Review article

**Bacillus thuringiensis**: a century of research, development and commercial applications

Georgina Sanahuja¹,†, Raviraj Banakar¹,†, Richard M. Twyman², Teresa Capell¹ and Paul Christou¹,³,*

¹Department of Plant Production and Forestry Science, ETSEA, University of Lleida, Lleida, Spain
²Department of Biological Sciences, University of Warwick, Coventry, UK
³Institució Catalana de Recerca i Estudis Avançats, Passeig Lluís Companys 23, Barcelona, Spain

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*Correspondence (fax +34 973 70 29 24; email christou@pvcf.udl.es)
†These authors contributed equally to this article.

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Summary

*Bacillus thuringiensis* (Bt) is a soil bacterium that forms spores during the stationary phase of its growth cycle. The spores contain crystals, predominantly comprising one or more Cry and/or Cyt proteins (also known as δ-endotoxins) that have potent and specific insecticidal activity. Different strains of Bt produce different types of toxin, each of which affects a narrow taxonomic group of insects. Therefore, Bt toxins have been used as topical pesticides to protect crops, and more recently the proteins have been expressed in transgenic plants to confer inherent pest resistance. Bt transgenic crops have been overwhelmingly successful and beneficial, leading to higher yields and reducing the use of chemical pesticides and fossil fuels. However, their deployment has attracted some criticism particularly with regard to the potential evolution of pest-resistant insect strains. Here, we review recent progress in the development of Bt technology and the countermeasures that have been introduced to prevent the evolution of resistant insect populations.

Introduction

The insecticidal properties of Bt were recognized many years before the bacterium was identified, with some accounts suggesting that Bt spores may have already been in use in ancient Egypt. In the modern era, the bacterium was isolated in 1901 by the Japanese biologist Shigetane Ishiwatari during an investigation into wilt disease in silk worms, and he named it *Bacillus sotto*. Ten years later, the same bacterium was isolated by Ernst Berliner from a diseased Mediterranean flour moth (*Ephestia kuehniella*) in the German province of Thuringia, and it was named *Bacillus thuringiensis* (Siegel, 2000). The defining feature of Bt is its ability to produce proteinaceous crystals during sporulation. Bt is a member of the *Bacillus cereus* group of Gram-positive, spore-forming soil bacteria, and occasionally the bacteria lose their ability to form crystals and then become indistinguishable from *B. cereus* itself. Similarly, *B. cereus* can be transformed into Bt, and investigations into the transformation mechanism led to the discovery that crystal formation is conferred by genes carried on a plasmid. The genes, which encode Cry/Cyt proteins, become active during sporulation because they are controlled by a dedicated RNA polymerase that is also synthesized specifically while spores are forming. Up to 20% of the spore protein content is represented by these Cry/Cyt toxins (Aronson, 2002).

The insecticidal properties of the crystals were discovered when dead flour moth caterpillars were found to be loaded with spores and crystals. Direct contact between the spores/crystals and healthy caterpillars had no effect, but when the spores and crystals were coated onto leaves, the caterpillars stopped feeding and died. After recognizing the potential of Bt as an insecticide, Mattes (1927) isolated the Bt strain discovered by Ernst and subsequent field trials against the European corn borer (*Ostrinia nubilalis*) gave promising results (Husz, 1930). This work eventually led to the development of Sporeine, a commercial Bt insecticide, which was used for the first time in 1938.
The biology of Bt toxins

Sporeine targeted lepidopteran insects, and the active ingredient was initially thought to be a food-borne invasive pathogen. However, this theory was discarded when direct injection of crystals into caterpillars was shown to have no effect. Careful time-course analysis of the guts of caterpillars feeding on contaminated food revealed the disruption of cilia on the brush border of midgut epithelial cells shortly after ingestion of the crystals, followed by cell swelling and lysis. The gut contents (including bacterial spores) were thus released into the body cavity, allowing bacteria to breed. Once the supply of nutrients was exhausted, the bacteria formed spores that could spread to a new host feeding on either the cadaver or the contaminated vegetation.

These early experiments showed that Bt toxins needed to be activated in the gut, and it was soon discovered that the critical factors were an alkaline environment and the presence of specific proteases, which cleaved the innocuous pro-toxin into its active form. Once activated by proteolysis, each toxin binds to receptors in the brush border membrane and causes pores to open, disrupting the movement of solutes across the gut epithelium and causing the influx of water. The toxins were shown to be orally lethal to caterpillars in pure form, and the pro-toxins could be converted into active toxins in vitro, using specific proteases under alkaline conditions. The requirement for alkaline conditions, specific proteases and specific receptors explains why Bt is harmless to mammals (which have an acidic gut and lack the corresponding receptors) and why each toxin has a narrow host range.

Toxin structure and specificity

Many researchers have attempted to introduce taxonomic classification systems for Bt, using various criteria such as serotyping, phage susceptibility and plasmid profiles, and this has resulted in the classification of approximately 100 subspecies. Although there is a good correlation between Bt subspecies and insect host range at the family level, the relationship tends to break down at the genus and species levels because most Bt strains can synthesize more than one toxin, resulting in complex and overlapping host profiles. For example, most Bt kurstaki strains are specific for lepidopteran insects (butterflies and moths), whereas israelensis strains are specific for dipterans (flies) and morrisoni strains are specific for coleopterans (beetles). Other strains are not active against insects at all, but are toxic towards different invertebrates. For example, Bt strains containing only Cry5- and Cry6-type toxins are active against nematodes.

At the genus and species level, it is more useful to classify Bt strains functionally on the basis of which toxin proteins they produce, as this is a more logical way to define the host range. The toxins can be described in terms of their amino acid sequences, protein structures and modes of activity (Crickmore et al., 1998). Cry toxins interact with specific receptors located on the surface of midgut epithelial cells and are activated by host proteases following receptor binding, resulting in the formation of a pre-pore oligomeric structure that is insertion competent. In contrast, Cyt toxins directly interact with membrane lipids and insert into the membrane. The known Cry and Cyt proteins now fall into 32 sets including Cyt1, Cyt2 and Cry1 to Cry67 (Crickmore et al., 2010; Figure 1).

