

# Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control

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The area devoted to growing transgenic plants expressing insecticidal Cry proteins derived from *Bacillus thuringiensis* (*Bt*) is increasing worldwide. A major concern with the adoption of *Bt* crops is their potential impact on nontarget organisms including biological control organisms. Regulatory frameworks should advocate a step-wise (tiered) approach to assess possible nontarget effects of *Bt* crops. Laboratory and glasshouse studies have revealed effects on natural enemies only when *Bt*-susceptible, sublethally damaged herbivores were used as prey or host, with no indication of direct toxic effects. Field studies have confirmed that the abundance and activity of parasitoids and predators are similar in *Bt* and non-*Bt* crops. In contrast, applications of conventional insecticides have usually resulted in negative impacts on biological control organisms. Because *Bt*-transgenic varieties can lead to substantial reductions in insecticide use in some crops, they can contribute to integrated pest management systems with a strong biological control component.

Microbial insecticides containing  $\delta$ -endotoxins (Cry proteins) from *Bacillus thuringiensis* (*Bt*) have been used as an alternative to conventional chemical insecticides for almost 60 years. They are regarded as environmentally friendly and highly selective and only a few adverse effects of *Bt* products on nontarget species have been reported<sup>1</sup>. However, *Bt* products constituted <2% of the overall world insecticide market until the genes expressing Cry proteins were engineered into plants and commercialized in 1996 (ref. 2). Now, this once minor insecticide has become a major control tactic, with *Bt* maize and *Bt* cotton grown on 22.4 million hectares worldwide in 2004, a 25% increase from the previous year<sup>3</sup>.

Cry1-expressing maize and cotton are protected from attack by lepidopteran pests like corn borers (mainly *Ostrinia nubilalis*) in maize and the budworm-bollworm complex (*Heliothis virescens*, *Helicoverpa* spp., *Pectinophora gossypiella*) in cotton<sup>2</sup>. In 2003, *Bt* maize for control of *Diabrotica* spp. (corn rootworms) was commercialized, expressing the coleopteran-specific Cry3Bb toxin. Potato plants expressing Cry3Aa to control *Leptinotarsa decemlineata* (Colorado potato beetle) were on the market from 1996 but taken off in 2001 because of marketing issues, consumer concerns and the introduction of a novel insecticide that controls the beetle and aphids<sup>2</sup>. New transgenic plants expressing novel

Cry or other insecticidal proteins, stacked genes or fusion proteins will increase in importance in the coming years<sup>4</sup>.

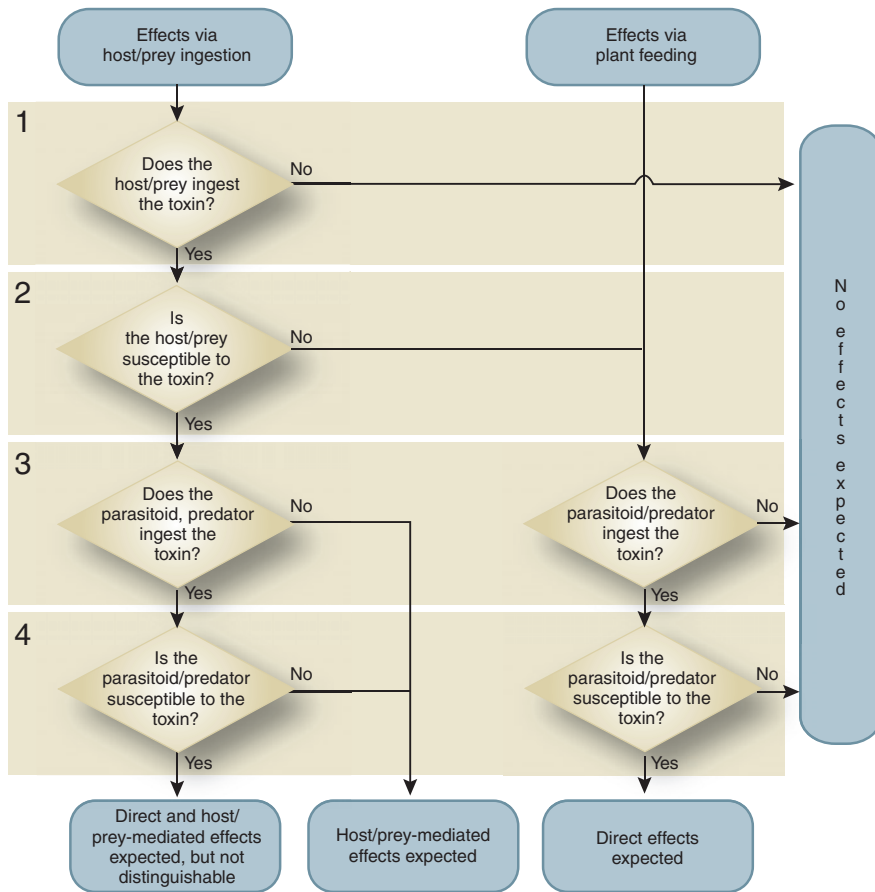
An advantage of insect-resistant transgenic plants is the reduced need for conventional insecticides, providing benefits for human health and the environment<sup>2,5</sup>. For example, in US cotton, the average number of insecticide applications used against the budworm-bollworm complex decreased from 4.6 in 1992–1995 to 0.8 in 1999–2001, largely owing to the introduction of *Bt* cotton<sup>6</sup>. In China, *Bt* cotton plants have provided a 60–80% decrease in the use of foliar insecticides<sup>5</sup>.

