Review

Impacts of *Bt* crops on non-target invertebrates and insecticide use patterns

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Abstract

The ubiquitous nature of *Bacillus thuringiensis* (*Bt*), a Gram-positive bacterium capable of producing crystal proteins with insecticidal activity during sporulation, is now being mirrored in major crops plants that have been engineered through recombinant DNA to carry genes responsible for producing these crystal proteins and providing host plant resistance to major lepidopteran and coleopteran pests. In 2007, the 11th year of commercial production, *Bt* maize and *Bt* cotton were commercially produced on a total of ≈42 million hectares in 20 countries. Assessment of environmental safety has been and continues to be a key element of transgenic crop technology. This review focuses on two environmental elements, effects on non-target invertebrates and changes in insecticide use patterns since the adoption of *Bt* maize and cotton. Meta-analyses of the extant literature on invertebrate non-target effects reveals that the pattern and extent of impact varies in relation to taxonomy, ecological or anthropomorphic guild, route of exposure and the non-*Bt* control against which effects are gauged. Hazards identified in the laboratory may not always manifest in the field and the minor negative effects of *Bt* crops demonstrated in the field pale in comparison with alternative pest suppression measures based on insecticides. The efficacy of *Bt* maize and cotton against major pest species has been associated with an estimated 136.6 million kg global reduction in insecticide active ingredient used between 1996 and 2006 (29.9% reduction). Benefits vary by country and region and are heavily weighted towards cotton production, which has historically been one of the largest users of insecticides in the world.

Keywords: *Bt* maize, *Bt* cotton, *Bacillus thuringiensis*, Integrated pest management, Biological control, Environmental risk

Review Methodology: A number of sources were used to gather information for this review including CAB Abstracts and Current Content (keyword search terms=transgenic & (*Bt* or *Bacillus* and *thuringiensis*)), as well as literature citations from numerous review articles and books on the topic, many of which are cited in this review. Finally, the meta-database of Marvier *et al*. [1] served to identify additional literature dealing with non-target issues. The literature search was current to late November 2008.

Introduction

The insecticidal properties of *Bacillus thuringiensis* (*Bt*) have been known for over a century and commercial products based on this organism have been available for 70 years, occupying >90% of the bio-pesticide market [2]. *Bt*, a Gram-positive bacterium capable of producing crystal proteins with insecticidal activity during sporulation, is ubiquitous in the environment, and the genes coding for these insecticidal proteins are now becoming ubiquitous in major crop plants throughout the world via recombinant DNA technology. *Bt* potatoes were first commercially produced in the USA in 1995, but issues with consumer acceptance led to their retraction from the market after 5 years [3]. In contrast, *Bt* cotton was first commercially produced in 1996 in Australia, Mexico and the USA and its adoption and use has spread to six additional countries. *Bt* cotton is currently grown on ≈14 million hectares worldwide, which represents ≈40% of all cotton production globally (Table 1; [4]). Similarly, *Bt*

Table 1 Summary production statistics for Bt maize and Bt cotton adopting countries, 2007

	Maize				Cotton			
Country	Yield (M kg)	Total ha (1000s)	% Bt	First *Bt* production	Yield (M kg)	Total ha (1000s)	% Bt	First *Bt* production
Argentina	21 755	2838	67	1998	153	310	49	1998
Australia	–	–	–	–	134	65	92	1996
Brazil	51 589	13 827	0	2008	1602	1077	45	2005
Canada	10 554	1361	49	1997	–	–	–	–
China	–	–	–	–	8055	6202	69	1997
Columbia	–	–	–	–	42	42	28	2002
Czech Republic	608	93	1.7	2005	–	–	–	–
France	13 107	1481	1.3	1998	–	–	–	–
Germany	3480	383	<1	2000	–	–	–	–
Honduras	555	362	<1	2001	–	–	–	–
India	–	–	–	–	5356	9554	66	2002
Mexico	–	–	–	–	135	110	52	1996
Phillippines	6730	2720	5	2003	–	–	–	–
Poland	1640	262	<1	2007				
Portugal	646	117	3.2	1999	–	–	–	–
Slovakia	675	158	<1	2006	–	–	–	–
South Africa	7338	2551	44	1997	11	15	85	1997
Spain	3647	365	23	1998	–	–	–	–
Uruguay	210	50	44	2003	–	–	–	–
USA	332 092	35 022	57	1996	4181	4246	63	1996

Compiled from [4, 89, 109, 110]. See Hellmich *et al*. [31] and Naranjo *et al*. [32] for a summary of Cry proteins and events.

maize, first commercially produced in the USA (1996) and Canada (1997) is now grown on a total of ≈28 million hectares in 15 countries representing ≈19% of global maize production (Table 1; [4]).

Overall, genetically engineered (GE) crops with either insect resistance, herbicide tolerance, or both traits, were grown on ≈114.3 million hectares worldwide in 2007 [4]. GE soybean with herbicide tolerance was the leading crop, comprising about 51% of the global GE crop area. There are a number of *Bt* vegetable crops under development and evaluation including broccoli, cabbage, cauliflower and eggplant [5]. *Bt* potatoes are likely to be re-introduced, probably in Asia, Africa and Eastern Europe, in the future [3], and *Bt* rice is being evaluated in several countries [6]. Malone *et al*. [7] review potential transgenic enhancements to crops based on non-*Bt* approaches. At present, the only *Bt* crops being commercially produced are maize and cotton.

Foliar-applied *Bt* products have been extensively evaluated for safety (see [2, 8, 9]), but the process of genetic engineering along with the continual, season-long expression of *Bt* proteins in transgenic plants and other biological and sociological issues have raised additional concerns about their environmental safety and benefits that continue to be addressed by researchers. An extensive base of literature has amassed and addressed various aspects of environment risk including evolution of resistance in targeted pests, genetic drift, effects on soil structure and decomposition, effects on non-target organisms and shifts in pest management strategies – mainly insecticide usage patterns. A wealth of review, synthesis and interpretive articles, and books, too extensive to cite explicitly, have been written on these environmental issues. This review will attempt to synthesize and generalize the literature bearing on invertebrate non-target effects of transgenic *Bt* crops through meta-analyses of extant studies, and provide an overview of changes in patterns of insecticide use as a result of *Bt* crop production worldwide. This approach will hopefully provide a somewhat unique perspective that does not directly duplicate the many excellent review articles, syntheses and books already available (e.g. [2, 10–27]). For coverage of the other environmental risk issues associated with GE crops, the reader is directed to several recent reviews on pest resistance and management [28], gene flow [29] and soil ecosystem effects [30].

Bt Crops Within the Context of Integrated Pest Management (IPM)

The current suite of commercially available *Bt* crops has been engineered for resistance to several of the most significant and most difficult pests to control. The target pests for lepidopteran-active *Bt* maize are primarily the European corn borer, *Ostrinia nubilalis* and several other stem-boring pests such as *Diatraea* spp. and *Sesemia nonagroides*; for coleopteran-active *Bt* maize, the target is corn rootworm, *Diabrotica* spp. [31]. In the cotton system, the primary targets are the bollworm/budworm complex (*Helicoverpa* and *Heliothis* spp.), the pink bollworm, *Pectinophora gossypiella* and other bollworms (*Earias* spp.) [32]. The primary target of *Bt* potato when it was cultivated in the USA was the Colorado potato beetle,

Leptinotarsa decemlineata. Both maize, and especially cotton, production are impacted by a wide range of additional pests not affected by *Bt* proteins, many of which can be yield limiting if left uncontrolled [31, 32]. Thus, while *Bt* crops represent an important tactic for managing critical key pests, they must be integrated into a more comprehensive IPM programme in order to attain successful management of the entire pest complex. Examples of how this is being achieved have been recently detailed for maize, cotton and other *Bt* crops that may become available in the future [3, 5, 6, 31, 32].