Despite their sequence diversity, all Cry proteins share a similar overall tertiary structure, as exemplified by the six structures solved thus far by X-ray crystallography (Cry1Aa, Cry2Aa, Cry3Aa, Cry3Bb, Cry4Aa and Cry4Ba) (Figure 2). The C-terminal portion is involved in crystal formation but is not part of the mature toxin, as it is cleaved off in the insect gut. The N-terminal portion is the toxin itself, and it comprises three domains. Domain I is a bundle of seven α-helices, six of which are amphipathic encircling the seventh hydrophobic helix, and this domain is responsible for membrane insertion and pore formation. Domain II consists of three anti-parallel β-sheets with exposed loop regions, and domain III is a β-sandwich. Both domains confer receptor binding specificity thus helping to define the host range (Boonserm et al., 2006).

A current model suggests that domains II and III initially bind to primary receptors (cadherins) which cleave the toxin within domain I and induce oligomerization, which in turn promotes binding to high-affinity secondary receptors tethered to the membrane via C-terminal glycosylphosphatidylinositol anchors (Soberón et al., 2009). The requirement for oligomerization has recently been confirmed through the isolation of dominant-negative mutations of Cry1Ab (Rodríguez-Almazán et al., 2009). An alternative model (Zhang et al., 2006) suggests that initial binding triggers a Mg²⁺-dependent signalling cascade that causes G-protein dependent cAMP accumulation and the activation of protein kinase A. Phylogenetic analysis has established that the diversity of the Cry family evolved by the independent evolution of the three domains and by swapping of domain III among toxins.
Figure 1 Phylogenetic trees representing (a) three-domain Cry proteins, and (b) related proteins (Cyt, Bin and Mtx).

Source: http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/. The phylogenetic trees are modified from a TREEVIEW visualization of NEIGHBOR treatment of a CLUSTALW multiple alignment and distance matrix of the full-length toxin sequences, as described by Crickmore et al. (2010). The grey vertical bars demarcate the four nomenclature ranks.
Development of Bt topical pesticides

Although Sporeine was used from 1938 in France, it was not registered as a pesticide in the United States until 1961. By this time, other Bt products such as Thuricide were already on the market, most based on *kurstaki* strain HD1 and active against lepidopteran pests (Baum et al., 1999). Despite their beneficial properties, these early Bt products did not compete well against chemical pesticides because of their poor performance. Commercial activities concentrated on two strategies to overcome these challenges, namely process development to increase the efficiency of the Bt products, and strain improvement to increase the intrinsic toxicity of the bacteria.

Process development began with improved fermentation and harvesting procedures, but perhaps the most important aspect was formulation. Liquid suspensions were the most convenient to handle, but tended to deteriorate in storage, while powders were easier to store and transport but drying was expensive and reformulation more complicated for the end user. These challenges were addressed by the addition of excipients such as suspension agents to prevent suspensions settling and preservatives to increase shelf life, and in the case of powders by adding chemicals to improve pouring and wetting. UV screening agents were also used, to prevent rapid photolysis after spraying (Burges and Jones, 1998a). These improvements, in addition to more rigorous quality control and the standardiza-
tion of potency testing, led to a sixfold or more increase in efficacy in the field (Burges and Jones, 1998b).

Strain improvement in the 1960s led to the replacement of many of the early products with new Bt strains that were up to 10-fold more potent than their predecessors, and the search for new and better strains continues to this day. Most Bt products are derived from kurstaki HD1 (e.g. Biobit, Dipel and Thuricide) although other strains are used to tackle lepidopteran pests (kurstaki SA-11, kurstaki SA-12), diptera (israeliensis) and coleoptera (tenebrionis) (Kaur, 2000). A selection of Bt topical pesticides is listed in Table 1.

Strain search and assessment programs initially involved bioassays and biochemical testing, but this has been replaced by PCR testing for specific toxin signatures. This can determine whether increased potency is achieved by higher toxin expression levels or the presence of a novel toxin, the latter offering the prospect of controlling different ranges of pests (Kuo and Chak, 1996; Porcar and Juárez-Pérez, 2003). Tailoring the host range can be achieved not only through the discovery of new toxins, but also by creating new bacterial strains carrying previously unknown combinations of existing toxins, a process that can be implemented by conjugation or direct transformation (González et al., 1982; Kronstad et al., 1983; Carlton and Gonzalez, 1985) (Table 2). Examples of combination pesticides produced by conjugation include

Table 1 Bt topical products based on natural strains (Kaur et al., 2000). Bt kurstaki HD-12 has been renamed SA-11

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Subspecies and strain</th>
<th>Target insect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biobit</td>
<td>Bt kurstaki HD-1</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Dipel</td>
<td>Bt kurstaki HD-1</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Florbac</td>
<td>Bt aizawai</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Costar</td>
<td>Bt kurstaki SA-12</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Delfin</td>
<td>Bt kurstaki SA-11</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Thuricide</td>
<td>Bt kurstaki HD-1</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Tekar</td>
<td>Bt israeliensis</td>
<td>Diptera</td>
</tr>
<tr>
<td>Javelin</td>
<td>Bt kurstaki SA-11</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Bactimos</td>
<td>Bt israeliensis</td>
<td>Diptera</td>
</tr>
<tr>
<td>Vectolex GC</td>
<td>Bacillus sphaericus</td>
<td>Diptera</td>
</tr>
<tr>
<td>Bactospine</td>
<td>Bt kurstaki HD-1</td>
<td>Lepidoptera</td>
</tr>
<tr>
<td>Acrobe</td>
<td>Bt israeliensis</td>
<td>Diptera</td>
</tr>
<tr>
<td>Novodor</td>
<td>Bt tenebrionis</td>
<td>Coleoptera</td>
</tr>
<tr>
<td>Trident</td>
<td>Bt tenebrionis</td>
<td>Coleoptera</td>
</tr>
</tbody>
</table>