Detailed analysis of any potential effects of transgenic crops on the environment and human health is crucial before commercial release<sup>7–9</sup>. Key concerns are potential ecological consequences on nontarget organisms, including natural enemies of pests, which provide an economically important service in pest suppression. Most regulatory systems have adopted a comparative risk assessment approach in which the transgenic crop is compared with the corresponding nontransgenic crop<sup>8–10</sup>, taking into account nontransgenic agricultural practice, including conventional pest control in the case of *Bt* crops. This approach also considers similar constructs and traits and the variation among commercial varieties that have a history of safe use. The applicant is required to conduct a detailed analysis of the chemical composition as well as a detailed agronomic assessment under field conditions<sup>10–12</sup>. When this approach is used, any major changes due to plant transformation will be detected, and plants with such changes can be eliminated during variety selection that precedes the commercialization of any new variety (whether conventionally bred or transgenic)<sup>13</sup>. This selection process allows the risk assessment to focus on the intended change, the introduced trait<sup>13</sup>.

Ecological risk assessment for regulatory purposes is commonly organized in a step-wise (tiered) approach, where the assessment increases in complexity and realism based on the knowledge gained during previous tests<sup>14–16</sup>. In the case of nontarget risk assessment of insecticidal transgenic crops, early tier (laboratory) tests are conducted to determine whether an organism is susceptible to the toxin under worst case conditions, that is, organisms are directly exposed to high doses of the toxin. These tests are relatively simple in design, easy to standardize, repeatable and the results are easy to interpret. The risk assessment can stop here if risks under these worst case conditions are considered negligible or acceptable. However, if risks have been identified or cannot be ruled out with some certainty, higher tier tests will follow that expose nontarget organisms to the toxin under more realistic conditions. Eventually, specific questions regarding the impact of a toxin on certain indicator organisms or ecological functions such as biological control might have to be addressed in field studies. The major goals of this step-wise approach are to maximize the possibility that hazardous proteins are identified early, and to prevent excessive testing of substances that pose

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**Figure 1** Decision tree to determine nontarget effects of *Bt* plants on natural enemies (parasitoids and predators) feeding on either host/prey species or directly on plant material (e.g. pollen) in studies under confined conditions.

negligible hazard, so that higher tier testing can concentrate on plants that potentially do pose risks. Despite the adoption of this approach by regulatory agencies and the absence of indicated hazards of the Cry toxins on biological control agents, some regulatory agencies have required additional field studies to obtain further evidence of safety<sup>17</sup>. The tiered approach has been challenged by some<sup>18,19</sup> who propose long-term, complex ecological field assessments of transgenic plants before commercial release in any case. The rationale for this alternative approach is the notion that there may be more subtle potential direct and indirect effects due to the expression of a Cry protein and the plants' interaction with the environment.

Since their introduction ten years ago, several studies have addressed the potential effects of *Bt* crops on arthropod biological control agents (parasitoids and predators) in the laboratory or glasshouse as well as under semi-field and field conditions. In this article, we compile the data from these experiments and evaluate all published peer-reviewed studies. On the basis of the available information, we believe some general conclusions can be drawn that will improve future risk assessment of *Bt* plants and help direct the research agenda to ensure their safety.

**Laboratory and glasshouse studies**

Well-designed studies under confined conditions can be particularly suitable for understanding the cause of effects of insect-resistant transgenic plants on natural enemies. We ask four questions regarding exposure and susceptibility of host/prey species and natural enemies to the expressed insecticidal proteins (Fig. 1). If these questions were addressed

in multitrophic studies, effects on the higher trophic level could be predicted.

Direct effects of the toxin can be expected only if it is ingested and the parasitoid/predator is susceptible. This requires that the toxin is taken up through direct feeding on plants (e.g., pollen) or that it is passed on in a biologically active form by the host/prey. Indirect, host/prey-quality mediated effects can be expected if susceptible herbivores ingest the toxin. Affected herbivores are often smaller, develop slower, behave differently and/or have an altered tissue composition compared to healthy individuals. Such changes may influence the quantity of prey and their nutritional quality for the next trophic level. Effects on natural enemies include lethal effects, sublethal effects (for example, prolonged development, reduced weight), altered behavior (for example, reduced parasitization rate, changes in prey choice) or no effects at all.

Results from studies that do not address the questions proposed in Figure 1 are difficult to interpret because the cause of observed effects remains unclear. We have therefore determined whether the published studies on nontarget effects of *Bt* plants have addressed these questions (Tables 1 and 2). Knowledge gaps are, whenever possible, supplemented with additional information.