The prevailing perspective is that *Bt* crops represent host plant resistance [33] and as such provide a foundation for suppressing key pests with little additional management input. Nonetheless, a fundamental component of modern IPM is the use of sampling and economic thresholds to determine the need for control actions. Clearly, the decision to employ *Bt* crops for pest control is made at planting time before the assessment of pest populations is possible. In general, the decision to use *Bt* crops is associated with production areas where key caterpillar or beetle pests are a perennial threat. Cotton producers in the San Joaquin Valley of California, USA, for example, do not have issues with caterpillar pest and thus do not use *Bt* cotton in that region [32]. Beyond recognition of historical pest distribution patterns, the deployment of *Bt* cotton by growers is based on their experience, their aversion to risk, and the anticipated benefits and costs of the technology. For caterpillar-resistant *Bt* maize, researchers have developed an evaluation system (http://www.Btet.psu.edu), which potentially allows growers to make more informed decisions about use of the technology based on pest distribution, climate, and various agronomic and economic considerations. Hellmich *et al*. [31] suggest that the criticism that *Bt* crops do not conform to the basic prescriptive use principles of IPM sets a double standard because use of resistant host plants derived from conventional methods is viewed as a fundamental tactic in IPM. Further, tactics such as biological control, another fundamental element of IPM, have been facilitated by *Bt* crops through broad-scale reductions in insecticide use (discussed in a later section). Ultimately, the decision to employ *Bt* crops rests with the grower. However, the growing number of traits (insect resistance and herbicide tolerance) being engineered into cotton and maize by the evolving crop biotechnology industry are making it increasingly difficult for growers to choose the best cultivars for their circumstances without also deploying unnecessary traits [33]. This has consequences for managing both pests and insecticide resistance.

Non-target Effects of *Bt* Crops

The potential impact of *Bt* crops, or GE crops in general, on non-target organisms and biodiversity was a concern well before the commercial production of these crops

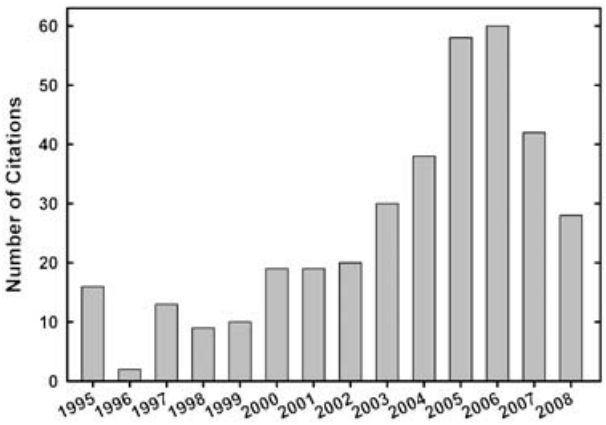

Figure 1 Distribution of original studies in the scientific literature addressing non-target effects of transgenic *Bt* crops on invertebrates. The data for 1995 includes all studies up to that year. Compiled from CAB Abstracts, the non-target database of Marvier *et al*. [1] and the author's personal database. Not included are a number of studies performed by industry as part of the commercial registration process with regulatory agencies

[16] and continues to be a well-researched topic today. As of late 2008, over 360 original research articles have addressed non-target effects in a number of *Bt* crops: mainly maize, cotton and potato. This does not include the many field and laboratory studies conducted by industry as part of the registration process with regulatory agencies such as the US Environmental Protection Agency. Several studies were published before commercialization of *Bt* crops and there was a steep upward trend in research effort during the late 1990s through to 2006 (Figure 1). The pace of research appears to have lessened a bit the last couple of years, but still remains significant. In addition, as noted above, a number of review and synthesis articles have attempted to collate and summarize these studies.

Risk Assessment Considerations

There continues to be debate about the most appropriate approaches to assessing risk to non-target organisms in transgenic crops ([18, 34–37]). Andow and Hilbeck [35] outline and discuss three general approaches to non-target risk assessment: (1) the ecotoxicology model, which is most often associated with the tiered approach used by regulatory bodies and focuses on acute toxicity; (2) the non-indigenous species model, which focuses on species that may be at risk from the introduction of a non-indigenous species, in this case the transgenic plant; and (3) the ecological model, which employs a tiered approach, but focuses on representative species belonging to functional groups that have both ecological and anthropocentric relevance, and attempts to measure longer-term fitness parameters based on potential routes of

exposure to toxins. Many regulatory bodies employ tier-based methodology to assess risk and there is general consensus in the scientific community that such an approach is most suitable and appropriate. Nonetheless, there remain differences of opinion on how best to employ risk assessment systems based on differing objectives and philosophies [36]. Most recently, Romeis *et al*. [37] offered an international initiative for risk assessment that capitalizes on some of the positive elements of both ecotoxicological and ecological approaches in an attempt to provide a general framework that adequately characterizes risk, but does not unduly hamper the introduction of important pest management technologies. In their approach, a problem formulation process is used to assess current knowledge and identify areas of concern or uncertainly, which then establishes risk hypotheses that are tested in a tiered approach. Further escalation through more complex and realistic tiers is only justified if the null hypothesis of no risk is rejected or other uncertainties exist. Focus is placed on important functional groups of non-target organisms, but based on practicality, the authors recognize the need for representative surrogate species in the testing process. Regardless of the process it is ultimately up to decision-making bodies to determine the balance of risks and benefits to society as a whole.

General Findings from Reviews and Syntheses

As noted, a large number of studies have addressed non-target effects in GE crops and several reviews and syntheses will be highlighted below. Recently, Lovei and Arpaia [21] reviewed the impact of transgenic crops (*Bt* and other) on a total of 32 species of natural enemies as evaluated in laboratory studies. They summarized multiple life history parameters and used a quasi-quantitative (vote-counting) approach to categorize statistical significance based on author-reported *P*-values. They concluded that 30% of studies for predators and nearly 40% of studies for parasitoids reported significant negative effects on multiple life history characteristics (47.5 and 33.6% neutral for predators and parasitoids, respectively). These values are inflated because of non-independence of multiple traits measured on given species in the same study. It also is important to note that over one-third of the studies included in their analysis involved GNA protein (snowdrop lectin [*Galanthus nivalis* agglutinin]), which is known to have broader toxicity than *Bt* [38]. Romeis *et al*. [25] summarized much of the same laboratory data for only *Bt* crops and concluded that such studies have only shown negative effects on natural enemies when susceptible and sublethally compromised herbivores feeding on *Bt* plant tissues or protein were offered as prey or hosts and that no direct toxic effects of *Bt* have been demonstrated. They further summarized a number of *Bt* crop field studies and concluded that neither the abundance of natural enemies nor their biological control function differed between *Bt* and non-*Bt* crops. These conclusions were based on author-reported statistics with unknown statistical power and in the case of some field studies amalgamated many different individual taxa.

