

2013

## *Bacillus thuringiensis*: Transgenic Crops

Julie A. Peterson

*University of Nebraska-Lincoln*, [julie.peterson@unl.edu](mailto:julie.peterson@unl.edu)

John J. Obrycki

*University of Kentucky*, [john.obrycki@uky.edu](mailto:john.obrycki@uky.edu)

James D. Harwood

*University of Kentucky*

Follow this and additional works at: <http://digitalcommons.unl.edu/entomologyfacpub>

 Part of the [Biological Engineering Commons](#), [Entomology Commons](#), [Environmental Indicators and Impact Assessment Commons](#), and the [Environmental Monitoring Commons](#)

---

Peterson, Julie A.; Obrycki, John J.; and Harwood, James D., "*Bacillus thuringiensis*: Transgenic Crops" (2013). *Faculty Publications: Department of Entomology*. 467.

<http://digitalcommons.unl.edu/entomologyfacpub/467>

This Article is brought to you for free and open access by the Entomology, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications: Department of Entomology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

# ***Bacillus thuringiensis*: Transgenic Crops**

Julie A. Peterson, John J. Obrycki, and James D. Harwood

Department of Entomology, University of Kentucky, Lexington, Kentucky, U.S.A.

## **Abstract**

*Bacillus thuringiensis* (Bt) crops, genetically modified to express insecticidal toxins that target key pests of corn, cotton, rice, potato, and other crops, have been rapidly adopted and have become dominant fixtures in agroecosystems throughout the world. Due to the constitutive nature of Bt toxin expression, insecticidal proteins may be found in nearly all plant tissues, presenting multiple sources for Bt toxins to enter the environment, thus creating complex direct and indirect pathways for non-target organisms to be exposed to insecticidal proteins. The environmental impacts of Bt crops have been widely debated, although both benefits and risks do exist. Benefits of Bt crop adoption include reduced risks to non-target organisms when compared with conventional spray applications of insecticides, as well as economic savings to growers and increased global food security. Conversely, impacts on non-target organisms, presence in the human food supply, pleiotropic effects of genetic transformation, and gene escape to wild plant populations are all considered as viable risks of Bt technology. To address the potential risks of Bt crop technology, proposed approaches to the environmental management of Bt crops are discussed, including within-plant modifications, reduction in Bt toxin and transgene escape, and large-scale integration into integrated pest and resistance management programs. Additionally, continued study of the effects of Bt toxins on non-target organisms at multiple tiers is necessary for intelligent use of this valuable pest management tool. The global area planted to Bt crops is expanding, and new Bt products and combinations are in various stages of development. Although Bt technology may offer an environmentally superior alternative to many insecticide applications, further risk assessment research addressing the impacts of Bt crops on agroecosystem function are needed to promote environmental safety.

## **Introduction**

Genetically modified organisms have been widely adopted in many parts of the world, prompting debate about the implications that this technology may have for environmental health. Transgenic crops have been genetically engineered to incorporate genes derived from another species that confer nutritional and agronomic benefits, such as resistance to insect pests, viruses, herbicides, or environmental conditions, such as low water availability. Among insect-resistant transgenic crops, the most widespread are those that express Bt toxins, coded for by genes from the naturally occurring soil bacterium *Bacillus thuringiensis*. Commercialized Bt crops include corn, cotton, and rice that are protected against Coleoptera and Lepidoptera pests. Bt toxins are recognized as having a narrower range of toxicity than many insecticides, including pyrethroids and neonicotinoids, and may therefore pose less risk to non-target organisms; however, potential environmental impacts of Bt toxins need to be examined and documented. This entry

will therefore examine the environmental risk assessment of Bt crops, focusing on sources and fate of Bt toxins in exposure pathways for non-target organisms, impact of Bt crops on the environment, and approaches to environmental management of Bt crops.

## **What Are Bt Crops?**

Transgenic Bt crops are genetically engineered to express insecticidal proteins that cause mortality of several common agricultural pests. The genes that code for these proteins, from a naturally occurring bacterium, *Bacillus thuringiensis* (Berliner) (Bacillaceae: Bacillales), are inserted into the genome of the desired crop plant. Genetic transformation is achieved by insertion of the target gene, its promoter and termination sequences, and a marker gene into the crop genome using the microprojectile bombardment method (“gene gun”) or the *Agrobacterium tumefaciens* (Smith and Townsend) (Rhizobiales: Rhizobiaceae) bacterium (vector-mediated transformation).

## Bt Toxins

*Bacillus thuringiensis* bacterial strains can produce a series of different toxins; however, only a few have been bioengineered into agricultural crops, including crystalline (Cry) and vegetative insecticidal (VIP) proteins.<sup>[1,2]</sup> These Bt toxins vary in their range of toxicity to invertebrates, with targeted pests dominated by larval insects in the orders Lepidoptera (moths) and Coleoptera (beetles). The insecticidal mode of action occurs when the Bt toxins bind to receptors on the midgut lining of susceptible insects, causing lysis of epithelial cells on the gut wall and perforations in the midgut lining. This damage to the insect's digestive tract induces cessation of feeding and death by septicemia. An important component of the insecticidal mechanism is its specificity, which is greater than that of many currently used insecticides. Additionally, Bt toxins degrade rapidly in the digestive tract of vertebrates,<sup>[3]</sup> contributing to their selective nature.

## Bt Crops and their Targeted Pests

Many crop plants have been genetically engineered to express Bt toxins, including field and sweet corn, cotton, potato, rice, eggplant, oilseed rape (canola), tomato, broccoli, collards, chickpea, spinach, soybean, tobacco, and cauliflower. However, only corn and cotton have seen widespread commercialization. Bt potatoes were grown commercially in the United States starting in 1995, but were withdrawn from the market in 2001 following pressure from anti-biotechnology groups and the decision of the global fast-food chain McDonalds to ban the use of genetically modified potatoes in their products.<sup>[4]</sup> This crop may see a resurgence in planting in Russia and eastern Europe in the near future,<sup>[5]</sup> as small-scale and subsistence farmers in these regions seek alternatives to expensive insecticide applications.<sup>[4]</sup> Bt rice has also been approved in certain regions of China,<sup>[5]</sup> thereby facilitating increased production worldwide.

## Global Prevalence

The planting of Bt crops has increased dramatically since the mid-1990s, becoming a prevalent component of agroecosystems worldwide<sup>[5–10]</sup> (Table 1). For example, Bt cotton and corn in the United States comprised just 1% of total area planted in 1996, their first year of commercial release; however, planting rates have increased rapidly, with areas of Bt cotton and corn in 2010 comprising 73% and 63% of total U.S. production, respectively.<sup>[11]</sup> Genetically modified crops are grown on 134 million hectares of land in 25 countries by 14.0 million farmers<sup>[5]</sup>; approximately 40% of that area is planted to corn and cotton expressing Bt insecticidal toxins.<sup>[12]</sup>

## Sources and Fate of Bt Toxins in the Environment

Toxin distribution and expression levels within a transgenic plant vary depending on the type of Bt protein, transformation event, gene promoter used, crop phenology, and environmental and geographical effects.<sup>[13–17]</sup> Most Bt crops employ a constitutive promoter, such as the cauliflower mosaic virus (CaMV 35S), that expresses insecticidal proteins throughout the life of the plant in nearly all tissues, which may include foliage, roots and root exudates, phloem, nectar, and pollen, creating the potential for a multitude of sources for environmental exposure. These pathways to exposure of non-target organisms include, but are not limited to, direct consumption of Bt toxins via ingestion of live or detrital plant material, as well as indirect consumption of Bt toxins via soil contamination from root exudates and persistence in the soil, or consumption of Bt-containing prey in tritrophic interactions (Figure 1). These pathways allow for multiple routes to exposure, even potentially within a given taxonomic group, such as ground beetles (Coleoptera: Carabidae), which have been documented to take up Bt toxins in the field.<sup>[18]</sup> Certain agronomic practices may also create unexpected routes to exposure. For example, following harvest in China, cottonseed hulls may be used as substrate for growing edible oyster mushrooms before being incorporated into cattle feed.<sup>[19]</sup> Other cotton gin by-products from transgenic plants are used in a variety of ways, including as catfish feed,<sup>[20]</sup> mulch, and fuel for wood-burning stoves.<sup>[21]</sup> Although transfer of Bt toxins from cottonseed hulls into mushrooms or cattle feed was not detected,<sup>[22]</sup> investigation of these complex and non-conventional pathways for Bt toxin movement is critically important.

## Direct Consumption of Bt Toxins

### Consumption of Live Plant Tissue

Ingestion of plant material, including foliage, roots, phloem, nectar, or pollen may be the most obvious pathway to Bt toxin exposure for targeted pests species, as well as non-target herbivores and natural enemies. Uptake of Bt toxins by herbivores feeding on transgenic plants is well documented (e.g., Dutton et al.,<sup>[23]</sup> Harwood et al.<sup>[24]</sup> Meissle et al.,<sup>[25]</sup> Obrist et al.,<sup>[26]</sup> and Obrist<sup>[27]</sup>). However, ingestion of Bt crop tissue may not always result in exposure to toxins. For example, phloem-feeding insects and their honeydew have tested positive for Bt toxins in some transgenic agroecosystems, including certain rice, oilseed rape, and corn events,<sup>[28–30]</sup> while failing to take up toxins from selected Bt corn events.<sup>[31]</sup> Exposure pathways of Bt toxins to herbivorous arthropods in transgenic agroecosystems are variable and may therefore be difficult to predict.