Figure 2 Structure of three-domain Cry proteins. (a) Primary structure, showing domain organization of representative members of each Cry family. (b) Conserved tertiary structure, showing the positions of the three domains. Source: de Maagd et al. (2001).
Using standard cloning methods and that plasmid chia coli genes can be inserted onto a different plasmid in as they would be incompatible. In such cases, the relevant plasmids with similar origins of replication in the same cell, particularly if this results in the coexistence of two It is not always possible to introduce plasmids by conjugation, soybean crops, and this is the active ingredient of Condor. larly active against specific lepidopteran pests that infest Ecogen) synthesizes a combination of Cry proteins particu- (Arantes and Lereclus, 1991). Strain EG2348 (created by ton and Gawron-Burke, 1993). Strain EG2348 (created by Ecogen) synthesizes a combination of Cry proteins particu- larly active against specific lepidopteran pests that infest soybean crops, and this is the active ingredient of Condor. It is not always possible to introduce plasmids by conjugation, particularly if this results in the coexistence of two plasmids with similar origins of replication in the same cell, as they would be incompatible. In such cases, the relevant genes can be inserted onto a different plasmid in Escheri- chia coli using standard cloning methods and that plasmid can be introduced into an existing Bt strain by artificial transformation (Arantes and Lereclus, 1991). Today, the Bt biopesticide market is dominated by Abbott Laboratories (Chicago, IL) (since the acquisition in 1995 of Novo- Nordisk’s biopesticide business) and Novartis (created through the merger in 1996 of Ciba and Sandoz), together accounting for >70% of global production. The other 30% is divided among approximately 30 companies with over 100 different Bt product formulations, most containing a single Bt toxin but some combining up to five.

Advantages and disadvantages of topical Bt pesticides

Topical Bt sprays are advantageous in terms of their safety, specificity and potency compared to chemical sprays, and are also biodegradable, which provides for a large and competitive market (Table 3). However, this last ‘advantage’ needs to be cited with caution, because Bt is only effective when present on the plant organs on which insects feed. Usually Bt is applied when early instar larvae are present, because older larvae are more tolerant. Bt sprays persist for only a few days on the leaf surface because UV light, weather, the chemical environment of the leaf surface and the presence of proteinases contrib- ute to the degradation of Cry proteins. Many spores are washed off the leaf surface into the soil. There is no evi- dence to suggest Bt is dangerous to humans and other mammals, and indeed the studies performed thus far sug- gest Bt is one of the safest microbial products known. Given its extensive use, it is remarkable that only a single injury sustained from the use of Bt products in the field has been reported, the identification of Bt spores in the corneal ulcer of a farmer whose face was splashed with Dipel (Burges, 2001). Laboratory mice can survive subcuta- neous injections of 10⁶ spores, or 10⁷ spores administered intranasally (Siegel, 2001), and although limited mortality is evident when 10⁸ spores are delivered intranasally, this is equivalent to an exposure level of 10¹² in humans, or one billion times higher than the maximum ever encoun- tered in the field during spraying. Even so, concerns about the environmental persistence of Bt spores in soil and water have encouraged research into different formula- tions, including pure protein crystals that are applied in the same way as the spores. As these are even more vul- nerable to degradation than the spores, Cry proteins have been encapsulated in the bacterium Pseudomonas fluores- cens (e.g. in the Mycogen products MVP which targets lepidopteran pests and M-Trak which targets coleopteran pests). This encapsulation strategy protects the Cry protein

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Bt topical products based on transconjugant and recombinant strains (Baum et al., 1999)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product</td>
<td>Strain/genes</td>
</tr>
<tr>
<td>Transconjugant strains</td>
<td></td>
</tr>
<tr>
<td>Agree</td>
<td>aizawai</td>
</tr>
<tr>
<td>Condor</td>
<td>kurstaki</td>
</tr>
<tr>
<td>Cutlass</td>
<td>kurstaki</td>
</tr>
<tr>
<td>Design</td>
<td>aizawai</td>
</tr>
<tr>
<td>Foil</td>
<td>kurstaki</td>
</tr>
<tr>
<td>Recombinant strains</td>
<td></td>
</tr>
<tr>
<td>Raven</td>
<td>Cry1Ac (x2), Cry3A</td>
</tr>
<tr>
<td>CRYMAX</td>
<td>Cry1Ac (x3), Cry2A</td>
</tr>
<tr>
<td>Lepinox</td>
<td>Cry1Aa, Cry1Ac (x2), Cry2A</td>
</tr>
<tr>
<td></td>
<td>Cry1F-1Ac (imported)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Current pesticides based on Bt (modified from Kaur, 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade names</td>
<td>Bt subspecies and strain</td>
</tr>
<tr>
<td>Able, Bactospirina, Condor, Costar, CRYMAX, Cutlass, Futura, Lepinox, Thuricide, Steward</td>
<td>kurstaki</td>
</tr>
<tr>
<td>Florbac, Agree, Design, Xentari</td>
<td>aizawai</td>
</tr>
<tr>
<td>Costar</td>
<td>kurstaki/SA-12</td>
</tr>
<tr>
<td>Foil, Raven</td>
<td>kurstaki</td>
</tr>
<tr>
<td>Thuricide, Biobit, Dipel, Foray, Javelin, Vault</td>
<td>kurstaki/HD-1</td>
</tr>
<tr>
<td>M-Trak</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Mattch, MVP</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Novodor, Trident</td>
<td>tenebrionis</td>
</tr>
</tbody>
</table>

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from UV light and chemical degradation, and allows large amounts of each Cry protein to be produced using high-yielding expression constructs, but the bacteria do not persist in soil or water for as long as Bt spores. Crop Genetics International developed a similar strategy using *Clavibacter xyli* var. *cynodontis*, an endophytic bacterium that can penetrate the vascular system of plants, and this confers resistance to the European corn borer (Lampel et al., 1994). The advantages and disadvantages of topical application are listed in Table 4 (Kaur, 2000).

**Bacillus thuringiensis** subspecies *israelensis* in mosquito control

Bt var *israelensis* was isolated in Israel in 1976, and was shown to be effective against dipteran larvae including blackflies and mosquitoes (Goldberg and Margalit, 1977). Therefore, whereas most Bt strains have been developed as topical pesticides for use in agriculture, Bt subsp. *israelensis* has been applied to water courses and stagnant pools to prevent the spread of malaria (Margalith and Ben-Dov, 2000; Fillinger et al., 2003). The toxins are encoded by a megaplasmid called pBtoxis which contains the genes for four Cry proteins (Cry4Aa, Cry4Ba, Cry10Aa, Cry11Aa) and two Cyt proteins (Cyt1Aa and Cyt2Ba) (Berry et al., 2002). The Cyt proteins interact directly with the lipid membrane in the larval midgut and act as receptors for the Cry toxins, so that the two act in synergy. Product development has shown that formulations containing smaller particles are the most effective because they remain suspended for longer and are easily ingested by the filter-feeding larvae. Heavier particles sink and are covered in mud, quickly becoming ineffective, so specialist products that facilitate slow release have the best performance, e.g. products with the bacteria suspended in ice chips, or formulations including *Bacillus sphaericus*.