Meta-analysis is one approach that can be used to overcome the weaknesses of these types of vote-counting syntheses by quantitatively combining the results of multiple studies using standardized effect sizes that take into account the variability, sample sizes and the magnitude of differences in comparative studies. Marvier and colleagues collated the extant literature on the non-target effects of *Bt* crops on invertebrates (mainly arthropods) in early 2006 (http://delphi.nceas.ucsb.edu/btcrops) and published [1] the first general meta-analyses of 42 field-based non-target studies focused on *Bt* maize and cotton. Their analyses showed that the abundance of all non-target invertebrates combined was slightly lower in *Bt* compared with non-*Bt* crops, but that abundances were much higher in *Bt* crops compared with non-*Bt* crops that had been treated with insecticides, mainly to control *Bt* targeted pests. They further concluded that taxonomic affiliation did not appear to alter these general findings and that it was unclear if the observed reductions of abundance in *Bt* crops were the result of direct toxicity or changes in prey/host availability in the case of natural enemies.

Wolfenbarger *et al*. [39] conducted a follow-up study using a modified version (45 field studies) of the Marvier *et al*. database that focused on partitioning the taxa into ecological function guilds and examined maize, cotton and potato. They found that predators as a group were slightly less abundant in *Bt* cotton compared with non-*Bt* cotton when neither received insecticide treatments, a pattern largely driven by a reduced abundance of nabid predators that was in turn likely the result of reductions in prey represented by target pests. They also found that parasitoids were much less abundant in *Bt* maize compared with unsprayed non-*Bt* maize, a pattern entirely caused by reduced abundance of *Macrocentris grandii*, a specialist exotic parasitoid of the primary *Bt* maize target, the European corn borer. Other functional guilds (herbivores, omnivores and detritivores) were unaffected in either *Bt* maize or cotton in comparison with untreated non-*Bt* controls. Predators and herbivores were slightly more abundant in *Bt* potatoes compared with an unsprayed non-*Bt* control and additional analyses of the potato metadata by Cloutier *et al*. [40] suggested that the increased presence of sucking herbivores on *Bt* potato may directly affect the predators feeding on these prey. Multiple functional guilds were more abundant in all *Bt* crops when compared with non-*Bt* crops treated with a variety of insecticides for control of *Bt* targeted pests. For studies in which both the *Bt* and non-*Bt* crops were sprayed with insecticides for non-target prey (cotton and potato), the abundance of all functional guilds were similar [39]. These authors further examined effects on several individual species that have been the topic of debate in the literature. For example, the effect of *Bt* proteins on *Chrysoperla*

carnea has been intensely debated in the literature [41–46], but the effect size based on field abundance of this species was essentially zero in both cotton and maize studies where no insecticides were used. In addition, Wolfenbarger *et al.* [39] found no effects of *Bt* cotton on *Lygus* spp. plant bugs, which have been noted (e.g. [47, 48]) or predicted [49] to be more problematic in *Bt* cotton.

Finally, Duan *et al.* [50] compiled an independent database of 25 laboratory studies on the honey bee, *Apis mellifera*, and a meta-analysis of these studies revealed that *Bt* proteins found in either lepidopteran- or coleopteran-resistant crops had no effect on the survival of larvae or adults.

Reassessment of Extant Non-target Studies – A Meta-analysis

Since the Marvier *et al.* [1] meta-database was developed in early 2006, there have been dozens of new non-target invertebrate studies published on *Bt* crops (see Figure 1). For this review, the author has added a total of 39 new laboratory-based studies and 14 new field-based studies to a modified version of the original database (described below). The laboratory-based database now includes 135 studies on nine *Bt* crops and 22 different *Bt* Cry protein or protein combinations from 17 countries. The field-based database contains 63 studies on five *Bt* crops and 13 *Bt* proteins from 13 countries. Not surprisingly, the bulk of all studies were from the USA (47%) and China (13%) where *Bt* crop adoption rates have been very high; however, very few if any non-target studies have come from Argentina, Canada and India (4% collectively), where adoption of *Bt* crops also has been high. In Europe, a comparatively large number of studies have come from Switzerland (9%), where there is no commercial production of *Bt* crops, followed by Spain, France and the UK (11% collectively). The vast majority of studies have focused on *Bt* maize and cotton, both commercially grown since 1996; many fewer studies were available on *Bt* potato that was grown for only 5 years in the USA, or rice and eggplant that are not presently produced commercially. A summary of the updated database is provided in Table 2 and a full listing of references included in the database is provided in the Further Reading section.

Methodology

Following the standards established by Marvier *et al.* [1], studies included in the database were selected based on the following criteria: (1) crop plants that were genetically modified to express one or more Cry proteins from *Bt* (three field studies also include vegetative insecticidal proteins [Vip] from *Bt*); (2) studies that measured the effect of the *Bt* crop or pure *Bt* Cry proteins on some aspect of the life history or abundance of non-target invertebrate taxa relative to a non-*Bt* control; and (3) were published in English. An additional criterion used to develop the updated and modified database used here is that studies had to have provided some measure of variance (SEM, SD) and sample size along with means on reported characteristics. This was necessary to calculate the weighted effect-size estimator used in this analysis (see below). A surprising number of studies did not provide measures of variance in the original database [39] and several new field and laboratory studies were not added to the new database as a result of these omissions by study authors. Finally, the issue of independence in the dataset is critical to the conduct of a robust meta-analysis. Both Marvier *et al.* [1] and Wolfenbarger *et al.* [39] describe the screening process and analytical methods used to eliminate non-independent data and analyses and those rules were applied to the 53 additional studies added here.

The meta-analyses described here used Hedges' *d*, a weighted mean effect size estimator that is calculated as the difference between an experimental (*Bt*) and control (non-*Bt*) mean response divided by a pooled standard deviation and multiplied by a small sample size bias correction term [51, 52]. In essence, the effect size is a standardized measure that accounts for levels of variation and replication in individual studies and can be used in analyses independent of the original experiment and its associated interpretations. In analyses, the effect size is then weighted by the reciprocal of the sampling variance [51]. The effect size was estimated such that a negative effect size would indicate either a lower abundance in field studies or a lower performance (slower development, lower survival or fecundity) in laboratory studies with the *Bt* crop or Cry protein compared with the non-*Bt* control, while a positive effect size would indicate the opposite. All analyses were performed using Meta-Win [52].

Meta-analyses of laboratory studies

With the exception of the honey bee meta-analysis discussed above, laboratory studies in the database have not yet been examined through meta-analysis. Many interesting questions could be addressed with the data, but the focus here is on comparing and contrasting generalized effects when organisms are exposed directly to *Bt* plant tissues (including pollen) or pure Cry protein (bi-trophic), or in the case of natural enemies, through their prey or hosts that have fed on *Bt* plant tissues or Cry proteins in diets (tri-trophic). Because these represent very different routes of exposure and because prey or host-mediated quality issues are known to affect tri-trophic interactions (e.g. [53–55]), these exposure routes were examined separately. Further, the emphasis here is on general patterns relative to *Bt* proteins and so studies on both lepidopteran-active and coleopteran-active proteins have been pooled.