**Effects on predators**

Eleven studies have investigated the effects of *Bt* plants on predators in a plant-herbivore-predator (tritrophic) system (Table

1). Deleterious effects on mortality, longevity or development of the predator have only been reported in studies using lepidopteran larvae as prey that ingested the toxin and were susceptible to it. Such effects are expected (Fig. 1). Even so, using susceptible prey does not always cause the expected effects on the predators<sup>20,21</sup>. To separate direct from indirect effects, it should be determined whether the predator is ingesting the *Bt* toxin when feeding on the prey and whether it is susceptible. One can predict with some accuracy if a predator is exposed to the toxin based on its mode of feeding. Predators with chewing mouthparts, such as lady beetles (Coccinellidae), are expected to ingest the toxin when preying on *Bt*-fed arthropods because they ingest the gut where most of the toxin is located. For predators with sucking mouthparts such as predatory bugs (Hemiptera), this is likely but less clear because they might selectively feed on body tissues that do not contain the toxin. Until now, toxin uptake by predators has only been measured directly by means of an immunological test (enzyme-linked immunosorbent assay (ELISA)) in two studies<sup>22,23</sup>.

Studies using purified Cry proteins (produced in recombinant microbes, e.g., *Escherichia coli*) can help to elucidate insect susceptibility to the toxin<sup>16</sup> and can be used to assess the risk of *Bt* plants, provided studies are done to demonstrate the biological similarity of the microbial and plant-expressed proteins. Direct feeding studies have, for example, been conducted with larvae of *Chrysoperla carnea* (green lacewing). Feeding predator larvae with high concentrations of pure Cry1Ab and Cry1Ac toxin revealed no direct toxicity<sup>24,25</sup>, contradicting earlier results (F.B. and colleagues<sup>26</sup>). Recent studies indicate that

Table 1 Studies under confined conditions examining effects of *Bt* plants on predators and parasitoids via tritrophic interactions

Crop	Toxin	Predator/parasitoid species	Prey/host order	Does the prey/host ingest the toxin?	Is the prey/host susceptible to the toxin?	Does the predator/parasitoid ingest the toxin?	Reported effects	Reference	
<b>Predators feeding on prey reared on <i>Bt</i> plants</b>									
Maize	Cry1Ab	<i>Chrysoperla carnea</i> (Neuroptera; Chrysopidae)	Lepidoptera	✓	✓	✓ <sup>a</sup>	↓	28, 29	
			Acari	✓	0	✓ <sup>a</sup>	0	29	
			Hemiptera	0	? <sup>b,c</sup>	0	0	29	
			Hemiptera	0 <sup>d</sup>	? <sup>b,c</sup>	0	0	30	
		<i>Orius majusculus</i> (Hemiptera; Anthocoridae)	Thysanoptera	✓ <sup>e</sup>	?	?	0	31	
		<i>Poecilus cupreus</i> (Coleoptera; Carabidae)	Lepidoptera	✓	✓	✓	↓	22	
Cotton	Cry1Ac	<i>Orius tristicolor</i> (Hemiptera; Anthocoridae)	Lepidoptera	✓	✓ <sup>f</sup>	?	↓	20	
			<i>Geocoris punctipes</i> (Hemiptera; Lygaeidae)	Lepidoptera	✓	✓ <sup>f</sup>	?	↓	20
			<i>Nabis</i> sp. (Hemiptera; Nabidae)	Lepidoptera	✓	✓ <sup>f</sup>	?	0	20
			<i>Zelus renardii</i> (Hemiptera; Reduviidae)	Lepidoptera	✓	✓ <sup>f</sup>	?	0	20
Potato	Cry3Aa	<i>Hippodamia convergens</i> (Coleoptera; Coccinellidae)	Hemiptera	?	?	?	0	84	
			<i>Coleomegilla maculata</i> (Coleoptera; Coccinellidae)	Coleoptera	✓	✓	✓ <sup>g</sup>	0	21
			<i>Coccinella septempunctata</i> (Coleoptera; Coccinellidae)	Hemiptera	?	? <sup>c</sup>	?	0	85
			<i>Propylea quatuordecimpunctata</i> (Coleoptera; Coccinellidae)	Hemiptera	?	? <sup>c</sup>	?	0	86
Rice	Cry1Ab	<i>Cyrtorhinus lividipennis</i> (Hemiptera; Miridae)	Hemiptera	0 <sup>h</sup>	? <sup>b,c</sup>	?	0	36	
<b>Parasitoids developing in hosts reared on <i>Bt</i> plants</b>									
Maize	Cry1Ab	<i>Cotesia marginiventris</i> (Hymenoptera; Braconidae)	Lepidoptera	✓	✓	✓	↓	46	
			Lepidoptera	✓	✓	?	↓	87	
			Lepidoptera	✓	✓	✓	↓	45	
			Cry9C	<i>Parallorhagus pyralophagus</i> (Hymenoptera; Braconidae)	Lepidoptera	✓	✓ <sup>i</sup>	?	↓
Cotton	Cry1Ac	<i>Cotesia marginiventris</i> (Hymenoptera; Braconidae)	Lepidoptera	✓	✓	?	↓	89	
			<i>Copidosoma floridanum</i> (Hymenoptera; Encyrtidae)	Lepidoptera	✓	✓	?	↓	89
			<i>Campoletis chloridae</i> (Hymenoptera; Ichneumonidae)	Lepidoptera	✓	✓	?	↓	90
Potato	Cry3Aa	<i>Aphidius nigripes</i> (Hymenoptera; Braconidae)	Hemiptera	?	? <sup>k</sup>	?	↓	48	
Oilseed rape	Cry1Ac	<i>Diaeretiella rapae</i> (Hymenoptera; Braconidae)	Hemiptera	0 <sup>l</sup>	? <sup>b,c</sup>	0 <sup>l</sup>	0	91	
			Lepidoptera	✓	0 <sup>m</sup>	?	0 <sup>n</sup>	43, 44	
		<i>Cotesia plutellae</i> (Hymenoptera; Braconidae)	Lepidoptera	✓	✓	?	↓	44	