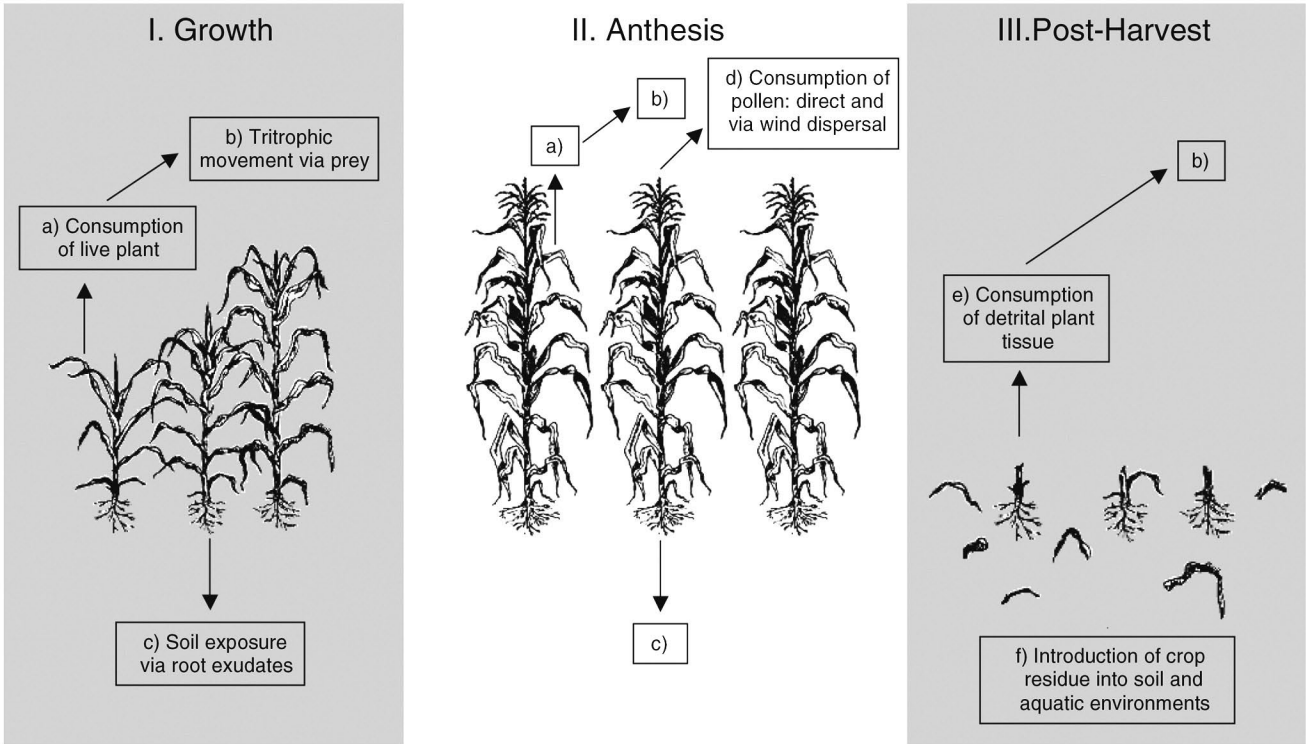
**Table 1.** Commercialized Bt crops, years marketed, Bt toxins most commonly expressed in commercial lines, their targeted pests, and countries that have adopted this technology.

Crop	Marketed	Bt toxins expressed	Targeted pest/s	Countries
Corn	1996–present	Cry1Ab, Cry1A.105, Cry1F, Cry2Ab2, Cry9C (withdrawn in 2000), VIP3A	European corn borer <i>Ostrinia nubilalis</i> Hubner, southwestern corn borer <i>Diatraea grandiosella</i> Dyar (Lepidoptera: Pyralidae), corn earworm <i>Helioverpa zea</i> (Boddie), fall armyworm <i>Spodoptera frugiperda</i> Smith (Lepidoptera: Noctuidae)	United States, Brazil, Argentina, Canada, South Africa, Uruguay, Philippines, Spain, Chile, Honduras, Czech Republic, Portugal, Romania, Poland, Egypt, Slovakia
	2003–present	Cry3Bb1, Cry34Ab1, Cry35Ab1, Cry3Aa	Corn rootworm <i>Diabrotica</i> spp. (Coleoptera: Chrysomelidae)	
Cotton	1996–present	Cry1Ac, Cry1F, Cry2Ab, VIP3A	Bollworm complex: <i>Heliothis</i> , <i>Helicoverpa</i> (Lepidoptera: Noctuidae), and <i>Pectinophora</i> (Lepidoptera: Gelechiidae)	United States, Brazil, Argentina, India, China, South Africa, Australia, Burkina Faso, Mexico, Colombia, Costa Rica
Potato	1995–2000	Cry3Aa	Colorado potato beetle <i>Leptinotarsa decemlineata</i> Say (Coleoptera: Chrysomelidae)	United States, Canada, Romania

Source: Data from James<sup>[5]</sup> and Duan et al.<sup>[146]</sup>

Many natural enemies are facultatively phytophagous during some or all of their life stages, consuming plant material or feeding on plant liquids to meet their nutritional and moisture requirements (reviewed in Lundgren).<sup>[32]</sup> Despite an abundant supply of moisture and prey items, many predatory insects,

including ground beetles (Coleoptera: Carabidae), damsel bugs (Hemiptera: Nabidae), stink bugs (Hemiptera: Pentatomidae), and ladybird beetles (Coleoptera: Coccinellidae) will also ingest plant leaf tissue, nectar, or phloem to supplement a prey-based diet.<sup>[33]</sup>



**Figure 1.** Sources for Bt toxin movement in a transgenic corn agroecosystem over the course of a growing season, including (I) growth, (II) anthesis, and (III) post-harvest time periods.



## Pollen Feeding

Another potential route of Bt toxin flow in the environment is through direct pollen feeding or consumption of pollen-contaminated material. Pollen is a component of the diets of many organisms, including springtails (Collembola)<sup>[34,35]</sup> and Western corn rootworms *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae),<sup>[36]</sup> as well as natural enemies, including ladybird beetles (Coleoptera: Coccinellidae),<sup>[37]</sup> ground beetles (Coleoptera: Carabidae),<sup>[38]</sup> green and brown lacewings (Neuroptera: Chrysopidae, Hemerobiidae),<sup>[39]</sup> hoverflies (Diptera: Syrphidae),<sup>[40]</sup> and spiders (Araneae).<sup>[41,42]</sup> In wind-pollinated Bt crops, such as corn, pollen is an abundant resource during anthesis and is deposited in large quantities (up to 1400 grains/cm<sup>2</sup> on plant surfaces<sup>[43]</sup> and more than 250 grains/cm<sup>2</sup> in ground-based spider webs<sup>[44]</sup>). Some pollen-feeding omnivores, such as *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), may also maximize their exposure to Bt toxins by aggregating at corn silks and leaf axils, where pollen grains accumulate during anthesis.<sup>[45,46]</sup> Pollen consumption can therefore represent a significant direct and indirect (through consumption of pollen-feeding prey) route of exposure for both predators and prey in transgenic agroecosystems, particularly during periods of crop anthesis.

## Consumption of Detritus

Bt toxins can persist in plant detritus beyond a single growing season<sup>[47,48]</sup> thereby exposing detritivores, such as earthworms, slugs, nematodes, protozoa, bacteria, and fungi, to Bt toxins through the consumption of such litter.<sup>[49–51]</sup> Crop detritus may also enter aquatic environments; for example, in agricultural systems where crop detritus is left in the field to prevent erosion, plant residues may account for up to 40% of non-woody vegetation entering streams.<sup>[52]</sup> Bt-containing crop tissue may then be consumed by aquatic detritivores, such as larval caddisflies (Trichoptera), crane flies (Diptera: Tipulidae), midges (Diptera: Chironomidae), and isopods. However, the bioactivity of Bt toxins in senesced plant material may be relatively short; lepidopteran-specific toxins were absent after 2 weeks in aquatic systems, while coleopteran-specific toxins decayed in as few as 6 days.<sup>[53]</sup> The harsh environmental conditions and constant physical abrasion experienced by plant tissue in flowing water were suggested as mechanisms stimulating such rapid breakdown.<sup>[54]</sup> Thus, while detritus provides a potential route of exposure, the functional consequence of Bt toxins in detritivore food webs remains unclear. However, what is evident is the persistence of Bt toxins in the environment following harvest and the possibility for long-term exposure of non-target organisms to this material.

## Indirect Consumption of Bt Toxins

### Soil Contamination via Root Exudates

One potential pathway of indirect exposure to Bt toxins is through contamination of the soil and therefore to soil-dwelling arthropods via root exudates. Bt corn, potato, and rice all release transgenic proteins from their roots during plant growth.<sup>[55,56]</sup> The soil-dwelling fauna, including beneficial non-target organisms, may therefore be exposed to Bt toxins via their secretion in plant root exudates. Bt toxin exposure to epigeal predators, ground beetle larvae and adults, and certain spiders [e.g., wolf spiders (Araneae: Lycosidae)] may also occur because of their feeding habits. Several studies have quantified the persistence of Bt toxins in the soil,<sup>[47,57–59]</sup> with results indicating persistence of these insecticidal proteins ranging from 2 to 32 weeks after introduction into the soil. This wide discrepancy may partially reflect differences in microbial activity of soils,<sup>[57,60,61]</sup> which is in turn affected by pH and mineral content.<sup>[59]</sup> Bt toxins may bind to humic acids, organic supplements, or soil particles, protecting the toxins from degradation by microbes and extending the persistence of insecticidal activity in the soil.<sup>[2]</sup> Thus, the persistence of Bt toxins may vary significantly due to their differential rate of decay based on microbial activity, soils, and environmental factors.