**Table 4** Advantages and disadvantages of Bt sprays

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potent insecticidal activity</td>
<td>Rapidly inactivated by UV light, heat and extreme pH</td>
</tr>
<tr>
<td>Specific host range</td>
<td>Susceptible to proteases in leaf exudates</td>
</tr>
<tr>
<td>Harmless to humans and other mammals</td>
<td>Easily removed from plant surface by wind and rain</td>
</tr>
<tr>
<td>Biodegradable</td>
<td>Therefore needs to be reapplied for full effect</td>
</tr>
</tbody>
</table>

The development of Bt transgenic crops

**Early development**

One of the major disadvantages of topical Bt pesticides is their short window of effectiveness, and the fact that inclement weather can render Bt sprays useless within a matter of hours. Topical Bt sprays must therefore be reapplied several times in a growing season to reach the entire larval pest population, increasing the amount of product that needs to be used and the fuel needed for spraying. Furthermore, topical sprays have little impact on so-called cryptic pests, i.e. sap sucking insects and larvae that feed near the roots.

A potential solution to this problem was developed in the mid-1980s when scientists introduced Bt *cry* genes into tobacco and tomato plants and expressed the proteins directly in plant tissues (Table 5). The Belgian company Plant Genetic Systems pioneered commercial interest in Bt transgenic technology, but no Cry protein could be detected in the first generation of experimental transgenic plants (Fischhoff et al., 1987; Vaeck et al., 1987; Perlak et al., 1991). Experiments to determine the cause of low expression levels concentrated on differences between prokaryotic and eukaryotic systems because recombinant Cry proteins were expressed at high levels in heterologous bacteria.

A large difference in average GC content was noted between Bt and plant DNA, plus differences in codon preference that could account for low-efficiency protein synthesis. The bacterial genes also contained frequent ATTTA sequences, which in plants act as signals to increase the rate of mRNA turnover. Perlak et al. (1991) modified the sequence of the *cry1Ab* and *cry1Ac* genes to increase the GC content, replace sequences with four or
more consecutive adenine or thymine residues and shift codon preference towards that favoured by plants, increasing protein levels by up to 100-fold and achieving total yields equivalent to 0.02% total soluble protein (TSP). This was still insufficient for adequate pest control, but expression levels could be increased still further using stronger promoters, more efficient polyadenylation and termination signals, and by including a heterologous intron in the expression construct. The development of synthetic cry genes optimized for expression in plants meant that Cry proteins were soon expressed at levels of 0.2–1% TSP (Koziel et al., 1993) and this increased to over 5% when cry genes were introduced into the chloroplast genome (McBride et al., 1995).

Field trials and early commercial crops

After successful results in laboratory tests, the first field trials with Bt transgenic tobacco were conducted in the United States and France in 1986. The plants expressed a truncated gene encoding the N-terminal (toxic) portion of Cry1A from Bt var kurstaki HD-73 under the control of the constitutive Cauliflower mosaic virus 35S promoter and protected the plants from leaf damage caused by Helicoverpa zea, a pest known variously as cotton bollworm, corn earworm or tomato fruitworm, depending on the crop it infests (Hoffmann et al., 1992). Whereas it was never likely that the Bt tobacco variety would be developed for commercial exploitation, transgenic potato plants expressing Cry3A from Bt var. tenebrionis were shown to protect the crop against Colorado potato beetle in the field much more efficiently than Cry3A topical sprays and were earmarked for commercial development (Perlak et al., 1993). Trials with cotton, maize and rice soon followed (Table 6).

In 1995, the US Environmental Protection Agency (EPA) approved the first registration of Bt potato, corn and cotton crops. The first to reach the market was Monsanto’s NewLeaf potato variety expressing Cry3A, swiftly followed by two transgenic corn hybrids expressing Cry1Ab to protect against the European corn borer, i.e. KnockOut by Syngenta (Basel, Switzerland) andaturaGard by Mycogen (both containing event 176). Monsanto also released the cotton varieties Bollgard and Ingard (events 531, 757 and 1076) expressing a modified Cry1Ac toxin. Two additional Bt corn varieties expressing Cry1Ab were released shortly thereafter, namely Agrisure CB by Northrup King (event Bt11) and the widely discussed YieldGard variety (event MON 810) by Monsanto. The early landscape of the biotech crop industry had thus been established (Box 1). NewLeaf potato and its successors (NewLeaf Y and NewLeaf Plus) were withdrawn from the market in 2002 (Box 2), and corn varieties containing event 176 were later withdrawn and replaced with more profitable products.

Growth and diversification of Bt crops

By 1998, the uptake of Bt crops had increased significantly as data became available showing the positive impact of Bt transgenic technology on agriculture and the environment (see below). In 1998, the EPA approved an insect-resistant tomato line (event 5345) expressing Cry1Ac, and in 2001 the Herculex corn variety jointly developed by Pioneer Hi-Bred (Johnston, IA) and Dow AgroSciences (Indianapolis, IN) (event TC 1507) expressing Cry1F and protecting plants against black cutworm (Agrotis ipsilon), fall armyworm (Spodoptera frugiperda) and the European corn borer. A new landmark was achieved in 2002, with the approval of Monsanto’s Bollgard II cotton (event 15985), which expressed two Bt toxins, Cry1Ac and Cry2Ab, and later YieldGard Rootworm (event MON 863), expressing a synthetic variant of the cry3Bb1 gene from Bt subsp. kumamotoensis, providing resistance against the western corn rootworm (Diabrotica virgifera virgifera). The first stacked variety developed by crossing two previously released Bt varieties was also released in 2003. This was Monsanto’s YieldGard Plus (event MON 810 + MON 863), which expressed Cry1Ab1 and Cry3Bb1. During this period, there was significant turbulence in the market as both large and small industry players manoeuvred to acquire strategic patents and technologies (Box 3). Tables S1–3 show, respectively, the current status of Bt patents, the companies currently engaged in the commercial development of Bt and the commercial status of different Bt crops.