Information lacking in the original studies was extracted from other sources whenever possible (indicated by footnotes). ?, not determined; ✓, yes; 0, no/no effects; ↓, negative effects. <sup>a</sup>See ref. 23. <sup>b</sup>Not relevant as prey only ingest traces of toxin. <sup>c</sup>Prey unaffected by the *Bt* plant. <sup>d</sup>See refs. 29,32. <sup>e</sup>See F.B. and colleagues<sup>92</sup> for another thrips species. <sup>f</sup>See ref. 93. <sup>g</sup>Exposure of the predator not verified, but assumed due to feeding manner (chewing mouthparts). <sup>h</sup>Trace amounts of *Bt* toxin detected in prey honeydew. <sup>i</sup>Unpublished data, see ref. 88. <sup>l</sup>Plants also express cow-pea trypsin inhibitor (CpTI). <sup>k</sup>Hosts affected by *Bt* potato (ref. 47); whether this was caused by the *Bt* toxin is unknown. <sup>m</sup>See ref. 35. <sup>n</sup>Use of a Cry1Ac resistant host strain. <sup>o</sup>Inconsistent effects on male weight.

Cry1A toxins do not show specific binding to brush border membrane vesicles from the midgut of *C. carnea* larvae<sup>25</sup>, which is a prerequisite for toxicity<sup>27</sup>. When *C. carnea* larvae are fed lepidopteran larvae reared on Cry1Ab-expressing maize, our studies<sup>28,29</sup> indicate significantly prolonged larval development and increased mortality. However, from the binding studies noted above, it can be concluded that these effects were a consequence of sublethally intoxicated lepidopteran prey, apparently being of lower nutritional quality. This is supported by our study results that showed *C. carnea* larvae are not affected when feeding on unsusceptible *Tetranychus urticae* (two-spotted spider mite; J.R. & F.B. and colleagues<sup>29</sup>) containing large amounts of biologically active *Bt* toxin (J.R. & F.B. and colleagues<sup>23</sup>). *Chrysoperla carnea* larvae in the

field are known to mainly feed on aphids, whereas lepidopteran larvae are not considered an important prey. Because aphids are not harmed by *Bt* maize<sup>29,30</sup>, the risk that this crop poses for *C. carnea* larvae can be regarded as negligible<sup>16</sup>.

Whereas some tritrophic studies using sublethally affected prey have resulted in negative effects on the predator, this has not been observed for two species of predatory bugs<sup>20</sup> (*Nabis* sp. and *Zelus renardii*) and the lady beetle *Coleomegilla maculata*<sup>21</sup>. When fed unsusceptible prey containing Cry1Ab, F.B. and colleagues<sup>31</sup> have shown the pirate bug *Orius majusculus* is also not affected. The results suggest that these predators are not susceptible to the amount of toxin encountered in the prey. Predators attacking sap-feeding herbivores, such as aphids and planthoppers,

**Table 2 Studies under confined conditions examining effects of *Bt* plants on predators via direct feeding on plant material**

Crop	Toxin	Predator species	Plant food provided	Does the predator ingest the toxin?	Reported effects	Reference
Maize	Cry1Ab	<i>Chrysoperla carnea</i> (Neuroptera; Chrysopidae)	Pollen <sup>a</sup>	? <sup>b</sup>	0	94
		<i>Orius insidiosus</i> (Hemiptera; Anthocoridae)	Pollen	✓ <sup>c</sup>	0	94
			Silk <sup>a</sup>	?	0	95
		<i>Orius majusculus</i> (Hemiptera; Anthocoridae)	Leaf and pollen <sup>a</sup>	✓ <sup>c</sup>	0	96
		<i>Coleomegilla maculata</i> (Coleoptera; Coccinellidae)	Pollen <sup>a</sup>	✓ <sup>d</sup>	0	94
			Pollen	✓	0	39
	Cry3Bb	<i>Coleomegilla maculata</i> (Coleoptera; Coccinellidae)	Pollen <sup>a</sup>	✓	0	40
			Pollen <sup>a</sup>	✓	0	38
Potato	Cry3Aa	<i>Geocoris punctipes</i> (Hemiptera; Lygaeidae)	Leaf	? <sup>e</sup>	0	97
		<i>Geocoris pallens</i> (Hemiptera; Lygaeidae)	Leaf	? <sup>e</sup>	0	97
		<i>Orius tristicolor</i> (Hemiptera; Anthocoridae)	Leaf	? <sup>e</sup>	0	97
		<i>Nabis</i> sp. (Hemiptera; Nabidae)	Leaf	? <sup>e</sup>	0	97
		<i>Lygus hesperus</i> (Hemiptera; Miridae)	Leaf	? <sup>e</sup>	0	97
Rice	Cry1Ab	<i>Propylea japonica</i> (Coleoptera; Coccinellidae)	Anthers and pollen <sup>a</sup>	✓	0 <sup>f</sup>	41