When non-target invertebrates were exposed to *Bt* proteins directly through either plant tissues or pure proteins in artificial diets, responses were variable depending on the life history trait measured and on the guild into

Table 2 Summary of laboratory-based and field-based studies used in the meta-analyses presented in this review

	Laboratory-based			Field-based	
No. Studies[1]		135			63
No. Observations		871			3544
Crops	Cotton Potato Rice Eggplant Tobacco	Maize Broccoli Cabbage Canola		Cotton Maize Potato Rice Eggplant	
Bt proteins[2]	Cry1A Cry1Aa Cry1Ab Cry1Ac Cry1B Cry1Ba Cry1C Cry1F	Cry2A Cry2Ab Cry2Ac Cry2B Cry3A Cry3B Cry3Bb Cry3Bb1	Cry9C Cry1Ab & 1Ac Cry1Ab & 2Ab Cry1Ac & CpTI Cry1Ac & 1C Cry1Ac & 2Ab	Cry1A Cry1Ab Cry1Ac Cry3A Cry3B Cry3Bb Cry3Bb1	Cry1Ab & 1Ac Cry1Ac & 2Aa Cry1Ac & 2Ab Cry1Ac & CpTI Cry1Ab & Vip3A Vip3A
Countries	Bulgaria Canada China Czech Republic Denmark France Germany India Italy	Japan New Zealand Philippines South Korea Spain Switzerland UK USA		Australia Brazil China Czech Republic Denmark France Germany	Hungary India Italy Spain Switzerland USA
Study types	Pure protein exposure Bi-trophic exposure Tri-trophic exposure			*Bt* vs. non-*Bt* crops (both w/o insecticides) *Bt* vs. non-*Bt* crop w/insecticides *Bt* vs. non-*Bt* (both w/insecticides)	
Parameters	Development/Growth Survival/Mortality Reproduction Consumption/Nutrition			Abundance	
No. Phyla		3		2	
No. Classes		8		6	
No. Orders[3]		16		21+	
No. Families[3]		43		139+	
No. Genera[3]		79+		172+	
No. Species[3]		99+		185+	

[1]The studies included in the database are noted in the Further Reading section.
[2]Proteins as reported by study authors; CpTI=cowpea trypsin inhibitor, Vip=vegetative insecticidal protein (from *B. thuringiensis*).
[3]The + indicates that not all taxa were identified to the specified level.

which the organisms were classified (Figure 2). Within the natural enemy group predators showed a slight but significant reduction in developmental rate when exposed to *Bt* proteins directly compared with non-*Bt* controls. Conversely, *Bt* proteins had no affect on survival or reproduction of either predators or parasitoids. Herbivorous taxa that represent pests in cropping systems, but are not the specific target of *Bt* crops, showed varying responses depending on taxonomic affiliation (Figure 2). 'Non-susceptible' pests not belonging to the orders Lepidoptera or Coleoptera and exposed to either lepidopteran-resistant or coleopteran-resistant crops, respectively, were unaffected by *Bt* proteins. In contrast, 'susceptible' pests belonging to the targeted taxonomic orders showed significant reductions in developmental rates and survivorship when exposed to *Bt* proteins relative to non-*Bt* controls (Figure 2). Too few studies in this group were available to examine effects on reproduction. Thus, even though a particular pest may not be considered a target of *Bt* crops from a labelling standpoint, these species as a group appear to be sufficiently susceptible to *Bt* proteins to result in lowered life history performance. The valued arthropod category included insect pollinators, charismatic butterflies (e.g. monarchs, swallowtails) and moths of economic importance (e.g. silk moths). Pollinators were not affected by *Bt* proteins and reflect the findings of Duan *et al.* [50] who used a larger dataset based solely on honeybees. Conversely, both the developmental rates and survival of valued herbivores were significantly lower when exposed to *Bt* proteins directly (Figure 2). This group was dominated by the monarch butterfly, *Danaus plexippus*, but also included

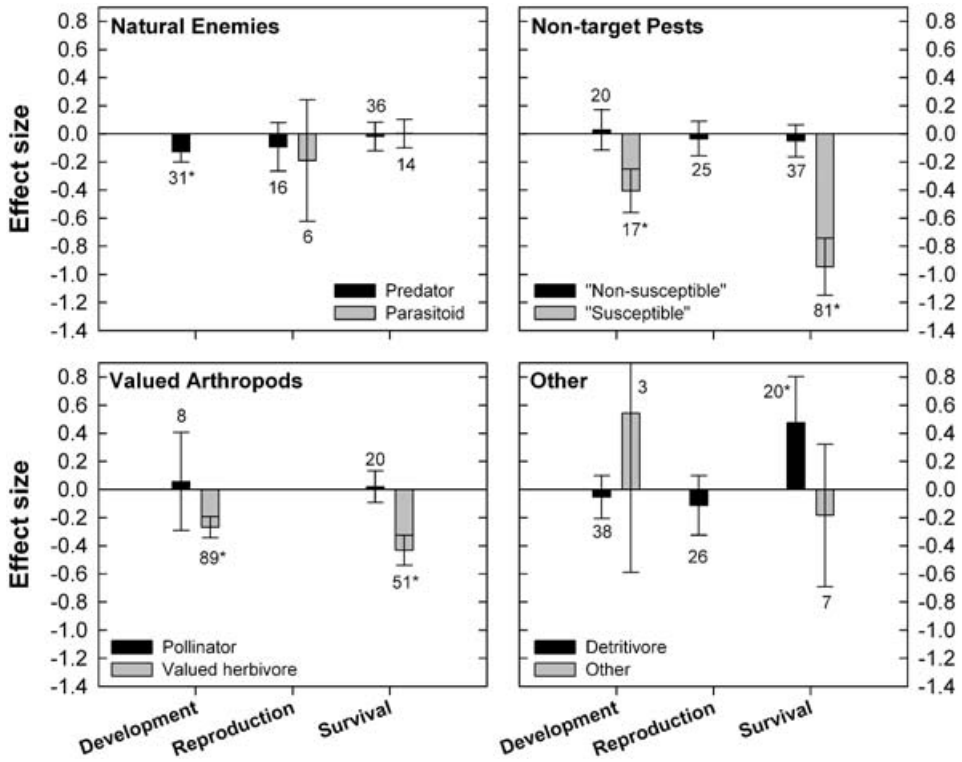

Figure 2 Meta-analyses of laboratory studies examining non-target effects of transgenic *Bt* crops on four general categories of invertebrates when organisms were directly exposed to either *Bt* proteins via transgenic plant materials or pure *Bt* proteins in artificial diets (bi-trophic exposure). Numbers above or below the bars indicate the total number of observations for each measured biological parameter and error bars denote 95% confidence intervals; error bars that do not include zero indicate significant effect sizes (*, $P < 0.05$). Negative effect sizes are associated with compromised performance on *Bt* compared with non-*Bt* controls. Natural enemies include arthropod predators and insect parasitoids; non-target pests includes those that are potentially susceptible (taxonomically related at the order level to the target of the *Bt* crop) or putatively non-susceptible based on taxonomic order. Valued arthropods include insect pollinators and herbivores with a subjective human or economic value (e.g. monarch and swallowtail butterflies, silk moths, caddis flies). The "other" category includes detritivores and other miscellaneous herbivores and omnivores not defined in other categories (e.g. daphnia, snails)

lepidopteran family members of Papilionidae, Saturniidae, Lycaenidae and Bombyxidae. This result is not surprising given the taxonomic affiliation of these species, but it should be noted that a thorough analysis of monarch butterfly showed that risk to this species in the field was negligible [56]. The remaining studies on detritivores and other herbivores and omnivores generally showed no affects of *Bt* proteins with the exception of significantly higher survival of detritivores as a group when exposed to *Bt* proteins, compared with a non-*Bt* control (Figure 2).