Table 6 Early field trials with Bt transgenic plants (data from Hilder and Boulter, 1999)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Bt toxin</th>
<th>Target pest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>Cry1A</td>
<td>Pinworm</td>
</tr>
<tr>
<td>Tobacco</td>
<td>Cry1A</td>
<td>Helicoverpa zea</td>
</tr>
<tr>
<td>Potato</td>
<td>Cry1A</td>
<td>Tuber moth</td>
</tr>
<tr>
<td>Cotton</td>
<td>Cry3A</td>
<td>Colorado potato beetle</td>
</tr>
<tr>
<td>Maize</td>
<td>Cry1A</td>
<td>Pink bollworm</td>
</tr>
<tr>
<td>Rice</td>
<td>Cry1A</td>
<td>European corn borer</td>
</tr>
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International expansion

The United States has enthusiastically embraced the development of Bt agriculture and has by far the largest amount of land in total used for Bt or Bt stacked crops. However, Bt crops are grown in 25 other countries and the number of countries adopting Bt crops and the amount of land set aside for their cultivation has shown a

Box 1 Portrait of an industry

The early pioneers of commercial Bt technology were agrobiotechnology companies such as Monsanto, DuPont, Calgene and Agracetus, which had collaborated with academic research groups to carry out field trials and develop potential commercial products in the mid-to-late 1980s. As the 1990s approached, these companies entered into agreements with major seed distributors such as Delta and Pine Land (DPL) to develop and test their enhanced transgenic varieties. The predominant role of Monsanto in the early commercial landscape reflected their exclusive agreement with DPL to market transgenic cotton seeds internationally. Later, Monsanto forged agreements with several local companies in developing countries particularly China and India (Huang et al., 2002a,b). Early in the 1990s, Monsanto purchased Dekalb Genetics, Asgrow and Holdens acquiring elite germplasm through which to commercialize its biotechnology products.

The Monsanto business model of assimilating companies with aligned strategic objectives was soon replicated by the other major players (see Figure below). Although the 1990s was characterized by aggressive positioning, mergers and acquisitions, this has now given way to a more collaborative industry. The recent approval of SmartStax corn, co-developed by Monsanto and Dow AgroSciences is one example, and others include the Greenleaf Genetics collaboration between Syngenta and Pioneer Hi-Bred International, and Monsanto licensing RoundupReady events to Syngenta so that the trait can be incorporated into their hybrids (Marra et al., 2010).

International expansion

The United States has enthusiastically embraced the development of Bt agriculture and has by far the largest

Figure in Box 1 Evolution of the commercial landscape for Bt crops. The five major companies currently selling Bt seeds have arisen through a series of mergers, acquisitions and spin offs/demerger as larger companies segregate their agribusiness interests. Monsanto Co., in its current incarnation, was an agribusiness spin-off from Pharmacia in 2002 following the merger of the original Monsanto Co. (established in 1901) with Pharmacia and Upjohn in 2000. Pharmacia created the new Monsanto as an agribusiness subsidiary in late 2000, and then established it as an independent company in 2002. Bayer CropScience is an agribusiness subsidiary of Bayer AG, formed following the acquisition of Aventis CropScience in 2000. Syngenta formed from the merger of Novartis and AstraZeneca in 2000, both of which were agribusiness spin-offs generated in previous mergers. Dow AgroSciences is a wholly owned subsidiary of Dow Chemical Co., formed when Dow Chemical Co. purchased Eli Lilly’s stake in Dow Elanco (an agribusiness spin-off from Dow Chemical Co. and Eli Lilly & Co. formed in 1989). Finally, Pioneer Hi-Bred International is now an agribusiness subsidiary of DuPont, which acquired 20% of the company in 1997 and the remaining 80% in 1999.

The Monsanto business model of assimilating companies with aligned strategic objectives was soon replicated by the other major players (see Figure below). Although the 1990s was characterized by aggressive positioning, mergers and acquisitions, this has now given way to a more collaborative industry. The recent approval of SmartStax corn, co-developed by Monsanto and Dow AgroSciences is one example, and others include the Greenleaf Genetics collaboration between Syngenta and Pioneer Hi-Bred International, and Monsanto licensing RoundupReady events to Syngenta so that the trait can be incorporated into their hybrids (Marra et al., 2010).
Box 2 The demise of NatureMark

NatureMark was a subsidiary of Monsanto created by the company in 1996 to market its new transgenic potato lines, beginning with NewLeaf (containing the gene for Cry3Aa) and subsequently NewLeaf Y (containing the Bt gene and an additional gene conferring resistance to Potato virus Y) and NewLeaf Plus (containing the Bt gene and an additional gene conferring resistance to Potato leaf roll virus). Despite rapid take up and hugely favourable responses from growers, all the potato lines and the NatureMark brand itself were abandoned in 2002 after a highly successful potato growing season in 2001. Why did this happen?

Figure in Box 2  NewLeaf Plus field (left) is protected from Potato leaf roll virus (PLRV) without sprays. No infection is evident. The conventional potato field on the right is 100% PLRV infected, despite sprays. Figure used with permission from Peter Thomas.

The NatureMark story shows how misguided activism can have disastrous effects. Activists began a very public campaign of misinformation against Monsanto’s potato products in 1999 and soon had the support of several major convenience food providers including McDonalds, which banned genetically engineered crops from its food to avoid negative publicity. This in turn put pressure on potato processors and growers, who had little choice but to cancel contracts for transgenic potatoes they would not be able to sell. The transgenic varieties had all received appropriate clearance from the regulators and were safe for human consumption, so it is clear that the entire chain of events was based on hysteria and not one shred of scientific evidence. Notwithstanding the above, Monsanto was forced to abandon NatureMark and all potato-related research and development as there would be no market for its products. The activists were successful, but it was a Pyrrhic victory. In their haste to save the environment by getting rid of ‘unnatural’ vegetables, they ensured that tons of chemical pesticides would be required for future potato crops and tons of fuel would be required to spray them; they ensured that farmers in North America would mourn the loss of a superior product, lose profit and make the agricultural sector as a whole less profitable; and they ensured that many additional beneficial crops would never see the light of day, or would be developed instead in other countries.