### Consumption of Prey Containing Bt Toxins

The movement of Bt toxins from plant tissue into herbivores and subsequently into their natural enemies has been well documented. Concentrations of Bt toxins typically decrease as they move through a food chain, indicating little evidence for bioaccumulation effects as seen in other insecticidal compounds<sup>[62]</sup>; however, two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), show evidence for the bioaccumulation of Bt toxins.<sup>[63]</sup> Although in a more typical example, Cry1Ac proteins expressed in transgenic cotton are ingested by beet armyworm caterpillars *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) and are also detectable, but at lower concentrations, in predatory stink bugs *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) when these prey are consumed.<sup>[63]</sup> However, not all tritrophic pathways facilitate the uptake of Bt toxins; Cry1Ab toxins are present in the marsh slug *Deroceras laeve* (Muller) (Pulmonata: Agriolimacidae) following consumption of Bt corn tissue, but are not taken up by the predatory ground beetle *Scarites subterraneus* (F.) (Coleoptera: Carabidae) in laboratory studies<sup>[64]</sup>; accordingly, field-collected specimens of this species did not test positive for Bt toxins.<sup>[18]</sup> Additionally, the concentration of Bt toxins transferred via trophic connections may vary based on the identity of the prey. In a laboratory experiment, two prey species of the wolf spider

*Pirata subpiraticus* (Bosenberg and Strand) (Araneae: Lycosidae), the striped stem borer *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae), and the Chinese brushbrown caterpillar *Mycalesis gotama* Moore (Lepidoptera: Nymphalidae) were allowed to feed on transgenic rice expressing Cry1Ab Bt toxins. These prey were subsequently fed to the wolf spider, and assays of each trophic level indicated that Bt toxins were transferred up the food chain; Cry1Ab concentration diminished with each additional trophic step, and the two prey species transferred Cry1Ab with significantly different efficiencies, having approximately 60 times the Cry1Ab concentration in brushbrown caterpillar-fed spiders compared with striped stem borer-fed spiders.<sup>[65]</sup> Adult ladybird beetles (Coleoptera: Coccinellidae) showed greatest uptake of Bt toxins in a corn agroecosystem post-anthesis, indicating that tritrophic movement of toxins was a greater pathway for toxin uptake than direct pollen consumption.<sup>[66]</sup> It is therefore clear that consumption of Bt-containing prey could be a major source of Bt toxin flow in non-target food webs, although the extent of toxin uptake and its concentration will depend on the strength of specific trophic pathways that occur within a given food web in the field.

### Impacts of Bt Crops on the Environment

Bt crops have become a dominant fixture in selected agroecosystems worldwide. Their planting on cultivated lands globally allows for large potential impacts of this technology on the environment. These impacts include both benefits and potential risks, the consequences to the environment of using Bt technology are intensely debated.

#### Benefits of Bt Technology

##### Reduced Risk Compared with Conventional Insecticides

The insecticidal toxins produced by transgenic Bt crops are considered to have fewer non-target effects than many insecticides due to their narrow range of toxicity and, therefore, to be advantageous to traditional methods of control. For example, populations of many natural enemies responded negatively to foliar applications of broad-spectrum pyrethroids compared with more selective insecticides, such as Bt toxins, indoxacarb, and spinosad, used to combat lepidopteran pests in sweet corn agroecosystems.<sup>[67]</sup> Field studies comparing Bt crops with their non-transgenic isolines that have been treated with broad-spectrum insecticides almost always reveal higher populations of beneficial arthropods in the Bt crops. A meta-analysis of these studies found that total non-target invertebrate abundance was higher in lepidopteran-targeting corn and cotton compared with non-transgenic crops managed with insecticides;

however, no differences for coleopteran-targeting corn were reported.<sup>[68]</sup> Non-transgenic control plots treated with insecticides had lower predator and herbivore abundance compared with unsprayed Bt fields; this result was particularly strong for predator populations in non-transgenic plots treated with pyrethroids, such as lambda-cyhalothrin, cyfluthrin, and bifenthrin.<sup>[69]</sup> Similarly, spiders were more abundant in Bt corn, cotton, and potato when compared with conventionally managed crops employing a range of insecticides, including foliar pyrethroid sprays, systemic neonicotinoid seed treatments, and organophosphate soil applications at planting.<sup>[70]</sup> Due to their selectivity, Bt crops are therefore safer for non-target organisms when compared with many insecticides, particularly those with broad-spectrum action.

##### Economic Savings

A reduction in the quantity and frequency of insecticide applications are economically beneficial, in addition to reduced exposure to chemical insecticides for farm workers and the environment. Bt cotton has significantly reduced insecticide inputs in numerous regions of the world, including the United States,<sup>[71,72]</sup> China,<sup>[73]</sup> and South Africa.<sup>[74]</sup> The adoption of Bt corn in the midwestern United States has provided an estimated \$6.9 billion in benefits to growers of both Bt and non-Bt corn in the past 14 years, due to area-wide suppression of European corn borer *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae), a key pest of this crop.<sup>[75]</sup> With more than 53 million hectares of Bt crops now planted worldwide, there are significant economic considerations, and it is evident that Bt-based production systems are not only more sustainable in the context of pest management but also have the capacity to enhance agricultural diversity through reduced chemical inputs.

##### Global Food Security

The human population is projected to reach 10 billion by 2050, and concomitant to this is the need for augmented global food security and production.<sup>[76]</sup> The employment of Bt crop technology may aid in this goal by increasing quantity and consistency of crop yields; for example, corn yields are increased or protected because of season-long control of European corn borer.<sup>[71]</sup> Additionally, stored corn grain is protected against lepidopteran pests<sup>[77]</sup> and mycotoxin levels, which pose a threat to the health of humans and livestock if introduced into the food supply,<sup>[78]</sup> are lower because of reduced feeding activity of European corn borer, which are associated with the fungal causal agents.<sup>[71,79]</sup> Bt crops may therefore confer significant beneficial effects for the global drive to increase agricultural productivity and safety.

## Potential Risks of Bt Crops

### Impacts on Non-Target Organisms

Despite the specificity of Bt toxins toward target pests, questions have been raised concerning their effects on abundance, diversity, or fecundity of some components of the non-target food web, including beneficial species such as pollinators, natural enemies, and/or detritivores. Given the important ecosystem services provided by the above-mentioned non-target organisms, the risk assessment of these groups is essential in the context of understanding environmental health. Lundgren et al.<sup>[17]</sup> identified four main approaches that risk assessment researchers have used to study the impact of Bt crops on non-target invertebrates: direct toxicity, tritrophic interactions, community level studies, and meta-analyses of data.

**Direct toxicity.** Feeding non-target organisms a diet that contains Bt toxins and measuring resulting parameters of development, fitness, and fecundity are done to examine the potential for directly toxic effects of Bt crops. The literature (reviewed in Lundgren et al.<sup>[17]</sup> and Lovei and Arpai<sup>[80]</sup>) provides contrasting evidence of non-target effects, ranging from no discernable effects of consumption of transgenic crops (e.g., Harwood et al.,<sup>[64]</sup> Pilcher et al.,<sup>[81]</sup> Al-Deeb et al.,<sup>[82]</sup> Lundgren and Wiedenmann,<sup>[83]</sup> and Anderson et al.<sup>[84]</sup>) to reports of a variety of negative effects (e.g., increased mortality, delay in development, reduction in weight gain, or changes in behavior) on beneficial organisms, such as pollinators,<sup>[85]</sup> predators,<sup>[86]</sup> parasitoids,<sup>[87]</sup> and other non-target arthropods.<sup>[50,88–91]</sup> Differing results of studies of direct toxicity of Bt toxins to non-target organisms exist for many groups; for example, in caddisflies (closely related to the target order Lepidoptera), studies have been published that report both sublethal negative effects<sup>[91]</sup> and the absence of negative impacts of Bt toxins.<sup>[54]</sup> Such laboratory toxicity studies may be extrapolated to the field, although toxicity studies should address all ecologically relevant routes to exposure for non-target organisms.<sup>[92]</sup>

**Tritrophic interactions.** These studies test for effects of Bt crops on natural enemies via consumption of Bt-containing prey; any observed effects may be due to ingestion of toxins or through prey-quality-mediated effects. Several studies have reported no tritrophic effects of Bt crops on natural enemies<sup>[63,93–95]</sup>; however, negative effects have been observed in other cases,<sup>[96,97]</sup> although these results are often attributed to prey-mediated effects whereas prey quality is lower when fed Bt crop tissue. Meta-analyses of tritrophic studies revealed that using prey items that were totally or partially susceptible to Bt toxins (and therefore were likely to be of lesser quality) had a negative effect on the performance of natural enemies, while using nonsusceptible prey (whose quality should be unaffected by consuming Bt toxins) had no effect on the performance of the natural enemies that consumed it.<sup>[98,99]</sup>

**Community level.** To study the effect of Bt crops on non-target organisms at the community level, arthropods are sampled from Bt and conventional crops to observe any differences in abundance, diversity, or community structure. Such studies have examined a variety of nontarget organism populations, including soil microarthropods, nematodes, decomposers, pollinators, and natural enemies.<sup>[81,100–110]</sup> Results of such studies often report no significant differences between populations of non-target organisms in Bt and non-Bt crops; however, a lack of taxonomic resolution in some studies can weaken these results.<sup>[70]</sup>