Information lacking in the original studies was extracted from other sources whenever possible (indicated by footnotes). ?, not determined; ✓, yes; 0, no effects. <sup>a</sup>Plant food supplemented with prey to improve predator survival. <sup>b</sup>Toxin in pollen confirmed, level of exposure unclear due to feeding manner of *C. carnea* larvae and provision of additional food, but probably low. <sup>c</sup>See ref. 34 for *O. majusculus*. <sup>d</sup>See ref. 39. <sup>e</sup>Some feeding likely, but ELISA tests failed to confirm exposure. <sup>f</sup>Inconsistent effects on female longevity.

are unlikely to be exposed because the Cry proteins do not appear to be transported in the phloem<sup>32</sup>. To date, only trace amounts of the Cry proteins have been detected in sap-feeders on different *Bt*-transgenic events of maize<sup>29,32–34</sup>, oilseed rape<sup>35</sup> and rice<sup>36</sup>. Thus, predators preferentially feeding on aphids, such as most lacewings and lady beetles, are unlikely to be at risk.

Arthropod predator species can be omnivorous as they may also feed selectively on certain plant tissues<sup>37</sup>. In eight studies, pollen, silk and leaf tissue from *Bt* plants was provided to predators (Table 2). For some of the predators studied, toxin uptake was either measured directly by ELISA (F.B. and colleagues<sup>34</sup>), its presence concluded from positive control treatments<sup>38</sup> or addressed indirectly by confirming the uptake and digestion of the plant material<sup>39–41</sup>. Studies that did not confirm the ingestion of the *Bt* toxins do not allow one to draw conclusions about the sensitivity of the predator to the toxin. However, compared with control plant material, *Bt* transgenic plants had no adverse effects, as measured by different life-table parameters (e.g., longevity or fecundity), on any of the predator species studied. The laboratory studies therefore indicate that direct feeding on *Bt* plant material poses a negligible risk for these predators.

### Effects on parasitoids

The effects of *Bt* plants on hymenopteran parasitoids developing in herbivores reared on transgenic plants have been investigated in ten studies (Table 1). As expected from the decision tree (Fig. 1), effects on mortality, development, weight or longevity were observed in all cases where *Bt*-susceptible lepidopteran herbivores were used as hosts. This is not surprising, given that host-parasitoid relationships are usually tight and parasitoids are very sensitive to changes in host quality<sup>42</sup>. Parasitoids developing in *Bt*-fed larvae of a resistant strain of *Plutella xylostella* (diamondback moth) were not affected<sup>43,44</sup>. This confirms that host quality was most likely the cause of effect in the other studies. To date, two studies suggest that toxin uptake by parasitoid larvae can differ among species because certain larvae may avoid the gut, where most of the toxin is concentrated<sup>45,46</sup>.

Ashouri *et al.*<sup>47</sup> reported lower adult weight and behavioral changes (increased flight incidence) of *Macrosiphum euphorbiae* (potato aphid),

when reared on *Bt* potato and consequently a higher mortality and reduced adult weight of the parasitoid *Aphidius nigripes*<sup>48</sup>. It is possible that unintended effects related to plant transformation caused the changes in aphid performance because secondary effects of gene expression are well known to occur in potato, whether transgenic or conventionally bred<sup>49</sup>. However, direct effects of the *Bt* toxin cannot be excluded, as toxin uptake by aphids on *Bt* potato and susceptibility of both hosts and parasitoids to Cry3Aa have not been investigated.

### Semi-field studies

Semi-field studies confine the test organisms together with plants in cages under close-to-field conditions. This allows a more accurate estimation of ecological risks because the organisms face more realistic levels of toxin and routes of exposure than in laboratory studies. Compared with full-scale field investigations, semi-field studies have the advantage of being conducted with a larger number of replicates and under more controlled conditions. Semi-field studies may thus be a powerful tool to refine the risk assessment in cases where laboratory or glasshouse studies cannot rule out with sufficient certainty a potential risk for selected nontarget species<sup>50,51</sup>. In specific cases, they have even been suggested as alternatives to laboratory testing<sup>50</sup>.