Species in the third trophic level can be exposed to plant-based constituents both through consumption of plant foliage, sap, pollen and nectar [57] as well as tri-trophically through consumption of herbivorous prey and hosts that have been exposed to these plant constituents. Many studies have examined the effects of *Bt* proteins on predators and parasitoids through this tri-trophic pathway using prey or hosts that are not susceptible to *Bt* proteins (high quality) and those that are sublethally affected in some manner after exposure to *Bt* proteins (low quality). High host or prey quality was generally determined by taxonomic affiliation relative to the *Bt* proteins examined, but several studies also used resistant lepidopteran hosts, which were completely unaffected by *Bt* proteins [54, 55, 58, 59]). Analyses revealed a clear and significant impact of host quality on the performance of parasitoids (Figure 3). Developmental rates, reproduction and survival of parasitoids as a group were reduced when they were provided with hosts that had been compromised by exposure to *Bt* proteins. When provided with high-quality hosts, parasitoid development and survival were equivalent on hosts exposed or not exposed to *Bt* proteins. There was even a slight increase in reproductive performance when parasitoids were provided with high-quality hosts exposed to *Bt* proteins, compared with non-*Bt* controls. Predators as a group showed slightly lower survivorship when provided compromised (low-quality) prey exposed to *Bt* proteins, but slightly faster developmental rates when provided unsusceptible (high-quality) prey exposed to *Bt* proteins (Figure 3). All other predator life history characteristics were unaffected by *Bt* proteins regardless of prey quality.

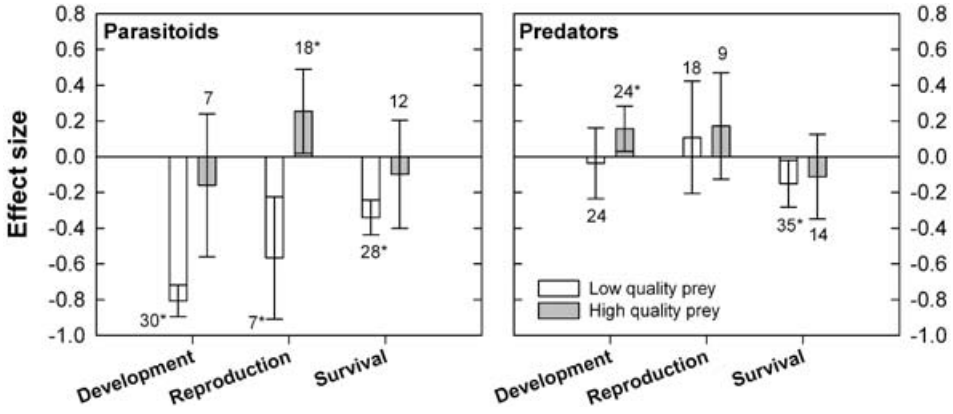

Figure 3 Meta-analyses of laboratory studies examining non-target effects of transgenic *Bt* crops on arthropod predators and insect parasitoids that were exposed to *Bt* proteins via prey or host that had fed on either transgenic plant materials or pure *Bt* proteins in artificial diets (tri-trophic exposure). Prey or hosts that were partially susceptible to *Bt* proteins and thus experienced reduced vigour were considered low quality prey. Numbers above or below the bars indicate the total number of observations for each measured biological parameter and error bars denote 95% confidence intervals; error bars that do not include zero indicate significant effect sizes (*, $P < 0.05$). Negative effect sizes are associated with compromised performance on *Bt* compared with non-*Bt* controls

Meta-analyses of field studies

In general, field studies were structured in such a way that three different and independent hypotheses could be tested. One type of experiment compared *Bt* with non-*Bt* fields, neither of which received any additional insecticide treatments and allows testing of the hypothesis that the proteins or other characteristics in the *Bt* plant affected arthropod abundance either directly or indirectly. The second set of studies that compare unsprayed *Bt* crops with non-*Bt* crops receiving insecticide treatments tests the hypothesis that arthropod abundance is influenced by the method used to control the pest targeted by the *Bt* crop. A third comparison, in which both the *Bt* and the non-*Bt* crop were treated with insecticides, tests the hypothesis that arthropod abundance is affected when other pests in the system not suppressed by *Bt* require additional insecticide treatments. This latter scenario is common for cotton, which harbours a large diversity of potential pests [32].

The addition of 14 new studies did not qualitatively alter the patterns for ecological functional guilds observed by Wolfenbarger *et al.* [39], regardless of the hypothesis tested (Figure 4A–C). This result was predicted by Wolfenbarger *et al.* [39], based on a cumulative meta-analysis that allows patterns in effect sizes to be examined over time as new studies are added. For all groups except parasitoids, they found that the trajectory for effect size was clear and unlikely to be altered by additional studies based on the same suite of *Bt* proteins. Field studies on parasitoids have been limited in all crops except maize; however, even there most of the observations have been on *M. grandii*, an exotic specialist of European corn borer. Additional field studies on a broader array of parasitoid taxa in both maize and cotton may be warranted given the results of laboratory studies (see Figure 3). However, it should be noted that the target pest, and in turn its specialist parasitoid, would be affected by any pest control method. Analyses of two crops, rice and eggplant, not previously reported indicates that no arthropod functional guild was affected by *Bt* in comparison with an unsprayed control (Figure 4A). The number of studies on these crops is still very limited and there was insufficient data to test hypotheses regarding the other types of control treatments.

From a pest management perspective, two of the key non-target concerns of *Bt* crops has been effects on potential biological control agents and on other pests in the system that are not specifically targeted by the transgene products. If the data are now organized to examine these two groups explicitly, slightly different patterns emerge (Figure 5). Natural enemies are significantly less abundant in *Bt* cotton compared with untreated non-*Bt* cotton, but much more abundant in *Bt* cotton when compared with non-*Bt* cotton sprayed with insecticides. The large differential for parasitoids in unsprayed maize (Figure 5A) is moderated by the abundance of predators in this crop making the overall effect of *Bt* maize on natural enemies insignificant. Keep in mind that the parasitoid group is largely represented by *M. grandii* and that effects on other parasitoids in both *Bt* maize and cotton have not been well documented in the field. The abundance of the subset of herbivores that represent pests putatively non-susceptible to *Bt* proteins in the cropping systems examined is in fact unaffected by most *Bt* crops. Non-target pests are higher in *Bt* potato, and as noted, Cloutier *et al.* [40] suggests this is largely a result of higher populations of sucking pests such as aphids. Insecticides applied to non-*Bt* crops for pest control is effective, leading to higher non-target pest populations in unsprayed *Bt* crops (Figure 5B). General predator-to-prey ratios were examined by Wolfenbarger *et al.* [39], based on studies in which both predators and herbivores were measured in the