Box 3 War and peace

Many aspects of Bt technology fall under the scope of intellectual property and can be protected with patents, although many of these aspects (processing and formulation in the case of Bt sprays, gene transfer and expression in the case of Bt crops) are not specific to Bt and can have wider ramifications. Many companies and academic departments working on Bt technology have sought patents, and in 1996 when the first Bt crops were commercialized these were divided more or less equally between the ‘old guard’ companies that had developed Bt topical products and the ‘new wave’ of companies expressing Bt genes in plants. Nearly 60% of the approximately 400 Bt patents were owned by Mycogen, Novartis, Abbot, Toa, AgEvo, Ecogen, Monsanto and Zeneca. It is noteworthy that the five major companies involved in Bt crop development today have, since 1996, engaged in many cooperative research and development agreements and this has continued to the present day. However, they have also litigated extensively to prevent patent infringements. The figure below shows current and past collaborations and licensing deals whereas the arrows show litigation pursued either by the company as it exists today, or one of its predecessors. The current Bt patent landscape is summarized in Table S1.

Figure in Box 3  Early patent wars (arrows show litigation) and collaborations (dotted lines) in the commercialization of Bt.
continued upward trend for 15 years (Figure 3). Bt agriculture is expanding on every continent except Europe, which persists with its absurdly byzantine approach to all genetically engineered crops. Remarkably, the small African nation of Burkino Faso grows more Bt crops than the whole of Europe. The total global area devoted to Bt crops in 2009 was >50 million hectares (36% of all biotech crops), made up of 21.7 million hectares of Bt-only crops and 28.7 million hectares of crops with Bt stacked with herbicide tolerance (James, 2010).

Although Argentina and Brazil currently hold second and third place in the global rankings for Bt agriculture, China and India have seen the most rapid adoption. This is because both are major growers of cotton, and China in particular is a major grower of rice. Field trials of Bt rice were first conducted in China in 1998. A series of transgenic Bt rice lines transformed with modified cry1A, cry1Ab and cry1Ac genes were assessed in large-scale trials in 2007 (Huang et al., 2007) and were approved for commercial release in November 2009, although large-scale cultivation is still pending.

**Ecological aspects of Bt crops**

**Potential for the evolution of resistant insect populations**

All insecticides create selection pressure on target populations, and the mode of action of Bt toxins (binding to a specific receptor on midgut epithelial cells) presents a clear opportunity for pests to evolve resistance. The first evidence of this process was observed in 1985, when resistant mealmoths (*Plodia interpunctella*) were found in grain stores that had been sprayed with Bt spores. The selection pressure was recreated in the laboratory, showing the evolution of resistant strains after 15 generations of sublethal selection (McGaughey, 1985). Resistance was also observed in wild populations of the diamondback moth (*Plutella xylostella*) feeding on watercress in Hawaii that had been sprayed with Bt up to 400 times (Liu and Tabashnik, 1997). Laboratory experiments were able to produce Bt-resistant varieties of several additional species that had not evolved resistance in the wild, suggesting that the intensive use of single Cry proteins was likely to result in the evolution of resistant strains (Tabashnik et al., 2005).

The Bt crop industry is aware of the danger of resistant pests, and many seed vendors insist on customer agreements that mandate the use of preventative measures, particularly the refugia strategy in which a proportion of any field containing Bt crops must be planted with the nontransgenic variety to encourage the breeding of nonresistant pests. The widespread use of this strategy is probably responsible for the remarkable lack of resistant populations even in areas devoted to high-intensity Bt agriculture for 15 years. Tabashnik and coworkers have studied pest populations on Bt sites in the United States, Australia, China and Europe and have found that among six major insect pests, field resistance occurred in only one species (*H. zea*) and only at a limited number of sites in Arkansas and in Mississippi, not in, for example, North Carolina, where the refuge areas are typically larger (Tabashnik et al., 2008). The prolonged efficacy of the first generation of Bt crops for more than a decade against nearly all targeted pest populations has exceeded the

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**Figure 3** Sharing out the Bt pie. More than 50 million hectares of Bt crops were grown commercially in 2008, the vast majority (>33 million hectares) in the USA. India, China, Argentina, Brazil, South Africa, Canada, Philippines, Australia and Uruguay were (in descending order by land area) the other countries to grow >100 000 hectares. The minor growers (in descending order by land area) were Spain, Mexico, Colombia, Honduras, Burkino Faso, Czech Republic, Romania, Portugal, Germany, Poland, Slovakia and Egypt. The data include Bt-only crops and Bt stacked with other traits. Source: Brookes and Barfoot (2010).
expectations of many entomologists working on population genetics (Bourguet, 2004). Although integrated pest management strategies have been carefully implemented by growers, the absence of resistant populations in the wild suggests that resistance may attract a fitness penalty in the absence of the toxin (Sanchis and Bourguet, 2008).

Although current refugia strategies have worked better than anticipated, a number of alternative or complementary approaches have been proposed to address the possibility of resistance evolving in target pests. High-dose and low-dose approaches aim in one case to overwhelm insect populations with immense toxin doses so that there is no chance to evolve resistance, and in the other case merely to make them more susceptible to predators, but both make many assumptions and would be difficult to implement in the field (Gould, 1994). Similarly, targeted expression (e.g. wound-inducible expression) or temporally restricted expression would provide the same advantages of a refugia but would be more difficult to implement. A more robust approach is resistance pyramiding, i.e. the stacking of multiple genes in the same plant that target the same pest via different mechanisms. In theory, resistance against one Cry protein could arise through a single point mutation in the gene encoding its receptor. Experiments have shown that mutations affecting the secondary, high-affinity Cry toxin receptors do not induce resistance, but multiple resistance alleles can be identified in the cadherin genes encoding the primary receptors (Soberón et al., 2009; Zhao et al., 2010). However, the chances of two mutations arising simultaneously in the receptors for two independently acting toxins would be much lower. This is probably why mosquito strains resistant to Bt var israelensis have not evolved despite many years of deployment—the toxin crystals in this case comprise five different toxins, one of which is Cry1A which, as discussed above, has a different action mechanism to the three-domain Cry toxins. Pyramiding resistance crops are therefore likely to require smaller refuges (Shelton et al., 2002).