Despite their usefulness, only three semi-field studies on the effects of *Bt* plants on natural enemies have been conducted. Field cage studies with *Bt* tobacco plants expressing low doses showed synergistic effects of the *Bt* toxin and a parasitoid resulting in increased mortality of *Heliothis virescens* (tobacco budworm) larvae<sup>52,53</sup>. On the other hand, studies with *Bt* sweet corn did not reveal conclusive results regarding the impact on predatory insects<sup>54</sup>.

### Field studies

More than 50 field studies varying greatly in size, duration and sampling effort have been conducted both in experimental and commercial fields to evaluate the impact of *Bt* crops on natural enemies (Table 3). Experimental field studies have only revealed minor, transient or inconsistent effects of *Bt* crops when compared with a non-*Bt* control (Table 3, columns 1 and 2). Exceptions were observed with specialist natural enemies, which were virtually absent in *Bt* fields due to the lack



of target pests as prey<sup>55</sup> or hosts<sup>56</sup> (Table 3, column 1, footnote c). Three studies in *Bt* crops revealed consistent reductions in the abundance of different generalist predators that were also associated with the reduced availability of lepidopteran prey<sup>57–59</sup>. However, in two of those studies<sup>58,59</sup>, declines were only reported for *Nabis* spp. (damselfly bugs) and at very low population levels making a robust assessment of these differences difficult (Table 3, column 1, footnote d).

*Bt* crops as a pest control measure are intended to replace or reduce applications of conventional insecticides commonly used in agriculture. Thus, a comparison of *Bt* crops with insecticides is a reasonable baseline for comparative risk assessment<sup>7–9</sup>. Several experimental field studies have included conventional insecticides as a treatment (Table 3). Direct comparison between *Bt* crops and insecticide treated non-*Bt* crops could not be performed because some of the studies have not statistically compared the two treatments. *Bt* crops and insecticides can therefore only be compared indirectly. Broad spectrum insecticides, such as pyrethroids and organophosphates, consistently reduced abundances of different groups of predators and hymenopteran parasitoids (Table 3, down arrows in column 3). Side effects of more selective insecticides such as indoxacarb (an oxadiazine) or spinosad (a macrolide) largely depended on the spray frequency whereas systemic insecticides (e.g., imidacloprid, a neonicotinoid) or *Bt* formulations were found to have no or little effect on natural enemies. Although some of the field studies lacked statistical power, because of limited replication and high variability in the data, and are affected by limitations in the spatial scale, they indicate clearly that nontarget effects of *Bt* crops are substantially lower than that of broad-spectrum insecticides. This has been confirmed by recent large-scale studies conducted in commercially managed *Bt* and non-*Bt* cotton fields in the United States<sup>60,61</sup> (Table 3).

Abundance and biodiversity data of natural enemies add to our understanding of agro-ecosystems, but most important for agriculture and the environment is the biological control function (predation and parasitization) that they provide. Surprisingly few studies have compared the function of natural enemies in *Bt* and conventional crops (Table 3, columns 2 and 4). Parasitization rates of naturally occurring or sentinel larvae of sensitive (target) lepidopteran species in *Bt* crops have often been reported to be lower compared with control plots<sup>53,62–64</sup> (Table 3, column 2, footnote c). This reduction in parasitism is not surprising, given that host populations were significantly decreased by the *Bt* crop. Predation rates on sentinel lepidopteran eggs or larvae were measured in *Bt* sweet corn and in *Bt* cotton. They did not differ between *Bt* and untreated non-*Bt* fields<sup>65–67</sup> but were significantly reduced by the application of broad-spectrum insecticides<sup>66</sup> (Table 3, column 4). Studies in commercially managed cotton fields revealed much higher predation rates in *Bt* cotton than in non-*Bt* fields, where more insecticides were applied<sup>61</sup> (Table 3, up arrow in column 2).

A six-year field study in *Bt* cotton on the abundance of 22 arthropod natural enemy taxa indicates that the exposure to the *Bt* toxin over multiple generations does not cause any chronic long-term effects<sup>57</sup>. A companion study during five years revealed no effects on the function of the natural enemy community toward pests targeted by the *Bt* crop as well as a nontarget herbivore, *Bemisia tabaci* (tobacco whitefly)<sup>65</sup>.

As yet, there is little evidence that secondary pest outbreaks in *Bt* crops have emerged as a problem requiring significant use of insecticides. This confirms that overall biological control function is not negatively affected by the use of *Bt* plants<sup>5,59,65</sup>. Observed decreases in aphid populations have even been linked to an increased biological control activity in *Bt* crops in the absence of insecticides<sup>68–70</sup>. The regional appearance

**Table 3 Field studies examining abundance of natural enemies and biocontrol function in *Bt* crops compared to non-*Bt* controls**