**Meta-analysis data.** This quantitative method addresses effects of Bt crops across multiple published studies and has been widely used to infer the consequence of Bt crops on a series of different parameters. For example, a meta-analysis of 42 field experiments revealed that the overall mean abundance of non-target invertebrates was significantly lower in lepidopteran-targeting Bt corn fields compared with non-transgenic fields when neither is treated with insecticides; no differences were found between coleopteran-targeting Bt and non-transgenic corn.<sup>[68]</sup> Unsurprisingly, the abundance of non-target arthropods was significantly higher in transgenic corn versus non-transgenic corn that had been treated with insecticides.<sup>[68]</sup> Additional meta-analyses have reported the effects of Bt crops on functional guilds of non-target organisms,<sup>[69]</sup> honey bees,<sup>[111]</sup> and spiders,<sup>[70]</sup> generally finding no differences in non-target arthropod populations between Bt and non-Bt crops. When examined at further taxonomic resolution, such analyses may reveal differential responses of functionally distinct taxa to Bt crops, as is the case with spider families. Meta-analysis revealed positive effects of Bt crops on the abundance of certain groups (Clubionidae, Linyphiidae, Thomisidae), no effect on others (Lycosidae, Oxyopidae, Araneidae), and negative effects on several families (Anyphaenidae, Philodromidae) relative to non-transgenic crops untreated with insecticides.<sup>[70]</sup>

### Presence in Human Food Supply

Concerns about the presence of Bt toxins in the human food supply do not stem from any direct toxic effects, as vertebrates lack the midgut receptors for binding of Bt toxins, but from the possibility that a portion of the population will exhibit an allergic reaction to ingestion of Bt proteins.<sup>[112]</sup> Most Bt toxins will readily break down in the acidic environment of a vertebrate digestive tract.<sup>[3]</sup> Bt corn expressing Cry9C proteins, marketed under the commercial name StarLink™, was planted in the United States from 1998 to 2000, but approved only for animal feed and ethanol production due to the persistence of Cry9C in the vertebrate gut.<sup>[113]</sup> When traces of Cry9C proteins were found in cornmeal destined for human consumption, several food items were recalled, including Taco Bell® taco shells, and



StarLink was voluntarily removed from the market.<sup>[114]</sup> However, no confirmed allergenic reactions due to Cry9C contamination were reported. Despite the lack of evidence for any true risk to humans based on consumption of Bt food products, sentiment against transgenic agricultural products destined for human consumption exists, especially in Europe, and has influenced the commercial acceptance of some products such as Bt potatoes.<sup>[4]</sup> Therefore, despite these limited effects on the human (and vertebrate) population, safeguards need to be in place to prevent the presence of unapproved genetically modified products entering the human food chain.

### Pleiotropic Effects of Genetic Transformation

Insertion of a Bt gene complex into a crop plant may result in unpredicted and unintended pleiotropic effects that change the plant from its non-transgenic counterpart in ways beyond just the expression of Bt toxins.<sup>[49,115–117]</sup> For example, a reported pleiotropic effect in Bt corn is an increase in the lignin content in transgenic plant tissue,<sup>[49]</sup> a trait that could lead to reduced decomposition rates in the soil.<sup>[118]</sup> However, other studies have contested this conclusion and shown no differences in rate of decomposition.<sup>[119]</sup> An additional pleiotropic effect of transformation in Cry1F corn may be an increase in attractiveness as an oviposition site for corn leafhoppers *Dalbulus maidis* (DeLong and Wolcott) (Hemiptera: Cicadellidae), a pest that is not targeted by Bt toxins, possibly due to altered plant traits that influence oviposition, such as leaf vein characteristics, foliar pubescence, or plant chemistry.<sup>[120]</sup> There is a lack of understanding of how these pleiotropic effects will affect ecosystem processes, although the potential consequences merit further examination in the context of their environmental impacts.

### Gene Escape

The transfer of genes from populations of domesticated crops into wild plants has been documented for many years.<sup>[121]</sup> The “escape” of Bt transgenes into wild plants could have undesirable effects by reducing genetic diversity and fecundity in wild populations or increasing fecundity and creating an invasive weed through reduction or elimination of herbivory. The presence of transgenic material from the CaMV 35S promoter used in Bt crops was reported in native maize landraces grown in remote areas of Oaxaca, Mexico, in 2001.<sup>[122]</sup> However, these results have been highly debated<sup>[123–125]</sup> and additional studies are conflicting, reporting both the presence<sup>[126]</sup> and absence<sup>[127]</sup> of transgenic DNA in traditional maize lines in Mexico. Additionally, transgene escape into weedy rice may increase the fecundity of this plant, as well as its ecological interactions with surrounding organisms.<sup>[128]</sup> The implications of transgene escape are yet to be fully understood, particularly in the context of ecological risk assessment.

## Approaches to Environmental Management of Bt Crops

To safely incorporate Bt crop technology into agroecosystems, approaches to environmental management should address the issue at multiple scales. These include engineering at the level of the individual plant genome, field- and farm-level modifications to reduce exposure of Bt toxins and escape of transgenes, and large-scale incorporation of Bt technology into integrated pest and resistance management programs. Finally, continued research concentrating on the non-target impacts of Bt crops should be conducted at multiple tiers across crop and toxin types, geographic regions, non-target organism taxa, and temporal and spatial scales, studying non-target organisms at the greatest taxonomic resolution possible. Regulation of transgenic crops in the effort to mitigate risk is complex; further recommendations and discussion of this topic can be found elsewhere.<sup>[129]</sup>

### Within-Plant Modifications

#### Selection of Low-Risk Promoters

As the gene promoter used in a transgenic event can have a strong impact on the eventual concentration and distribution of Bt toxins within the plant, the choice of promoter should be made within the context of environmental safety. Certain promoters have been identified as having greater non-target risks than others; for example, harmful effects of Bt corn event 176 on non-target Lepidoptera larvae [monarchs *Danaus plexippus* (L.) (Nymphalidae) and black swallowtails *Papilio polyxenes* F. (Papilionidae)] have been reported, while other events expressing the same Cry toxin (e.g., Bt11 and MON810) had no effect.<sup>[90]</sup> Event 176 has increased expression of Bt proteins in the pollen compared with the other events<sup>[130]</sup> and therefore poses a greater risk to non-target organisms.

#### Tissue- and Time-Specific Expression

The use of gene promoters that are tissue- or time-specific to express toxins only in plant tissues when they are susceptible to feeding has been introduced.<sup>[131]</sup> This technique has been employed in the transgenic expression of snowdrop lectin, a plant-derived protein with insecticidal properties, in rice. To target phloem-feeding pests such as brown planthoppers, lectins are selectively expressed in the vascular tissue.<sup>[132,133]</sup> Such selective expression of Bt toxins in tissue and time to target susceptible pests and reduce exposure to non-target beneficial arthropods could potentially increase environmental safety, thereby reducing the pathways for Bt toxin movement through non-target food webs.



### **Reduction in Bt Toxin and Transgene Escape**

At the field or farm level, management practices may be implemented that reduce the movement of Bt toxins or transgenes from their source (Bt plants) into surrounding habitats. Current practices may depend on the crop and agronomic aims of the grower; for example, large quantities of crop residue may be incorporated into the soil during the harvesting process, although this is not the practice when crop material is removed for ethanol production or under reduced-tillage practices.<sup>[134]</sup> Although Bt toxins may degrade quickly following the incorporation of Bt crop plant detritus into aquatic systems, this potential pathway for transgenic protein movement may be avoided through the employment of practices that prevent movement of transgenic crop tissue beyond field borders. The establishment of riparian buffer zones and filter strips may reduce the quantity of crop detritus and other compounds originating in cropland (e.g., fertilizers, insecticides) that enter nearby streams and waterways.<sup>[135]</sup> Similarly, reducing exposure pathways for gene flow into wild plant populations via physical methods, such as isolation of crops or plant destruction, may delay transgene escape. However, controlling gene flow via pollen and seeds in the environment can be very difficult; a physical separation of 200 m between transgenic corn still yields contamination levels of 0.1% between plant populations due to cross-pollination.<sup>[136]</sup> Seeds are additionally difficult to control owing to their persistence in the soil seed bank, as well as ability to sometimes germinate and persist outside of cultivated fields.<sup>[137]</sup> Management of the movement of Bt toxins and genetic material from cultivated fields into the surrounding environments warrants additional research. Interestingly, technology that could have reduced the spread of transgenes, called the “terminator gene,” was abandoned in 1999 because of the criticism that the gene prevents farmers from harvesting viable seed and thereby exclusively benefits the seed companies.<sup>[138]</sup>

### **Large-Scale Integrated Pest and Resistance Management**

Although Bt crops allow reductions in the application of certain insecticides compared with conventionally managed crops (while other insecticidal practices persist, such as neonicotinoid seed treatments on corn<sup>[139]</sup>), it should not be assumed that this technology will readily fit into integrated pest management practices.<sup>[17]</sup> Considerations of compatibility with biological control and delaying resistance in pest populations are also necessary.