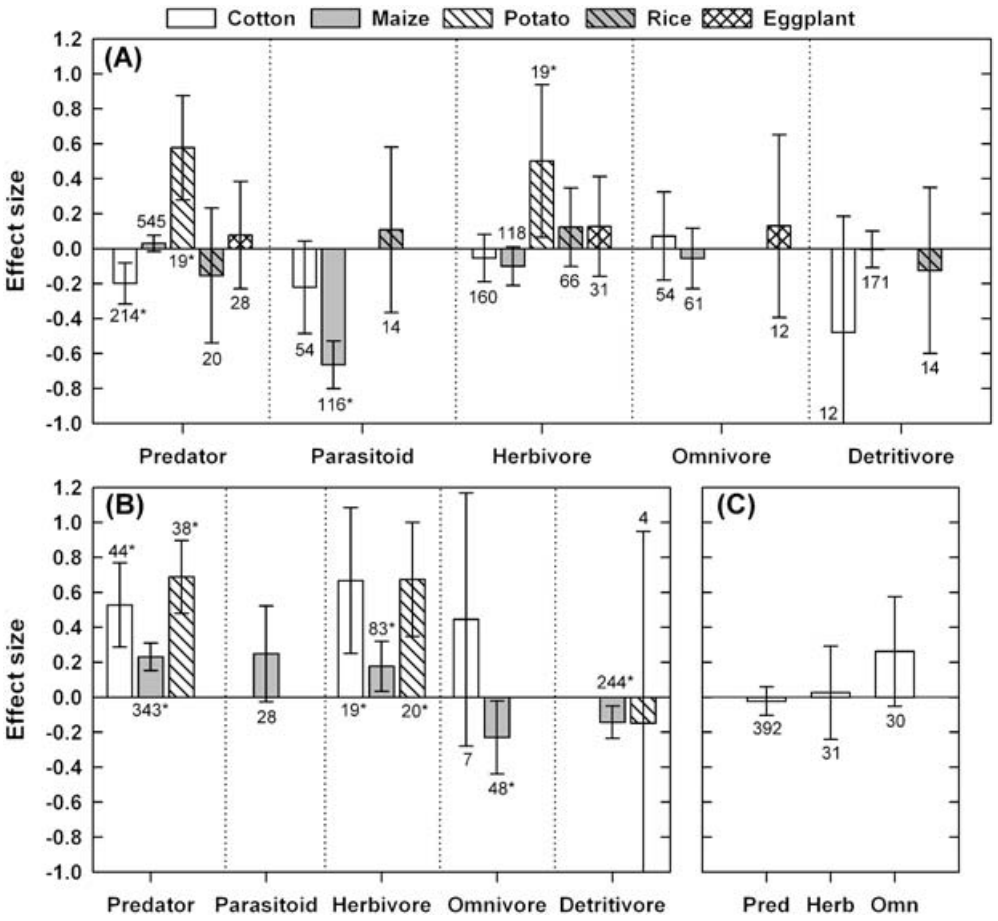

Figure 4 Meta-analyses of field studies examining the abundance of non-target invertebrates in transgenic *Bt* crops by ecological functional guilds. (A) *Bt* crops compared with non-*Bt* crops, neither treated with insecticides; (B) *Bt* crops compared with non-*Bt* crops treated with insecticides; (C) *Bt* crops compared with non-*Bt* crops both treated with insecticides. Numbers above or below the bars indicate the total number of observations and error bars denote 95% confidence intervals; error bars that do not include zero indicate significant effect sizes (*, $P < 0.05$). Negative effect sizes are associated with lower abundance on *Bt* crops compared with non-*Bt* controls

same study. They found that predator-to-prey ratios were unaffected by either *Bt* crops or insecticide use with the exception that higher ratios were noted in *Bt* maize when compared with insecticide-treated non-*Bt* maize.

Comparing laboratory and field studies

The correspondence of laboratory and field studies is somewhat difficult to gauge as different taxa were focused upon, protein exposures in laboratory studies were more controlled and often at levels in excess of field concentrations, and field abundance integrates and is governed by many interacting and uncontrollable factors. Even with these limitations there are some general patterns worthy of note. Within natural enemy guilds, laboratory studies showed negative effects of *Bt* on development and survival of predators and parasitoids through bi-trophic and/or tri-trophic exposure, both realistic routes of exposure in field populations. In turn, predator abundance was slightly lower in *Bt* cotton (but not *Bt* maize, rice or eggplant) and parasitoid abundance was much lower in *Bt* maize (but not in *Bt* cotton, rice or eggplant). Clearly, changes in developmental rates and survival would impact population growth, but the lack of consistent effects in the field would suggest that other variables are involved, most notably reductions in target prey or hosts. 'Valued herbivores' were not examined in the field studies examined here and very few studies included pollinators making analyses of these groups problematic [but see 56]. The enhanced survival of detritivores as a group in laboratory studies was not reflected in field abundance studies. Finally, the finding of no effect of *Bt* on non-susceptible, non-target pests in the laboratory is consistent with the lack of changes in abundance in *Bt* crop fields, compared with non-*Bt* controls. The laboratory studies in the database are representative of early tier tests required by various regulatory agencies (several studies were in fact tests provided by industry) to establish the potential for hazard. Some hazards to *Bt* proteins have indeed been demonstrated in laboratory studies, but in general their manifestation in the field has been limited based on available studies. Further, more detailed analyses to examine the ability of these laboratory tests to predict non-target

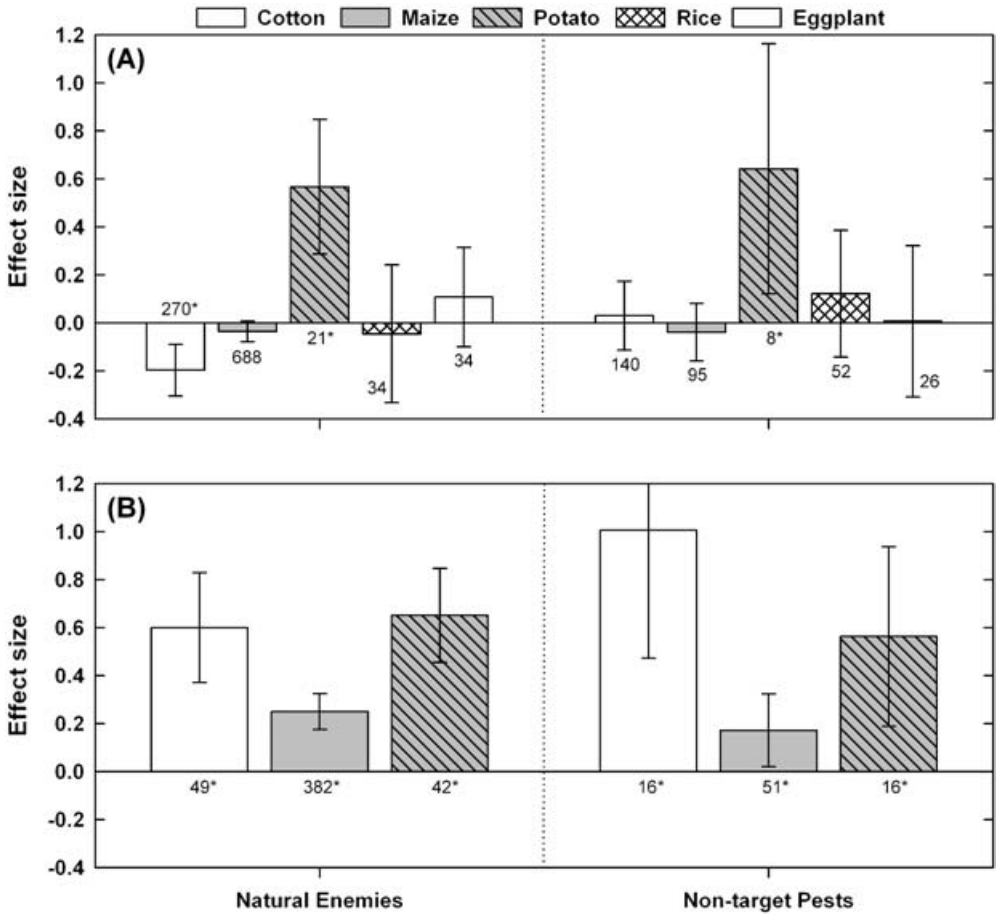

Figure 5 Meta-analyses of field studies examining the abundance of non-target arthropods in transgenic *Bt* crops within a pest management context. (A) *Bt* crops compared with non-*Bt* crops, neither treated with insecticides; (B) *Bt* crops compared with non-*Bt* crops treated with insecticides. Numbers above or below the bars indicate the total number of observations and error bars denote 95% confidence intervals; error bars that do not include zero indicate significant effect sizes (*, $P < 0.05$). Negative effect sizes are associated with lower abundance on *Bt* crops compared with non-*Bt* controls. Natural enemies include arthropod predators and insect parasitoids, and non-target pests include those that are putatively non-susceptible to *Bt* crops based on taxonomic order (i.e. non-Coleopteran or Lepidopteran pests relative to the target of the crop)

risk in the field is on-going (Duan, Lundgren, Naranjo and Marvier, unpublished).