Crop	Toxin	<i>Bt</i> plants versus control plants <sup>a</sup>		Insecticide-treated plants versus control plants <sup>a</sup>		
		Natural enemy abundance	Biocontrol function	Natural enemy abundance	Biocontrol function	
<b>Experimental field studies</b>						
Maize	Cry1Ab	No consistent effects	ND	ND	ND	95 <sup>b</sup>
		No consistent effects	↓ <sup>c</sup>	ND	ND	62
		No consistent effects	ND	Pyrethroid↓; microbial insecticide ( <i>Bt</i> formulation), no consistent effects	ND	75
		↓ <sup>d</sup>	ND	ND	ND	58
		No consistent effects	ND	ND	ND	98, 99
		No consistent effects	ND	ND	ND	100
		No consistent effects	ND	ND	ND	101
		No consistent effects	ND	Pyrethroid, no consistent effects	ND	102
		No consistent effects	ND	ND	ND	103
		ND	↓ <sup>c</sup>	ND	ND	64
	No consistent effects	ND	Pyrethroid↓	ND	104	
	No consistent effects	No consistent effects	Pyrethroid↓; oxadiazine↓; macrolide↓; microbial insecticide ( <i>Bt</i> formulation), no consistent effects	Pyrethroid↓; oxadiazine↓; macrolide, no consistent effects	66	
	No consistent effects	No consistent effects	ND	ND	105	
	No consistent effects	ND	ND	ND	94	
	↓ <sup>c</sup>	ND	ND	ND	56	
	ND	No consistent effects	ND	ND	106	
	No consistent effects	ND	Neonicotinoid <sup>e</sup> , no consistent effects	ND	76	
	No consistent effects	↓ <sup>c</sup>	ND	ND	63	
	No consistent effects	ND	ND	ND	107	
	No consistent effects	ND	Pyrethroid↓	ND	108	
No consistent effects	ND	ND	ND	54		
Cry3Bb	ND	ND	Pyrethroid <sup>e</sup> no consistent effects, neonicotinoid <sup>e</sup> , no consistent effects	ND	109	

Table 3 (continued)

Crop	Toxin	<i>Bt</i> plants versus control plants <sup>a</sup>		Insecticide-treated plants versus control plants <sup>a</sup>		
		Natural enemy abundance	Biocontrol function	Natural enemy abundance	Biocontrol function	
Cotton	Cry1Ab+VIP3A Cry1Ab Cry1Ac	No consistent effects	ND	No consistent effects <sup>f</sup>	ND	110
		No consistent effects	ND	Pyrethroid↓; neonicotinoid <sup>e</sup> , no consistent effects	ND	70, 111
		↓ <sup>g</sup>	ND	Pyrethroid↑↓ <sup>h</sup>	ND	112
		No consistent effects	ND	ND	ND	79
		No consistent effects	ND	ND	ND	113
		No consistent effects	ND	Pyrethroid, organophosphate↑↓ <sup>i</sup>	ND	114
		No consistent effects	ND	ND	ND	115
		No consistent effects	ND	Organophosphate↓	ND	116
		No consistent effects	ND	ND	ND	117
		ND	No consistent effects	ND	Chlorinated hydrocarbons, no consistent effects	118
		No consistent effects	ND	Pyrethroid, organophosphate↓	ND	119
		↓	ND	Pyrethroid, organophosphate↓	ND	57
		ND	No consistent effects	ND	ND	65
		No consistent effects	No consistent effects	ND	ND	67
Potato	Cry1Ac/Cry2Ab Cry3Aa	↓ <sup>d</sup>	ND	↓ <sup>i</sup>	ND	59
		No consistent effects	ND	Pyrethroid↓, organophosphate↓	ND	69
		No consistent effects	ND	Organophosphate↓	ND	116
		No consistent effects	ND	Pyrethroid↓, organophosphate <sup>d</sup> , no consistent effects; microbial insecticide ( <i>Bt</i> formulation), no consistent effects	ND	120
		No consistent effects	ND	Pyrethroid↓; pyrazole↓	ND	85
		No consistent effects	ND	Pyrethroid↓; organophosphate <sup>e</sup> , no consistent effects; microbial insecticide ( <i>Bt</i> formulation), no consistent effects	ND	68
		↓ <sup>c</sup>	ND	ND	ND	55
		No consistent effects	ND	ND	ND	121
		No consistent effects	ND	ND	ND	122
		No consistent effects	↓ <sup>c</sup>	ND	ND	53
Tobacco	Cry1Ac Cry1Ab	ND	No consistent effects	ND	ND	52
		ND	No consistent effects	ND	ND	123
		No consistent effects	ND	ND	ND	124
		No consistent effects	ND	ND	ND	124
Eggplant	Cry3B	No consistent effects	ND	ND	ND	124
<b>Studies in commercial fields</b>						
Cotton	Cry1Ac		↑	↑		61
			↑↓	ND		60

In experimental field studies, untreated non-*Bt* (control) crops were compared with *Bt* crops and with different insecticides. Studies in commercial fields compared *Bt* crops and non-*Bt* crops both under commercial production conditions (that is, both receiving insecticides according to the conventional practice). <sup>a</sup>Effects are noted as: (↓) consistent negative effects on one or more taxa; (↑) consistent positive effects; ND, not determined. <sup>b</sup>Data from Cry1Ab and Cry1Ac expressing plants pooled. <sup>c</sup>Natural enemy for which a consistent effect was observed is a specialist antagonist of the target pest. <sup>d</sup>Decrease in *Nabis* spp., but at very low population levels. <sup>e</sup>Systemic insecticides, seed treatment or in-furrow application. <sup>f</sup>Different transformation events and various soil insecticides have been tested. <sup>g</sup>Fewer immature stages of lacewings, probably due to a lower plant attractancy for ovipositing females. <sup>h</sup>Some families of parasitic hymenopterans increased after pesticide application. <sup>i</sup>Increased ladybird and lacewing abundance due to increased aphid populations after insecticide application. <sup>j</sup>Insecticides used according to conventional practice.