#### **Compatibility with biological control**

Integrated pest management practices attempt to incorporate mechanical, physical, chemical, and cultural controls; host

resistance (including transgenic crops); and autocidal, biochemical, and biological controls in a synergistic manner. Increased attention has focused on conservation biological control: the modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce effects of pests (e.g., Landis et al.<sup>[140]</sup> and Eilenberg et al.<sup>[141]</sup>). Natural enemies can be abundant in agricultural systems and often play an essential role in pest suppression. Maintenance of relevant natural enemy populations via conservation biological control is a practical and sustainable option for high-acreage field crops, such as corn and cotton,<sup>[142]</sup> which are dominated by Bt varieties. Any negative effects of Bt toxins on natural enemies could reduce their effectiveness as biological control agents and therefore limit natural pest suppression in agroecosystems. Understanding the potential impacts of transgenic crops on non-target arthropods is essential in order to provide a framework for integrating natural enemies into sustainable methods of pest control in the agricultural environment.

#### **Resistance Management Techniques**

The development of resistance to Bt toxins by pest populations is a major concern. Integrated resistance management programs must continue to be developed and followed to promote the sustainable use of Bt crops. This is of critical importance given that resistance to Bt sprays has occurred in multiple populations of the pestiferous diamond back moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)<sup>[143]</sup> and three instances of field-evolved resistance to transgenic Bt crops have been reported in moth larvae: *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) to Cry1F corn in Puerto Rico, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) to Cry1Ab corn in South Africa, and *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) to Cry1Ac in the southwestern United States.<sup>[144]</sup> Current resistance management employs structured refuges and high-dose toxin crops, as well as monitoring for resistance development in the field and monitoring for compliance of growers to refuge protocol. Additional attempts to delay resistance include creating transgenic plants that express more than one type of Bt toxin that targets the same pest, called gene pyramiding.<sup>[131]</sup> Improved resistance management would include increased education for growers and the public about the importance of resistance management and refuge compliance, as well as continued monitoring of field populations for the development of resistance. Future strategies to passively achieve resistance compliance include mixed seed refuges, in which transgenic and non-transgenic seeds are sold in combination within the bag.<sup>[145]</sup>

### **Conclusions**

The sources and fate of Bt toxins in the environment can be complex and variable depending on crop, transgenic event,

geography, and other environmental variables. The effects of Bt crops and their toxins on the environment have been widely debated, particularly the potential implications associated with ecological impacts such as gene escape and non-target risks. Approaches to the environmental management of Bt crops and their integration into integrated pest and resistance management systems warrant further study. Despite the concerns associated with Bt crops, significant reductions in chemical input are evident and this technology is environmentally safer when compared with many approaches to pest suppression, particularly those using broad-spectrum insecticides.

### Future of Bt Technology

The focus of current transgenic technology has been on stacking and pyramiding of events. Stacking incorporates multiple transgenic traits into the crop genome in order to express more than one type of insecticidal toxin, therefore targeting multiple pest species. Pyramiding of transgenes allows for the crop to express multiple types of Bt toxins that target the same pest. Additionally, several other Bt crops are expected to be approved for commercial availability by 2015, including potatoes for planting in eastern Europe and eggplant in India.<sup>[5]</sup> The global adoption of biotechnology in agriculture is projected to continue with estimates that genetically modified crops will reach 200 million hectares, grown by 20 million farmers in 40 countries by 2015.<sup>[5]</sup>

**Acknowledgments** — Funding for this project was provided by the U.S. Department of Agriculture Cooperative State Research Education and Extension Service Biotechnology Risk Assessment Grant 2006-39454-17446. JDH is supported by the University of Kentucky Agricultural Experiment Station State Project KY008043. This is publication number 10-08-132 of the University of Kentucky Agricultural Experiment Station.

### References

1. Yu, C.G.; Mullins, M.A.; Warren, G.W.; Koziel, M.G.; Estruch, J.J. The *Bacillus thuringiensis* vegetative insecticidal protein Vip3 lyses midgut epithelium cells of susceptible insects. *Appl. Environ. Microbiol.* 1997, *63*, 532–536.
2. Glare, T.R.; O'Callaghan, M. *Bacillus thuringiensis: Biology, Ecology, and Safety*; John Wiley & Sons, Ltd.: West Sussex, U.K., 2000.
3. Mendeelson, M.; Kough, J.; Vaituzis, Z.; Matthews, K. Are Bt crops safe? *Nat. Biotechnol.* 2003, *21* (9), 1003–1009.
4. Kaniewski, W.K.; Thomas, P.E. The potato story. *AgBioForum* 2004, *7*, 41–46.
5. James, C. *Global Status of Commercialized Biotech/GM Crops: 2009*; ISAAA Brief No. 41. ISAAA: Ithaca, NY, 2009.
6. Cannon, R.J.C. Bt transgenic crops: Risks and benefits. *Integr. Pest Manag. Rev.* 2000, *5*, 151–173.
7. Pray, C.E.; Huang, J.; Hu, R.; Rozelle, S. Five years of Bt cotton in China—The benefits continue. *Plant J.* 2002, *31*, 423–430.
8. Shelton, A.M.; Zhao, J.Z.; Roush, R.T. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annu. Rev. Entomol.* 2002, *47*, 845–881.
9. Lawrence, S. AgBio keeps on growing. *Nat. Biotechnol.* 2005, *63*, 3561–3568.
10. James, C. *Executive Summary of Global Status of Commercialized Biotech/GM Crops: 2006*; ISAAA Brief No. 36. ISAAA: Ithaca, NY, 2006.
11. United States Department of Agriculture National Agricultural Statistics Service. Acreage report, 2010; <http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2010.pdf> (Accessed, November 2010).
12. GMO Compass. *Rising Trend: Genetically Modified Crops Worldwide on 125 Million Hectares*, 2009, available at [http://www.gmocompass.org/eng/agri\\_biotechnology/gmo\\_planting/257.global\\_gm\\_planting\\_2008.html](http://www.gmocompass.org/eng/agri_biotechnology/gmo_planting/257.global_gm_planting_2008.html).
13. Fearing, P.L.; Brown, D.; Vlachos, D.; Meghji, M.; Privalle, L. Quantitative analysis of CryIA(b) expression in Bt maize plants, tissues, and silage and stability of expression over successive generations. *Mol. Breed.* 1997, *3* (3), 169–176.
14. Duan, J.J.; Head, G.; McKee, M.J.; Nickson, T.E.; Martin, J.H.; Sayegh, F.S. Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a nontarget ladybird beetle, *Coleomegilla maculata*. *Entomol. Exp. Appl.* 2002, *104*, 271–280.
15. Grossi-de-Sa, M.F.; Lucena, W.; Souza, M.L.; Nepomuceno, A.L.; Osir, E.O.; Amugune, N.; Hoa, T.T.C.; Hai, T.N.H.; Somers, D.A.; Romano, E. Transgene expression and locus structure of Bt cotton. In *Environmental Risk Assessment of Genetically Modified Organisms. Methodologies for Assessing Bt Cotton in Brazil*; Hilbeck, A., Andow, D.A., Fontes, E.M.G., Eds.; CAB International: Wallingford, U.K., 2006; 93–107.
16. Obrist, L.B.; Dutton, A.; Albajes, R.; Bigler, F. Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. *Ecol. Entomol.* 2006, *31* (2), 143–154.
17. Lundgren, J.G.; Gassman, A.J.; Bernal, J.; Duan, J.J.; Ruberson, J. Ecological compatibility of GM crops and biological control. *Crop Prot.* 2009, *28*, 1017–1030.
18. Peterson, J.A.; Obrycki, J.J.; Harwood, J.D. Quantification of Bt-endotoxin exposure pathways in carabid food webs across multiple transgenic events. *Biocontrol Sci. Technol.* 2009, *19* (6), 613–625.
19. Li, X.; Pang, Y.; Zhang, R. Compositional changes of cottonseed hull substrate during *P. ostreatus* growth and the effects on the feeding value of spent substrate. *Bioresour. Technol.* 2001, *80*, 157–161.

