to have higher sensitivity for detecting low levels of recessive alleles resistant to Bt toxins (Huang 1997).

6 Role of Insecticides after Stacked Bt Hybrids

Following the commercial release of Bt hybrids for corn borers and corn rootworms, there has been a marked shift in the use of insecticides in corn production (Pilcher et al. 2002; Wilson et al. 2005; Brookes and Barfoot 2006). A multi-state survey was conducted over three years on corn grower use of insecticides to control European corn borer in the Midwest Corn Belt (Pilcher et al. 2002). Pilcher et al. (2002) reported that the percentage of growers that decreased their insecticide use has nearly doubled from 13.2% in 1996 to 26.0% in 1998. Rice (2004) estimated that planting Bt hybrids against corn rootworms will alone result in about a 75% reduction in insecticide use targeting corn rootworms. In a 2001 survey conducted among corn growers across five states, Wilson et al. (2005) reported that the perceived benefits of using Bt hybrids were reduced grower exposure to insecticides (69.9%) and lower levels of insecticide active ingredient in the environment (68.5%). At the national level, the planting of Bt hybrids has resulted in a reduction of insecticide active ingredient by 0.6 million kilogram and an annual environmental impact quotient (EIQ) by 21 million field EIQ/ha units from 1996 to 2005 (Brookes and Barfoot 2006). EIQ is calculated in terms of field value per hectare using various toxicological and environmental impact data for each pesticide (Kovach et al. 1992).

Although planting Bt hybrids has resulted in a significant reduction in the use of conventional insecticides on corn, it has not totally eliminated the use of insecticides in the majority of corn growing areas of the U.S. In fact, a high percentage of commercial Bt hybrids today come with insecticide-protected coated seeds, which are primarily treated with systemic neonicotinoids targeting secondary soil insects (Mullin et al. 2005; Magalhaes et al. 2007). In those situations where growers decide not to plant Bt hybrids, it is recommended that growers use pre-plant sampling methods to identify fields at risk to secondary soil insects (Keaster and Riley 1999, McLeod et al. 1999; Youngman et al. 1993). The most common methods for managing secondary soil insects are soil-applied insecticides or planting insecticide-protected coated seeds (Andersch and Schwarz 2003). The rate and type of insecticides used in either method depends on the target insect. Insecticides belonging to the organophosphate, carbamate, pyrethroid, or phenylpyrazole classes have been the most commonly used as soil-applied insecticides (Andersch and Schwarz 2003).

Due to several disadvantages associated with the conventional soil-applied insecticides (Altmann 2003; Andersch and Schwarz 2003), planting insecticide-protected coated seeds to manage early season secondary soil insects is now increasing and becoming more widely adopted. Imidacloprid, clothianidin, thiamethoxam and tefluthrin are the main insecticides used by seed companies to treat corn seeds for protection against early season feeding injury to germinating seeds and newly emerging roots (Mullin et al. 2005; Magalhaes et al. 2007).

With the widespread use of neonicotinoid seed protectants on Bt hybrid corn seeds, there is a growing concern for monitoring resistance development in insects that are the target of these seed protectants (Magalhaes et al. 2007), in addition to evaluating the indirect effects of neonicotinoids on non-target organisms (Mullin et al. 2005). Development of resistance to neonicotinoids has been documented in several insect species. Specific examples include whiteflies *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood), brown planthopper *Nilaparvata lugens* (Stal), Colorado potato beetle *Leptinotarsa decemlineata* (Say) and mango leafhopper *Idioscopus clypealis* (Lethierry) worldwide (Elbert et al. 2008). The development of resistance to neonicotinoids in adults and larvae of the Colorado potato beetle (Zhao et al. 2000; Nauen and Denholm 2005) raises two points for concern with respect to the continued, widespread use of neonicotinoid seed treatments on Bt corn rootworm hybrid seeds. First, the Colorado potato beetle and corn rootworms belong to the same taxonomic family (Chrysomelidae); and second, the Colorado potato beetle and corn rootworms share a similar history of developing resistance to insecticides in the major insecticide classes: chlorinated hydrocarbons, organophosphates, and carbamates. Although, in most cases, management of corn rootworm larvae is not intended through the use of neonicotinoid seed treatments where Bt corn rootworm hybrids are planted. Nevertheless, neonicotinoids still serve as an important tool for managing corn rootworms in refuge plantings and areas where growers choose not to plant Bt hybrids for corn rootworms.

A study was conducted to examine the effects of seed treatments associated with Bt hybrids expressing Cry3Bb1 and Cry1Ab/c endotoxins on several species of carabid beetles (Mullin et al. 2005). They found that adult beetles representing 16 carabid species, which had fed on the pollen of Bt corn hybrids suffered no significant toxicity, whereas beetles representing 18 carabid species suffered nearly complete mortality when exposed to corn seedlings grown from imidacloprid, thiamethoxam or clothianidin treated seeds.

In order to manage the increasing selection pressure from using seed treatment insecticides, Magalhaes et al. (2007) provided a baseline tool for predicting and monitoring the early signs of resistance development among geographically distinct populations of western corn rootworm. High-dose and refuge strategy could be employed to delay resistance to neonicotinoids as suggested by Zhao et al. (2000) in their study reporting resistance development in *L. decemlineata* to imidacloprid. They reported that resistance to imidacloprid is an incompletely recessive trait. According to Elbert et al. (2005), efforts should be made to follow IRM guidelines for managing resistance development to neonicotinoid insecticides. The idea being to optimize the use of this technology against the target insects while simultaneously reducing their impact on non-target species.

7 Conclusion

Bt transgenic corn hybrids have become an integral part of corn production in most of the corn growing areas of the world (Bates et al. 2005; James 2008; Tabashnik 2008). Its continued adoption worldwide speaks of increasing confidence among corn growers for this technology. Higher returns from planting Bt hybrids as a result of increased yield and fewer insecticide applications (Pilcher et al. 2002; Wilson et al. 2005; Brookes and Barfoot 2006; James 2008) are the primary factors. Implementation of a robust IRM strategy, which is the first one of its kind to be implemented on such a large scale, further boosts the confidence of growers for the sustainable use of this technology. In addition, a regular monitoring plan for resistance development in the target pest is a necessary step against resistance development, the overall aim of which is to secure the long-term usefulness of the Bt technology. Implementation of these strategies has contributed much to the fact that no cases of failure in the Bt corn hybrid technology have been reported since its commercial introduction in 1995. In addition to Bt hybrids, the increasing trend in the use of insecticide-protected coated seeds, makes it imperative that IRM plans be developed and implemented for the target pests of this technology as well.

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