Overall, these meta-analyses are instructive in providing a more quantitative synthesis of the extant non-target data for *Bt* crops. Nonetheless, the interpretations derived are only as sound as the available data and several limitations have been noted relative to field studies of parasitoids, pollinators and arthropods of special human interest, and to studies on non-maize and cotton systems. Published studies vary in quality and comprehensiveness, particularly field studies. Several studies were rather inclusive in the taxa examined (e.g. [60–65]), while other studies focused on particular functional guilds such as predators (e.g. [66–70]), herbivores [23, 71] or detritivores [72, 73]. Some studies were conducted over multiple sites and years, while others for only a single year. Wolfenbarger *et al.* [39] examined the sensitivity of field-based meta-analysis to several experimental design issues including plot size, study duration, total sampling dates and found no consistent trends in effect sizes. They also noted no publication bias in field studies, indicating that negative as well as positive studies were reported equally, and they found no undue influence by individual studies with large numbers of observations. A similar analysis here of publication bias for the laboratory studies also suggests no publication bias. Clearly, field studies have been more inclusive taxonomically (see Table 2) than laboratory studies, but they have also tended to focus on arthropods, primarily insects and arachnids in crop fields. Whether this breadth of coverage is sufficient to document effects on biodiversity within agroecosystems is unknown and preliminary studies like Rosi-Marshall *et al.* [74] point to the potential for off-site effects. Nonetheless, the weight of considerable data from the field points to a consistent conclusion that *Bt* crops have only minor effects, if any, on a large number of taxa that are dwarfed in comparison with alternative pest control measures such as broader-spectrum insecticides.

Effects on Biological Control Function

One of the greatest concerns regarding *Bt* crops, or any pest control technology, from an IPM perspective is their impact on biological control, a critical ecosystem service that suppresses many potential pests in agricultural systems. This concern is emphasized by the large number of both laboratory and field non-target studies that have focused on arthropod natural enemies. While examination of effects of *Bt* proteins on life history characteristics in the laboratory and ultimately population density in the field are essential topics of investigation, perhaps more critical are impacts on biological control function – rates of predation and parasitism and effects on pest dynamics. However, relatively few studies have examined biological control function in the field. Schuler *et al*. [58] demonstrated in field simulators that *Bt* transgenic rape did not affect parasitoid/host interactions between *Myzus persicae* and its endoparasitoid *Diaeretiella rapae* or the ability of the parasitoid to suppress aphid populations. No differences in proportional parasitism of various aphid species between *Bt* maize and non-*Bt* maize were noted by Pons and Stary [75]. Predictably, Bourguet *et al*. [76] and Manachini [77] observed lower levels of proportional parasitism on the target European corn borer by specialist tachinid parasitoids in *Bt* compared with non-*Bt* corn, while Siegfried *et al*. [78] found reduced proportional parasitism by two specialist exotic parasitoids, *M. grandii* and *Eriborus terebans*, in corn borer collected from *Bt* maize. However, Orr and Landis [79] reported equal levels of proportional parasitism and predation of natural-deposited corn borer eggs, and parasitism of sentinel larvae by *M. grandii* and *E. terebans* was similar in *Bt* and non-*Bt* maize. Likewise, rates of predation on sentinel corn borer egg masses were similar in *Bt* and non-*Bt* sweet maize [68]. Predation on sentinel pink bollworm (target pest) egg masses [80, 81] and sentinel pink bollworm pupae [81] were the same in both *Bt* and non-*Bt* cotton. Additional field life-table studies on another key cotton pest, *Bemisia tabaci*, demonstrated that marginal rates of both parasitism and predation on nymphs were equivalent in *Bt* and non-*Bt* cotton [81]. This study also reported no changes in predator to prey ratios for either *B. tabaci* or *Lygus hesperus*, another key pest in western USA cotton systems, neither of which is susceptible to *Bt* cotton. Interestingly, this biological control function was sustained despite a ≈20% reduction in the abundance of five common generalist predators in *Bt* cotton (likely caused by reduction in prey targeted by *Bt* cotton) demonstrated in a companion 5-year study [69]. Finally, in studies conducted in commercial fields where both *Bt* and non-*Bt* cotton received differing insecticide applications, predation on sentinel *Helicoverpa zea* (target pest) eggs was higher in *Bt* fields at two field sites and unchanged at a third in the southeastern USA [82]. Overall, these studies suggest an expected general reduction in parasitism by specialists of *Bt*-targeted pests probably due to host reduction, but no influence of *Bt* crops on the biological control activity of primarily generalist arthropod predators, even if population densities of some species are reduced in *Bt* fields. In general, *Bt* crops appear compatible with biological control and may even enhance the effect of natural enemies within an IPM framework [83].

Insecticide Use Patterns

There has been considerable effort made in attempting to estimate the economic and environmental costs and benefits associated with GE crop production, and *Bt* crop production in particular, compared with conventional production systems (see reviews by [17, 84–86]). Of special interest has been the changing dynamics of insecticide use patterns associated with the increasing adoption of *Bt* crops by growers worldwide. In general, the cultivation of *Bt* maize and cotton has led to a reduction in insecticide use that has been realized by both large- and small-scale farmers [87]. This has been particularly important in countries such as China and India where there are millions of small-scale farmers. However, there are major differences in reductions between *Bt* cotton and *Bt* maize that are related largely to the respective pest complexes and the degree to which the *Bt* trait has replaced insecticide applications as a pest control measure.

Insecticide Use in Cotton

Cotton producers are among the largest user of insecticides in the world [87] and the crop harbours dozens of pests of which caterpillars are the most significant [32]. Brookes and Barfoot [85] have compiled perhaps the most comprehensive estimates of the impact of GE crops on pesticide use, crop production, economics and other environmental variables available over the past few years, based on comparative farm-level data in adopting countries. Their analyses for the period 1996–2006 estimate that *Bt* cotton production has reduced the total volume of insecticide active ingredient by 128.4 million kg globally, representing an overall reduction of 22.9%, the largest reduction in pesticide use by any GE crop. They further estimate that the environmental toxicity of the insecticides used, as measured by the environmental impact quotient (EIQ; [88]), has been reduced by 24.6% over this 11-year period. Reductions in the EIQ range from 33, 24 and 20% in China, Australia and the USA, respectively, to between 5 and 8% for the remaining *Bt* cotton adopting countries, including India which now grows the largest amount of *Bt* cotton in the world (see Table 1). Nonetheless, Brookes and Barfoot [85] estimate that the ratio of benefits in developing versus developed countries relative to reductions in insecticide EIQ is 6.8:1.