20. Li, M.H.; Hartnell, G.F.; Robinson, E.H.; Kronenberg, J.M.; Healy, C.E.; Oberle, D.F.; Hoberg, J.R. Evaluation of cottonseed meal derived from genetically modified cotton as feed ingredients for channel catfish, *Ictalurus punctatus*. *Aquac. Nutr.* 2008, *14* (6).
21. Robertson, R. *Cotton Gin Trash Now Valuable By-Product*, Southeast Farm Press, 2009, <http://southeastfarmpress.com/grains/cotton-gin-trash-now-valuable-product>
22. Jiang, L.; Tian, X.; Duan, L.; Li, Z. The fate of Cry1Ac Bt toxin during oyster mushroom (*Pleurotus ostreatus*) cultivation on transgenic Bt cottonseed hulls. *J. Sci. Food Agr.* 2008, *88*, 214–217. Accessed November 1, 2010.
23. Dutton, A.; Klein, H.; Romeis, J.; Bigler, F. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecol. Entomol.* 2002, *27* (4), 441–447.
24. Harwood, J.D.; Wallin, W.G.; Obrycki, J.J. Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: Molecular evidence from a transgenic corn agroecosystem. *Mol. Ecol.* 2005, *14* (9), 2815–2823.
25. Meissle, M.; Vojtech, E.; Poppy, G.M. Effects of Bt maize-fed prey on the generalist predator *Poecilus cupreus* L. (Coleoptera: Carabidae). *Transgenic Res.* 2005, *14* (2), 123–132.
26. Obrist, L.B.; Klein, H.; Dutton, A.; Bigler, F. Effects of Bt maize on *Frankliniella tenuicornis* and exposure of thrips predators to prey-mediated Bt toxin. *Entomol. Exp. Appl.* 2005, *115* (3), 409–416.
27. Obrist, L.B.; Dutton, A.; Romeis, J.; Bigler, F. Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. *BioControl* 2006, *51* (1), 31–48.
28. Raps, A.; Kehr, J.; Gugerli, P.; Moar, W.J.; Bigler, F.; Hilbeck, A. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. *Mol. Ecol.* 2001, *10* (2), 525–533.
29. Bernal, C.C.; Aguda, R.M.; Cohen, M.B. Effect of rice lines transformed with *Bacillus thuringiensis* toxin genes on the brown planthopper and its predator *Cyrtorhinus lividipennis*. *Entomol. Exp. Appl.* 2002, *102* (1), 21–28.
30. Burgio, G.; Lanzoni, A.; Accinelli, G.; Dinelli, G.; Bonetti, A.; Marotti, I.; Ramilli, F. Evaluation of Bt-toxin uptake by the non-target herbivore, *Myzus persicae* (Hemiptera: Aphididae), feeding on transgenic oilseed rape. *Bull. Entomol. Res.* 2007, *97* (2), 211–215.
31. Head, G.; Brown, C.R.; Groth, M.E.; Duan, J.J. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: Implications for secondary exposure risk assessment. *Entomol. Exp. Appl.* 2001, *99* (1), 37–45.
32. Lundgren, J.G. *Relationships of Natural Enemies and Non-prey Foods*; Springer International: Dordrecht, the Netherlands, 2009.
33. Hagen, K.S.; Mills, N.J.; Gordh, G.; McMurtry, J.A. Terrestrial arthropod predators of insect and mite pests. In *Handbook of Biological Control*; Bellows, T.S., Fisher, T.W., Eds.; Academic Press: San Diego, CA, 1999; 383–504.
34. Kevan, P.G.; Kevan, D.K.M. Collembola as pollen feeders and flower visitors with observations from the high Arctic. *Quaest. Entomol.* 1970, *6*, 311–326.
35. Chen, B.; Snider, R.J.; Snider, R.M. Food consumption by Collembola from northern Michigan deciduous forest. *Pedobiologia* 1996, *40*, 149–161.
36. Kim, J.H.; Mullin, C.A. Impact of cysteine proteinase inhibition in midgut fluid and oral secretion on fecundity and pollen consumption of western corn rootworm (*Diabrotica virgifera virgifera*). *Arch. Insect Biochem.* 2003, *52*, 139–154.
37. Lundgren, J.G.; Wiedenmann, R.N. Nutritional suitability of corn pollen for the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae). *J. Insect Physiol.* 2004, *50* (6), 567–575.
38. Laroche, A.; Larivière, M.C. *A Natural History of the Ground-Beetles (Coleoptera: Carabidae) of America North of Mexico*; Pensoft: Sofia, Bulgaria, 2003.
39. Canard, M. Natural food and feeding habits of lacewings. In *Lacewings in the Crop Environment*; McEwen, P.K., New, T.R., Whittington, A.E., Eds.; Cambridge University Press: Cambridge, U.K. 2001; 116–129.
40. Olesen, J.M.; Warncke, E. Predation and potential transfer of pollen in a population of *Saxifraga hirculus*. *Holarctic Ecol.* 1989, *12*, 87–95.
41. Smith, R.B.; Mommsen, T.P. Pollen feeding in an orbweaving spider. *Science* 1984, *226* (4680), 1330–1332.
42. Ludy, C. Intentional pollen feeding in the garden spider *Araeus diadematus*. *Newsl. Br. Arachnol. Soc.* 2004, *101*, 4–5.
43. Pleasants, J.M.; Hellmich, R.L.; Dively, G.P.; Sears, M.K.; Stanley-Horn, D.E.; Mattila, H.R.; Foster, J.E.; Clark, P.; Jones, G.D. Corn pollen deposition on milkweeds in and near cornfields. *Proc. Natl. Acad. Sci. U. S. A.* 2001, *98*, 11919–11924.
44. Peterson, J.A.; Romero, S.A.; Harwood, J.D. Pollen interception by linyphiid spiders in a corn agroecosystem: Implications for dietary diversification and risk-assessment. *Arthropod Plant Interact.* 2010, *4* (4), 207–217.
45. Isenhour, D.J.; Marston, N.L. Seasonal cycles of *Orius insidiosus* (Hemiptera: Anthracoridae) in Missouri soybeans. *J. Kans. Entomol. Soc.* 1981, *54*, 129–142.
46. Coll, M.; Bottrell, D.G. Microhabitat and resource selection of the European corn-borer (Lepidoptera, Pyralidae) and its natural enemies in Maryland field corn. *Environ. Entomol.* 1991, *20*, 526–533.
47. Zwahlen, C.; Hilbeck, A.; Gugerli, P.; Nentwig, W. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Mol. Ecol.* 2003, *12* (3), 765–775.



48. Zwahlen, C.; Andow, D.A. Field evidence for the exposure of ground beetles to Cry1Ab from transgenic corn. *Environ. Biosafety Res.* 2005, *4* (2), 113–117.
49. Saxena, D.; Stotzky, G. *Bacillus thuringiensis* (Bt) toxin released from the root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. *Soil Biol. Biochem.* 2001, *33* (9), 1225–1230.
50. Zwahlen, C.; Hilbeck, A.; Howald, R.; Nentwig, W. Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris*. *Mol. Ecol.* 2003, *12* (4), 1077–1086.
51. Harwood, J.D.; Obrycki, J.J. The detection and decay of Cry1Ab Bt-endotoxins within non-target slugs, *Deroceras reticulatum* (Mollusca: Pulmonata), following consumption of transgenic corn. *Biocontrol Sci. Technol.* 2006, *16* (1), 77–88.
52. Stone, M.L.; Whiles, M.R.; Webber, J.A.; Williard, K.W.J.; Reeve, J.D. Macroinvertebrate communities in agriculturally impacted southern Illinois streams: Patterns with riparian vegetation, water quality, and in-stream habitat quality. *J. Environ. Qual.* 2005, *34*, 907–917.
53. Prihoda, K.R.; Coats, J.R. Aquatic fate and effects of *Bacillus thuringiensis* Cry3Bb1 protein: Toward risk-assessment. *Environ. Toxicol. Chem.* 2008, *27* (4), 793–798.
54. Jensen, P.D.; Dively, G.P.; Swan, C.M.; Lamp, W.O. Exposure and nontarget effects of transgenic Bt corn debris in streams. *Environ. Entomol.* 2010, *39* (2), 707–714.
55. Saxena, D.; Stotzky, G. Insecticidal toxin from *Bacillus thuringiensis* is released from roots of transgenic Bt corn in vitro and in situ. *FEMS Microbiol. Ecol.* 2000, *33* (1), 35–39.
56. Saxena, D.; Stewart, C.N.; Altosaar, I.; Shu, Q.Y.; Stotzky, G. Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B-thuringiensis* corn, potato, and rice but not of *B-thuringiensis* canola, cotton, and tobacco. *Plant Physiol. Biochem.* 2004, *42* (5), 383–387.
57. Koskella, J.; Stotzky, G. Microbial utilization of free and clay-bound insecticidal toxins from *Bacillus thuringiensis* and their retention of insecticidal activity after incubation with microbes. *Appl. Environ. Microb.* 1997, *63* (9), 3561–3568.
58. Stotzky, G. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. *Plant Soil* 2004, *266* (1–2), 77–89.
59. Icoz, I.; Stotzky, G. Fate and effects of insect-resistant *Bt* crops in soil ecosystems. *Soil Biol. Biochem.* 2008, *40*, 559–586.
60. Palm, C.J.; Schaller, D.L.; Donegan, K.K.; Seidler, R.J. Persistence in soil of transgenic plant-produced *Bacillus thuringiensis* var. *kurstaki*  $\Delta$ -endotoxin. *Can. J. Microbiol.* 1996, *42*, 1258–1262.
61. Crecchio, C.; Stotzky, G. Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. *Soil Biol. Biochem.* 1998, *30*, 463–470.
62. Skarphedinsdottir, H.; Gunnarsson, K.; Gudmundsson, G.A.; Nfon, E. Bioaccumulation and biomagnification of organochlorines in a marine food web at a pristine site in Iceland. *Arch. Environ. Contam. Toxicol.* 2009, *58* (3), 800–809.
63. Torres, J.B.; Ruberson, J.R. Interactions of *Bacillus thuringiensis* Cry1Ac toxin in genetically engineered cotton with predatory heteropterans. *Transgenic Res.* 2008, *17* (3), 345–354.
64. Harwood, J.D.; Samson, R.A.; Obrycki, J.J. No evidence for the uptake of Cry1Ab Bt-endotoxins by the generalist predator *Scarites subterraneus* (Coleoptera: Carabidae) in laboratory and field experiments. *Biocontrol Sci. Technol.* 2006, *16* (4), 377–388.
65. Jiang, Y.-H.; Fu, Q.; Cheng, J.-A.; Zhu, Z.-R.; Jiang, M.-X.; Ye, G.-Y.; Zhang, Z.-T. Dynamics of Cry1Ab protein from transgenic Bt rice in herbivores and their predators. *Acta Entomol. Sin.* 2004, *47*, 454–460 [Chinese with English abstract].
66. Harwood, J.D.; Samson, R.A.; Obrycki, J.J. Temporal detection of Cry1Ab-endotoxins in coccinellid predators from fields of *Bacillus thuringiensis* corn. *Bull. Entomol. Res.* 2007, *97*, 643–648.
67. Musser, F.R.; Shelton, A.M. Bt sweet corn and selective insecticides: Impacts on pests and predators. *J. Econ. Entomol.* 2003, *96* (1), 71–80.
68. Marvier, M.; McCreedy, C.; Regetz, J.; Kareiva, P. A metaanalysis of effects of Bt cotton and maize on nontarget invertebrates. *Science* 2007, *316* (5830), 1475–1477.
69. Wolfenbarger, L.L.; Naranjo, S.E.; Lundgren, J.G.; Bitzer, R.J.; Watrud, L.S. Bt crop effects on functional guilds of non-target arthropods: A meta-analysis. *PLoS One* 2008, *3* (5), e2118.
70. Peterson, J.A.; Lundgren, J.G.; Harwood, J.D. Interactions of transgenic *Bacillus thuringiensis* insecticidal crops with spiders (Araneae). *J. Arachnol.*, 2011, *39*(1), 1–21.
71. Betz, F.S.; Hammond, B.G.; Fuchs, R.L. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. *Regul. Toxicol. Pharmacol.* 2000, *32* (2), 156–173.
72. Gianessi, L.P.; Carpenter, J.E. Agricultural biotechnology: Insect control benefits; National Center for Food and Agricultural Policy, 1999, available at <https://research.cip.cgiar.org/confluence/download/attachments/3443/AG7.pdf>
73. Pray, C.; Ma, D.; Huang, J.; Qiao, F. Impact of Bt cotton in China. *World Dev.* 2001, *29*, 813–825.
74. Thirtle, C.; Beyers, L.; Ismael, Y.; Piesse, J. Can GM technologies help the poor? The impact of Bt cotton in Makhathini Flats, KwaZulu-Natal. *World Dev.* 2003, *31*, 717–732.
75. Hutchinson, W.D.; Burkness, E.C.; Mitchel, P.D.; Moon, R.D.; Leslie, T.W.; Fleischer, S.J.; Abrahamson, M.; Hamilton, K.L.; Steffey, K.L.; Gray, M.E.; Hellmich, R.L.; Kaster, L.V.; Hunt, T.E.; Wright, R.J.; Pecinovsky, K.; Rabaey, T.L.; Flood, B.R.; Raun, E.S. Area-wide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science* 2010, *330*, 222–225.