The pyramiding strategy is supported by laboratory tests with cotton bollworm and the recent analysis of pink bollworm (Pectinophora gossypiella) populations in Bt cotton fields in India. The laboratory studies showed that cotton bollworm can evolve resistance to MVP, a commercial Bt formulation based on Cry1Ac, but not to DiPel or XenTari, which contain multiple Cry proteins (Akhurst et al., 2003). The resistant strain was also resistant to Cry1Ab (which binds to the same receptor as Cry1Ac in cotton bollworm) but not to Cry2Aa or Cry2Ab which bind different receptors (Liao et al., 2002). Field monitoring of pink bollworm in 2009 showed that the pest evolved resistance to Bollgard I cotton (Cry1Ac) in four areas of Gujarat: Amreli, Bhavnagar, Junagarh and Rajkot. However, no resistance was observed in fields growing the Bollgard II variety, which contains Cry1Ac and Cry2Ab (Monsanto, 2010).

Attempts have also been made to enhance toxin activity by coexpression or protein fusion. One example is the expression of Cry toxins along with fragments of their receptors, which can potentiate their activity by allowing them to assemble into pore-forming complexes immediately (Chen et al., 2007). The fusion of two or more toxins has been used to generate artificial hybrids with a host range differing from that of the parent toxins. For example, Naimov et al. (2003) created a fusion toxin comprising a truncated version of Cry1Ba and domain II from Cry1la.

Desiree potato plants expressing this recombinant toxin were the first Bt plants resistant to both coleopteran and lepidopteran pests (Colorado potato beetle, potato tuber moth (Phthorimaea operculella) and European corn borer) and the hybrid protein did not compete for binding sites with either of the parent toxins, indicating it bound to a distinct receptor. More recently, Walters et al. (2010) created a hybrid Cry1Ab/Cry3A toxin (eCry3.1Ab) which was toxic to the western corn rootworm, a pest unaffected by either of the parent toxins. In a related approach, Mehlo et al. (2005) fused the Cry1Ac toxin to the galactose-bind domain of the nontoxic ricin B-chain, again expanding its repertoire of potential receptors and therefore broadening its host range. Transgenic rice and maize plants expressing the fusion protein were significantly more toxic in insect bioassays than those containing the Bt gene alone. They were also resistant to a wider range of insects, including important pests that are not normally susceptible to Bt toxins.

Environmental impact

Although there is much debate both politically and publically concerning the environmental impact of genetically engineered crops, it is clear that Bt crops have provided immense environmental benefits. The deployment of Bt crops has reduced the use of pesticides, also saving on fossil fuels required for spraying, reducing CO2 emissions by limiting the need for ploughing, and conserving soil and moisture by encouraging no-tilling agriculture. The cumulative reduction in pesticide use for the period 1996–2008 was approximately 356 000 tonnes (8.4%), which is equivalent to a 16.1% reduction in the associated net environmental impact as measured by the environmental impact quotient.
These factors, together with average yield increases of up to 2–3 per growing season) and a concomitant reduction in the number of poisonings caused by chemical exposure. These factors, together with average yield increases of up to 10%, have raised net income by as much as 40% (Subramanian and Qaim, 2010).

Although the reduction in pesticide use has been beneficial to the environment and the economy, concern has been expressed that the use of Bt transgenic plants could affect beneficial insects, upset the balance of natural ecosystems and encourage the breeding of secondary pests. It is unclear why those raising such concerns are not equally concerned about the impact of topical Bt sprays and chemical pesticides on these populations, because sprays are the only current alternative to transgenic crops that will guarantee adequate food production.

**Beneficial insects**

The potential impact of Bt crops on beneficial insects was brought into focus by the now discredited Monarch butterfly study, which suggested Monarch larvae feeding on leaves covered in pollen shed from Bt maize plants (event Bt176) did not grow as rapidly as those feeding on uncontaminated leaves. This report was seized on by opponents of genetic engineering technology and is still routinely cited as an argument against the deployment of Bt crops despite follow-up studies finding no evidence for a statistically significant effect. What other evidence is there regarding the impact of Bt crops on nontarget insects?

Field studies on the NewLeaf potato (Cry3Aa) showed that the toxin specifically affects the Colorado potato beetle and has no deleterious effect on other insects in the potato field, including the beetle’s natural predators. In contrast, chemical sprays killed both the beetle and its predators, leading to an explosion in the population of vectors carrying viral pathogens, thus increasing the risk of potato virus diseases (Reed et al., 2001). Any impact on natural predators that normally keep pest populations in check could have knock-on effects throughout the food web, so careful studies of these effects are required (Dutton et al., 2002). One such study looked at nontarget arthropod predators in Bt maize fields (specifically events MON 810 expressing Cry1Ab, MON 88017 expressing Cry3Bb1 and a stacked variety MON 89034 × MON 88017, expressing Cry1A105, Cry2Ab2 and Cry3Bb1). The study showed that the predator and alternative prey populations naturally adjusted to reflect the absence of the targeted pest (Faria et al., 2007). Bt maize increased the population of corn aphid (Rhopalosiphum maidis) which resulted in more honey dew synthesis, which increased the number and longevity of the lepidopteran larval parasitoid Cotesia marginiventris. Bt cotton appears to have no effect on the cotton aphid (Aphis gossypii) population, and the Bt toxin was not detected in the honey dew, which is an energy source for many arthropod species including predators and parasitoids. Bt cotton therefore has no negative impact on beneficial insects in the cotton ecosystem (Lawo et al., 2009).

**Secondary pests**

A secondary pest is a pest species whose numbers are usually kept in check by the presence of a primary pest, such that no control measures are necessary. However, elimination of the major pest may elevate the secondary pest to primary status, perhaps even affecting surrounding crops that are not usually troubled by either the primary or secondary pest species. Cotton bollworm is a primary pest of cotton, and it suppresses the population of mirid bugs, i.e. homopteran insects that feed on plant sap. Bt cotton represents approximately 95% of all cotton in Northern China and is lethal to the cotton bollworm at the larval stage, so a study was carried out to look at any impact on mirid bug populations (Lu et al., 2010). The study showed that mirid bug populations have not increased in nontransgenic cotton because the species is controlled by broad-spectrum pesticides that are also used to kill bollworm larvae. In Bt cotton, the mirid bug population has increased every year from 1997 to 2008 and has gained the status of a primary pest, a phenomenon that is now impacting on unrelated crops such as dates, grapevine, apple, peach and pear. Although this is an undesirable outcome, it is somewhat balanced by the increased insect biodiversity observed in Bt cotton in China. Field studies revealed 31 insect species in Bt plots (23 beneficial) compared to 14 species in non-Bt plots, and only five of which were beneficial (Pray et al., 2002).