In Australia and the USA where insecticide use patterns are closely followed by the industry more detail is available. The use of single gene *Bt* cotton (Ingard™) in Australia between 1996 and 2004 resulted in 59% fewer applications and a 44% reduction in insecticide active ingredient compared with conventional cotton production [87]. From 2002 to 2006, the use of dual gene *Bt* cotton (Bollgard II™), which has been the only *Bt* cotton grown in Australia since 2004, has been associated with an 85% reduction in sprays and an associated 65–75% decrease in insecticide active ingredient. Overall insecticide use on cotton for all pests in the USA has declined over the past two decades. Based on average use during the 10 years before *Bt* cotton and the 11 years since adoption, the number of applications has decreased by about 40% [89]. This pattern cannot be completely attributed to *Bt* cotton as other factors are involved, including the introduction of newer and more effective insecticides, the near eradication of the boll weevil (historically one of the most significant pest of cotton) and better overall IPM practices [90]. Several pest groups, mainly plant bugs and stink bugs, have increased in prominence since the adoption of *Bt* cotton, mainly as a result of the reduced use of broad-spectrum sprays for caterpillars (see [32] for a discussion). Sprays for the major caterpillar pests have declined to an average of 0.79 per ha over the past 11 years (57.4% reduction), while those for the plant bug, *Lygus* spp., have increased to an average of 0.61 (74% increase). Insecticide applications to control stinkbugs were not tracked prior to 1992, but appear to have increased from 1996 to 2003 and then stabilized at around 0.33 applications per ha.

In China, the largest producer of cotton in the world, the number of insecticide applications is estimated to have been reduced by 59–66% resulting in a 61–80% reduction in total insecticide volume, compared with conventional production [91–93]. Likewise, in India it is estimated that the number of insecticide applications has been comparatively reduced by 38–42% and the volume of active ingredient has been reduced by 50–70% [94–96]. These figures vary within these vast countries with differing pest issues and production practices in different regions, making generalizations difficult [86, 87]. For example, based on farmer surveys in Northern China, Yang *et al.* [97] found that many growers continued to protect *Bt* cotton from pests much as they would conventional cotton, resulting in a large number of insecticide applications against the cotton bollworm that were probably unnecessary. However, those farmers trained in IPM methods realized greater reductions in insecticide use compared with those without an understanding of IPM principles [97]. Additional issues with black market seed of questionable quality [98] and the results of several alternative economic modelling analyses [99, 100] suggest that the benefits may not be uniformly positive. In South Africa, estimates suggest benefits to both small-scale dryland and large-scale irrigated production, with a comparative 40–66% reduction in applications and a 25–70% reduction in insecticide volume [101–105]. Insecticide applications in Argentina and Mexico have been estimated to have dropped by about 50% [106, 107].

Insecticide Use in Maize

Reductions in insecticide usage in maize systems have been much less dramatic compared with cotton. This is mainly because relatively few insecticide applications focused on the main target of *Bt* maize, the European corn borer, because such applications are generally ineffective [31]. *Bt* maize resistant to corn rootworms has only been available since 2003 and even then does not have the level of efficacy common in the lepidopteran-resistant events. Thus, their adoption has been limited. Nonetheless, new events are being introduced and the potential benefit of using *Bt* maize for rootworm control is huge because soil insecticides applied against this pest complex represent the largest single use of insecticides in the USA [108]. Based again on comparative farm-level data in adopting countries, Brookes and Barfoot [85] estimate that between 1996 and 2006 the use of *Bt* maize has been associated with an 8.2 million kg reduction in insecticide active ingredient and a 5.3% reduction in the EIQ. Reductions in the EIQ in other adopting counties have ranged from 60% in Canada, 33% in Spain, 26% in South Africa and 0% in Argentina. Estimated reductions in insecticide use in Argentina, South Africa, Spain and the USA are 0, 10, 63 and 8 %, respectively (see [86]). Also in contrast to *Bt* cotton, the ratio of benefit relative to EIQ in developing versus developed countries is estimated at 1:55.5 [85]. This reflects the large production of *Bt* maize in developed North American countries and the lack of benefit estimated for Argentina, a large maize-producing country. *Bt* maize was approved for commercial production in Brazil in 2008 and is being assessed for production in China, so the benefits in insecticide reduction are likely to grow in the future, particularly for developing nations.

The reader is directed to Fitt [87] and Qaim *et al.* [86] for a more detailed discussion of patterns of insecticide use and an overall economic assessment of *Bt* crops worldwide.

Conclusion/Summary

The production of *Bt* crops has grown from the cultivation of a few million hectares in three countries in 1996 to ≈42 million hectares in 20 countries as of 2007. *Bt* crops represent an important tactic in the IPM toolbox, providing effective control of certain key pests through host plant resistance, and contributing to the overall development of robust IPM systems. The assessment of environmental risk, including the evolution of resistance, genetic drift through gene flow, effects on soil structure and decomposition, effects on non-target organisms,

and shifts in pest management strategies, has been and continue to be topics of research and debate centred around transgenic crop technology. This review has focused on invertebrate non-target effects and changes in patterns of insecticide usage with the adoption of *Bt* crops. Over 360 original research articles have been published as of late 2008 that address non-target impacts of *Bt* crops on invertebrate organisms, mostly arthropods. General patterns from the extant literature were examined through meta-analyses of 135 laboratory-based non-target studies and 63 field-based studies. Collectively, laboratory studies have identified negative effects of *Bt* crops on several life-history characteristics within various taxonomic, functional and anthropomorphic groups when organisms are exposed directly to *Bt* proteins in plant tissues or through artificial diets. Some of these effects were expected based on the taxonomic affinity of the non-target organisms to the groups targeted by the *Bt* crops. In studies exposing natural enemies to *Bt* intoxicated prey or hosts via tri-trophic pathways, the quality of the prey or hosts was an important determinant of observed effect, with negative effects being associated with low quality, sublethally compromised prey or hosts. These effects disappear with unaffected, high quality prey or host indicating that host-mediated effects and not direct toxicity are involved. Collectively, few harmful non-target effects of *Bt* crops have been demonstrated in field studies, and in general greater levels of hazard have been identified in laboratory studies than have been manifested in the field. This may be explained partially by the fact that different taxa were examined in laboratory and field studies, protein exposures in laboratory studies were more controlled and often at levels in excess of field concentrations, and the fact that field abundance integrates and is governed by many interacting and uncontrollable factors, including prey and host abundance. The non-target effects of insecticides for control of target and non-target pests are much greater than *Bt* crops alone. Meta-analyses also point to gaps in knowledge of certain functional guilds, especially in field studies.

Field studies have demonstrated expected reductions in biological control function for specialist parasitoids of target pests in *Bt* maize systems, but no studies have shown any change in levels of predation of either target or non-target pests between *Bt* and non-*Bt* crops even when population densities of some predator species are lower in the *Bt* crop (e.g. [81]). Overall, the use of *Bt* crops has the potential to enhance the role of biological control in IPM systems.

The efficacy of *Bt* maize and cotton against major pest species has been associated with an estimated 136.6 million kg global reduction in insecticide active ingredient used between 1996 and 2006 (29.9% reduction). These benefits vary by country and region and are heavily weighted towards cotton production, which has historically been one of the largest users of insecticides in the world. Reduction in the vast quantities of soil insecticides used for control of corn rootworms is expected as coleopteran-resistant maize adoption grows in the future. Scientific interest and debate will continue in the area of environmental safety as new countries adopt GE crop technology and as new biotech crops are developed and cultivated.

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