76. Lutz, W.; Samir, K.C. Dimensions of global population projections: What do we know about future population trends and structures? *Philos. Trans. R. Soc. Lond. B* 2010, *365* (1554), 2779–2791.
77. Giles, K.L.; Hellmich, R.L.; Iverson, C.T.; Lewis, L.C. Effects of transgenic *Bacillus thuringiensis* maize grain on *B-thuringiensis*-susceptible *Plodia interpunctella* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 2000, *93* (3), 1011–1016.
78. Hussein, H.S.; Brasel, J.M. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* 2001, *167* (2), 101–134.
79. Munkvold, G.P.; Hellmich, R.L.; Rice, L.G. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. *Plant Dis.* 1999, *83* (2), 130–138.
80. Lovei, G.L.; Arpaia, S. The impact of transgenic plants on natural enemies: A critical review of laboratory studies. *Entomol. Exp. Appl.* 2005, *114*, 1–14.
81. Pilcher, C.D.; Obrycki, J.J.; Rice, M.E.; Lewis, L.C. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. *Environ. Entomol.* 1997, *26*, 446–454.
82. Al-Deeb, M.A.; Wilde, G.E.; Higgins, R.A. No effect of *Bacillus thuringiensis* corn and *Bacillus thuringiensis* on the predator *Orius insidiosus* (Hemiptera: Anthoridae). *Environ. Entomol.* 2001, *30* (3), 625–629.
83. Lundgren, J.G.; Wiedenmann, R.N. Coleopteran-specific Cry3Bb1 toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). *Environ. Entomol.* 2002, *31*, 1213–1218.
84. Anderson, P.L.; Hellmich, R.L.; Sears, M.K.; Sumerford, D.V.; Lewis, L.C. Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. *Environ. Entomol.* 2004, *33*, 1109–1115.
85. Ramirez-Romero, R.; Desneux, N.; Decourtye, A.; Chaffiol, A.; Pham-Delegue, M.H. Does Cry1Ab protein affect learning performances of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae)? *Ecotoxicol. Environ. Saf.* 2008, *70* (2), 327–333.
86. Hilbeck, A.; Moar, W.J.; Pusztai-Carey, M.; Filippini, A.; Bigler, F. Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environ. Entomol.* 1998, *27*, 1255–1263.
87. Ramirez-Romero, R.; Bernal, J.S.; Chaufaux, J.; Kaiser, L. Impact assessment of Bt-maize on a moth parasitoid, *Cotesia marginiventris* (Hymenoptera: Braconidae), via host exposure to purified Cry1Ab protein or Bt-plants. *Crop Prot.* 2007, *26*, 953–962.
88. Losey, J.E.; Rayor, L.S.; Carter, M.E. Transgenic pollen harms monarch larvae. *Nature* 1999, *399*, 214.
89. Jesse, L.C.H.; Obrycki, J.J. Field deposition of Bt transgenic corn pollen: Lethal effects on the monarch butterfly. *Oecologia* 2000, *1125*, 241–248.
90. Zangerl, A.R.; McKenna, D.; Wraight, C.L.; Carroll, M.; Ficarell, P.; Warner, R.; Berenbaum, M.R. Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proc. Natl. Acad. Sci. U. S. A.* 2001, *98* (21), 11908–11912.
91. Rosi-Marshall, E.J.; Tank, J.L.; Royer, T.V.; Whiles, M.R.; Evans-White, M.; Chambers, C.; Griffiths, N.A.; Pokelsek, J.; Stephen, M.L. Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *Proc. Natl. Acad. Sci. U. S. A.* 2007, *104* (41), 16204–16208.
92. Duan, J.J.; Lundgren, J.G.; Naranjo, S.E.; Marvier, M. Extrapolating non-target risk of Bt crops from laboratory to field. *Biol. Lett.* 2010, *6*, 74–77.
93. Zwahlen, C.; Nentwig, W.; Bigler, F.; Hilbeck, A. Tritrophic interactions of transgenic *Bacillus thuringiensis* corn, *Anaphothrips obscurus* (Thysanoptera: Thripidae), and the predator *Orius majusculus* (Heteroptera: Anthoridae). *Environ. Entomol.* 2000, *29* (4), 846–850.
94. Lundgren, J.G.; Wiedenmann, R.N. Tritrophic interactions among Bt (CryMbl) corn, aphid prey, and the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environ. Entomol.* 2005, *34* (6), 1621–1625.
95. Ferry, N.; Mulligan, E.A.; Majerus, M.E.N.; Gatehouse, A.M.R. Bitrophic and tritrophic effects of Bt Cry3A transgenic potato on beneficial, non-target, beetles. *Transgenic Res.* 2007, *16*, 795–812.
96. Hilbeck, A.; Baumgartner, M.; Fried, P.M.; Bigler, F. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environ. Entomol.* 1998, *27*, 480–487.
97. Hilbeck, A.; Moar, W.J.; Pusztai-Carey, M.; Filippini, A.; Bigler, F. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*. *Entomol. Exp. Appl.* 1999, *91*, 305–316.
98. Naranjo, S.E. *Risk Assessment: Bt Crops and Invertebrate Non-Target Effects—Revisited*; ISB News Report: Agricultural and Environmental Biotechnology, December 2009, 1–4.
99. Naranjo, S.E. Impacts of Bt crops on non-target invertebrates and insecticide use patterns. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* 2009, *4* (11), 1–23.
100. Orr, D.B.; Landis, D.A. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact of natural enemy populations in transgenic versus isogenic corn. *J. Econ. Entomol.* 1997, *90*, 905–909.
101. Reed, G.L.; Jensen, A.S.; Riebe, J.; Head, G.; Duan, J.J. Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: Comparative efficacy and non-target impacts. *Entomol. Exp. Appl.* 2001, *100*, 89–100.
102. Al-Deeb, M.A.; Wilde, G.E.; Blair, J.M.; Todd, T.C. Effect of Bt corn for corn rootworm control on nontarget soil microarthropods and nematodes. *Environ. Entomol.* 2003, *32*, 859–865.