**Environmental diversity**

In addition to insect populations, it is useful to study the impact of Bt on other parts of the ecosystem, particularly the soil as this is where Bt spores end up when washed from the plant surface, and is the destination of Bt toxins.
exuded from plant roots, released from decaying or residual plant biomass ploughed into the soil. Earthworms (oligochaetes) are good indicators of general soil health and comparisons of earthworm numbers in plots containing nontransgenic maize and Bt maize expressing cry1Ab (events Bt11 and MON 810) and cry3Bb1 (event MON 863) over 4 years showed no differences in development or biomass (Zeilinger et al., 2010).

More earthworms were found within the rows of maize plants than between them in all plots, perhaps because the soil is lighter and has more biological activity and therefore represents a better source of nutrients.

Looking to the future

The first generation of Bt crops has been extraordinarily successful, with a few examples of pest populations evolving resistance. These crops are already being supplanted with second-generation varieties with more resilient traits generated by stacking and pyramiding resistance genes. Even so, this is not the time to be complacent and the search for more efficacious and potent strains must continue (Christou et al., 2006; Crickmore, 2006). New strains of Bt are reported on a regular basis, especially now proteomics methods can be used to screen for novel toxins on a large scale.

Figure 4 Nine common pests of rice, cotton and maize that are controlled by Bt crops. Top row from left (rice pests): yellow stem borer (Scirpophaga incertulas), rice leaffolder (Lerodea eufala), striped stem borer (Chilo suppressalis). Middle row from left (cotton pests): pink bollworm (Pectinophora gossypiella), tobacco budworm (Heliothis virescens), American bollworm (Helicoverpa armigera). Bottom row from left (maize pests): European corn borer (Ostrinia nubilalis), fall armyworm (Spodoptera frugiperda), northern corn rootworm (Diabrotica barberi). Image sources from top left: (i) IRRI, (ii) IRRI, (iii) Bayer CropScience, (iv) USDA, (v) Cotton Corporation of India Ltd., (vi) USDA, (vii) Frank Peairs, Colorado State University, Bugwood.org, (viii) Canadian Biodiversity Information Facility, (ix) Plant Management Network.

mosquitocidal toxins, and Zhang et al. (2010) recently isolated strain LLP29 from the phylloplane of Magnolia denudate, identifying a novel toxin (Cyt1Aa6) which is lethal to mosquito larvae. Homologous genes have also been identified in related bacteria, reflecting the fact that Cry proteins are members of a diverse superfamily.

Strain engineering efforts have continued to extend the Bt host range. For example, Liu et al. (2010) recently reported the construction of strain BIOT185 from the original strains HBF-1 and BTO 185 that express Cry8ca2 and Cry8Ea1, respectively. The new strain is toxic towards scarab insects such as Atractaspis corpulenta. Similarly, Wang et al. (2008) constructed a new strain by introducing the cry3Aa7 gene into the UV17 strain, which produces Cry1Aa, Cry1Ac, Cry1Ca and Cry2Ab. The new strain was toxic to both lepidopteran and coleopteran insects.

The toxicity of Cry proteins can be enhanced by amino acid substitutions, the introduction of cleavage sites in specific regions of the protein and the deletion of small fragments from the N-terminal region (Pardo-López et al., 2009). For example, replacing residue N372 in Cry1Ab domain II with alanine or glycine increases its toxicity to the gypsy moth (Lymantria dispar) eightfold by inducing a fourfold increase in binding affinity to its receptor (Rajamohan et al., 2006). More recently, Muñoz-Garay et al. (2009) produced an engineered Cry1Amod toxin lacking helix α-1, which did not need to bind the receptor cadher-
in and therefore killed even insects that were resistant to the parent toxin, Cry1Ab.

The commercial environment for stacked and pyramid ing traits in Bt crops was given a boost in 2010 with the approval of SmartStax corn, codeveloped by Monsanto and Dow AgroSciences. Approvals were granted in the United States, Canada, Japan, Mexico, The Philippines, Taiwan and South Korea (Marra et al., 2010). SmartStax was created by crossing several existing varieties to stack all the events (MON 89034 × TC 1507 × MON 88017 × DAS-59122-7) in one line. SmartStax corn contains eight transgenes, including six Bt genes offering a broad spectrum of pest resistance above and below ground, while still retaining the specificity of each toxin. The specific toxins are Cry1A.105, Cry2Ab, Cry3Bb1, Cry34Ab1, Cry35Ab1 and Cry1Fa2, providing resistance to a long list of coleopteran and lepidopteran pests (Figure 4), including the European corn borer, south-western corn borer (Diatraea grandiosella), southern cornstalk borer (Diatraea crambidoides), corn earworm, fall armyworm, stalk borer (Papaipema nebris), lesser corn stalk borer (Elasmopalpus lignosellus), sugarcane borer (Diatraea saccharalis), western bean cutworm (Striacosta albicosta), black cutworm, western corn rootworm, northern corn rootworm (Diabrotica barberi) and Mexican corn rootworm (Diabrotica virgifera zeae), combining this with tolerance to two different broad-spectrum herbicides (glyphosate and glufosinate). The refuge requirement for SmartStax corn is just 5%.

The first 100 years of Bt topical sprays and transgenic crops have been extraordinarily successful and advantageous, with a strong record in terms of safety, efficacy and environmental beneficence, and the ability to bring economic prosperity in both developing countries and the industrialized west. Recent reports show that work continues to identify and create novel Bt strains and toxins with more potent and specific effects, and to generate transgenic plant lines that suppress agricultural pests and reduce opportunities for the evolution of resistant strains while conferring no harm on beneficial insects or soil organisms. This unique combination of benefits will continue to provide farmers and health professionals with the weapons they need to fight pests and insect-borne diseases, maintaining crop yields and improving the health of the world’s growing population.

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**Supporting information**

Additional Supporting information may be found in the online version of this article:

**Table S1** The distribution of Bt patents in 2010 (Updated from Krattiger (1996) with data from EU and US patent databases).

**Table S2** Companies engaged in the commercial development of Bt crops.

**Table S3** Commercial status of Bt crops. Data from Center for Environmental Risk Assessment (CERA) GM crop database.

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