103. Sisterson, M.S.; Biggs, R.W.; Olson, C.; Carriere, Y.; Dennehy, T.J.; Tabashnik, B.E. Arthropod abundance and diversity in Bt and non-Bt cotton fields. *Environ. Entomol.* 2004, *33*, 921–929.
104. Bhatti, M.A.; Duan, J.; Head, G.P.; Jiang, C.; McKee, M.J.; Nickson, T.E.; Pilcher, C.L.; Pilcher, C.D. Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected Bt corn on foliage-dwelling arthropods. *Environ. Entomol.* 2005, *34*, 1336–1345.
105. de la Poza, M.; Pons, X.; Farinos, G.P.; Lopez, C.; Ortego, F.; Eizaguirre, M.; Castanera, P.; Albajes, R. Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain. *Crop Prot.* 2005, *24*, 677–684.
106. Meissle, M.; Lang, A. Comparing methods to evaluate the effects of Bt maize and insecticide on spider assemblages. *Agric. Ecosyst. Environ.* 2005, *107*, 359–370.
107. Naranjo, S.E. Long-term assessment of the effects of transgenic Bt cotton on the abundance of nontarget arthropod natural enemies. *Environ. Entomol.* 2005, *34*, 1193–1210.
108. Naranjo, S.E. Long-term assessment of the effects of transgenic Bt cotton on the function of the natural enemy community. *Environ. Entomol.* 2005, *34*, 1211–1223.
109. Ludy, C.; Lang, A. A 3-year field-scale monitoring of foliage-dwelling spiders (Araneae) in transgenic Bt maize fields and adjacent field margins. *Biol. Control* 2006, *38*, 314–324.
110. Torres, J.B.; Ruberson, J.R. Abundance and diversity of ground-dwelling arthropods of pest management importance in commercial Bt and on-Bt cotton fields. *Ann. Appl. Biol.* 2007, *150* (1), 27–39.
111. Duan, J.J.; Marvier, M.; Huesing, J.; Dively, G.; Huang, Z.Y. A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). *PLoS One* 2008, *3* (1), e1415.
112. Bernstein, J.A.; Bernstein, I.L.; Bucchini, L.; Goldman, L.R.; Hamilton, R.G.; Lehrer, S.; Rubin, C.; Sampson, H.A. Clinical and laboratory investigation of allergy to genetically modified foods. *Environ. Health Perspect.* 2003, *111* (8), 1114–1121.
113. Environmental Protection Agency. *Cry9C Food Allergenicity Assessment Background Document*, 1999; <http://www.epa.gov/pesticides/biopesticides/cry9c/cry9c-peer-review.htm>
114. Carter, C.A.; Smith, A. Estimating the market effect of a food scare: The case of genetically modified StarLink corn. *Rev. Econ. Stat.* 2007, *89* (3), 522–533.
115. Picard-Nizou, A.L.; Pham-Delegue, M.-H.; Kerguelen, V.; Douault, P.; Marillau, R.; Olsen, L.; Grison, R.; Toppan, A.; Masson, C. Foraging behaviour of honey bees (*Apis mellifera* L.) on transgenic oilseed rape (*Brassica napus* L. var. *oleifera*). *Transgenic Res.* 1995, *4*, 270–276.
116. Birch, A.N.E.; Geoghegan, I.E.; Griffiths, D.W.; McNicol, J.W. The effect of genetic transformations for pest resistance on foliar solanidine-based glycoalkaloids of potato (*Solanum tuberosum*). *Ann. Appl. Biol.* 2002, *140*, 143–149.
117. Faria, C.A.; Wackers, F.L.; Pritchard, J.; Barrett, D.A.; Turlings, T.J.C. High susceptibility of Bt maize to aphids enhances the performance of parasitoids of lepidopteran pests. *PLoS One* 2007, *2*, e600.
118. Flores, S.; Saxena, D.; Stotzky, G. Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biol. Biochem.* 2005, *37*, 1073–1082.
119. Zurbrugg, C.; Honemann, L.; Meissle, M.; Romeis, J.; Nentwig, W. Decomposition dynamics and structural plant components of genetically modified Bt maize leaves do not differ from leaves of conventional hybrids. *Transgenic Res.* 2010, *19*, 257–267.
120. Virla, E.G.; Casuso, M.; Frias, E.A. A preliminary study on the effects of a transgenic corn event on the non-target pest *Dalbulus maidis* (Hemiptera: Cicadellidae). *Crop Prot.* 2010, *29* (6), 635–638.
121. Ellstrand, N.C.; Prentice, H.C.; Hancock, J.F. Gene flow and introgression from domesticated plants into their wild relatives. *Annu. Rev. Ecol. Syst.* 1999, *30*, 539–563.
122. Quist, D.; Chapela, I.H. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 2001, *414* (6863), 541–543.
123. Christou, P. No credible scientific evidence is presented to support claims that transgenic DNA was introgressed into traditional landraces in Oaxaca, Mexico. *Transgenic Res.* 2002, *11* (1), III–V.
124. Kaplinsky, N.; Braun, D.; Lisch, D.; Hay, A.; Hake, S.; Freeling, M. Biodiversity (communications arising): Maize transgene results in Mexico are artefacts. *Nature* 2002, *416* (6881), 601.
125. Quist, D.; Chapela, I.H. Maize transgene results in Mexico are artefacts—Reply. *Nature* 2002, *416* (6881), 602.
126. Serratos-Hernandez, J.-A.; Gomez-Olivares, J.-L.; Salinas-Arreortua, N.; Buendia-Rodriguez, E.; Islas-Gutierrez, F.; de Ita, A. Transgenic proteins in maize in the soil conservation area of Federal District, Mexico. *Front. Ecol. Environ.* 2007, *5* (5), 247–252.
127. Ortiz-Garcia, S.; Ezcurra, E.; Schoel, B.; Acevedo, F.; Soberon, J.; Snow, A.A. Absence of detectable transgenes in local landraces of maize in Oaxaca, Mexico (2003–2004). *Proc. Natl. Acad. Sci. U. S. A.* 2005, *102* (35), 12338–12343.
128. Xia, H.; Lu, B.R.; Su, J.; Chen, R.; Rong, J.; Song, Z.P.; Wang, F. Normal expression of insect-resistant transgene in progeny of common wild rice crossed with genetically modified rice: Its implication in ecological biosafety assessment. *Theor. Appl. Genet.* 2009, *119* (4), 635–644.
129. Committee on Genetically Modified Pest-Protected Plants, Board on Agriculture and Natural Resources, National Research Council. *Genetically Modified Pest-Protected Plants: Science and Regulation*; National Academy Press: Washington, D.C., 2000.
130. Wraight, C.L.; Zangerl, A.R.; Carroll, M.J.; Berenbaum, M.R. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proc. Natl. Acad. Sci. U. S. A.* 2000, *97* (14), 7700–7703.

131. Gould, F. Integrating pesticidal engineered crops into Mesoamerican agriculture. In *Transgenic Plants in Mesoamerican Agriculture: Bacillus thuringiensis*; Hruska, A.J., Pavon, M.L., Eds.; Zamorano Academic Press: Zamorano, Honduras, 1997; 6–36.
132. Shi, Y.; Wang, W.B.; Powell, K.S.; Van Damme, E.; Hilder, V.A.; Gatehouse, A.M.R.; Boulter, D.; Gatehouse, J.A. Use of the rice sucrose synthase-I promoter to direct phloem-specific expression of  $\beta$ -glucuronidase and snowdrop lectin genes in transgenic tobacco plants. *J. Exp. Bot.* 1994, *45*, 623–631.
133. Rao, K.V.; Rathore, K.S.; Hodges, T.K.; Fu, X.; Stoger, E.; Sudhaker, D.; Williams, D.; Christou, P.; Bharathi, M.; Brown, D.P.; Powell, K.S.; Spence, J.; Gatehouse, A.M.R.; Gatehouse, J.A. Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. *Plant J.* 1998, *15*, 469–477.
134. Giampietro, M.; Ulgiati, S.; Pimentel, D. Feasibility of large-scale biofuel production—Does an enlargement of scale change the picture? *Bioscience* 1997, *47*, 587–600.
135. Mayer, P.M.; Reynolds, S.K.; McCutchen, M.D.; Canfield, T.J. *Riparian Buffer Width, Vegetative Cover, and Nitrogen Removal Effectiveness: A Review of Current Science and Regulations*; EPA/600/R-05/118; U.S. Environmental Protection Agency: Cincinnati, OH, 2006.
136. National Academy of Sciences. *Genetically Modified Pest-Protected Plants: Science and Regulation*; National Academy Press: Washington D.C., 2000.
137. Pessel, F.D.; Lecomte, J.; Emeriau, V.; Krouti, M.; Messean, A.; Gouyon, P.H. Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. *Theor. Appl. Genet.* 2001, *102*, 841–846.
138. Terminator gene halt a ‘major U-turn.’ *BBC News* 1999, available at <http://news.bbc.co.uk/2/hi/science/nature/465222.stm>
139. Leslie, T.W.; Biddinger, D.J.; Mullin, C.A.; Fleischer, S.J. Carabidae population dynamics and temporal partitioning: Response to couples neonicotinoid-transgenic technologies in maize. *Environ. Entomol.* 2009, *38* (3), 935–943.
140. Landis, D.A.; Wratten, S.D.; Gurr, G.M. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annu. Rev. Entomol.* 2000, *45*, 175–201.
141. Eilenberg, J.; Hajek, A.; Lomer, C. Suggestions for unifying the terminology in biological control. *Biocontrol* 2001, *46*, 387–400.
142. Thorbek, P.; Sunderland, K.D.; Topping, C.J. Reproductive biology of agrobiont linyphiid spiders in relation to habitat, season and biocontrol potential. *Biol. Control* 2004, *30*, 193–202.
143. Tabashnik, B.E. Genetics of resistance to *Bacillus thuringiensis*. *Annu. Rev. Entomol.* 1994, *34*, 47–79.
144. Tabashnik, B.E.; Patin, A.L.; Dennehy, T.J.; Liu, Y.-B.; Carriere, Y.; Sims, M.A.; Antilla, L. Frequency of resistance to *Bacillus thuringiensis* in field populations of pink bollworm. *P. Natl. Acad. Sci. U. S. A.* 2000, *97* (24), 12980–12984.
145. Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division. *Optimum AcreMax Bt Corn Seed Blends: Biopesticides Registration Action Document*, 2010; [http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech\\_docs/brad\\_006490\\_oam.pdf](http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006490_oam.pdf)
146. Duan, J.J.; Head, G.; Jensen, A.; Reed, G. Effects of *Bacillus thuringiensis* potato and conventional insecticides for Colorado potato beetle (Coleoptera: Chrysomelidae) management on the abundance of ground-dwelling arthropods in Oregon potato ecosystems. *Environ. Entomol.* 2004, *33*, 275–281.