of mirids (Miridae) and stinkbugs (Pentatomidae) as pests in *Bt* cotton has not been attributed to a disturbance of the biological control function but to the fact that those pests had earlier been under control from broad spectrum insecticides applied against lepidopteran pests<sup>71,72</sup>. This is regarded as a risk of insecticidal transgenic crops by some authors<sup>19</sup>. However, this phenomenon is often observed when broad-spectrum pest control is replaced by a more specific and targeted method, whether chemical, biological or transgenic.

### Conclusions and recommendations

Although the effects of *Bt* plants have been investigated for a limited number of predator and parasitoid species under confined conditions, two general trends are evident: first, there is no indication of direct effects of *Bt* plants on natural enemies, either in direct plant feeding assays or when natural enemies have been provided with unsusceptible hosts/prey containing the Cry toxin; second, adverse effects on

natural enemies have been observed only in studies with susceptible herbivores as hosts/prey. These effects are most likely due to reduced host/prey quality. One exception to this is the reported impact of *Bt* potatoes on an aphid parasitoid<sup>48</sup>, which was probably due to affected aphid hosts<sup>47</sup>.

The data available to date do not allow us to predict the safety of other insecticidal proteins that might be expressed in future crops. Compounds, such as lectins or protease inhibitors, have different modes of action compared with Cry proteins and are known to cause effects on biological control agents<sup>73</sup>. However, if the questions proposed in **Figure 1** would be addressed in future studies, the cause of observed effects could be elucidated, thus reducing uncertainties in the risk assessment of novel insecticidal transgenic plants. It is obvious that not all four questions have to be answered in every study. For example, if herbivores do not ingest the toxin, their sensitivity cannot be assessed but the exposure of the natural enemy can be regarded as being zero.

Laboratory studies have been criticized as being ecologically unrealistic and not able to predict large-scale, long-term effects<sup>18,19,74</sup>. However, the *C. carnea* example shows that well-designed laboratory studies can, with a high degree of certainty, exclude negative effects in the field. *C. carnea* larvae were found not to be sensitive to Cry1 toxins<sup>24,25</sup>. However, our studies<sup>28,29</sup> have shown *C. carnea* is affected when fed with *Bt* maize-reared lepidopteran larvae but not when they have received *Bt* maize-reared aphids<sup>29,30</sup>, their predominant prey. Collectively, these studies indicated that *Bt* maize poses no or a negligible risk for *C. carnea*<sup>16</sup>, which has been confirmed by a number of field studies<sup>56,57,62,75,76</sup>. This example also shows that care must be taken when designing laboratory studies to ensure that the results are relevant for the field situation. Predators should not be forced to feed exclusively on prey species that they do not ordinarily attack in the field because this can lead to ecologically irrelevant results<sup>16</sup>.

The reduction of pests is the obvious goal of any crop protection method. Therefore, it is questionable if studies using herbivores that are targeted by the toxin in *Bt* crops are relevant to assess the risks for natural enemies. Surviving, sublethally affected herbivores are likely to be altered in nutritional quality and this will have potential consequences for higher trophic levels. Most predators are able to feed on a prey spectrum and they can switch to alternative prey when target pests are scarce. Specialist predators and parasitoids are likely to be most sensitive to changes in host/prey quality or quantity. Negative effects on natural enemies that depend on the target pests have been listed as one of the risks of insecticidal transgenic crops by some authors<sup>19</sup>. However, such effects on natural enemies, that are a consequence of an intended effect (that is, control of a pest), are common for all pest control methods, including insecticides, biological control and conventional host-plant resistance<sup>77,78</sup> and are generally not regarded as a risk. Although region-wide suppression of target pests could indeed cause a regional loss of a specific natural enemy, this is likely to be a rare event given that the natural enemy is usually able to survive on host/prey populations that thrive in the non-*Bt* refuge or on alternative host plants<sup>79,80</sup>.

For ecological risk assessment one needs to evaluate if statistically significant findings from laboratory, semi-field and field studies are of ecological relevance. Various risk assessment frameworks, including Annex III of the Cartagena Protocol on Biosafety<sup>81</sup>, refer to the importance of assessing risks of transgenic crops in the context of the risks posed by the conventional agricultural practice<sup>7-9</sup>. Insecticide treatments, the most dominant current pest control strategy, should be considered as one baseline for risk assessment. Alternative control methods (e.g., biological control by released natural enemies) or no pest control should be included in comparison only if they are of practical relevance. Field studies to date have revealed that predator and parasitoid abundance and biological control function are similar in *Bt* and untreated non-*Bt* crops, whereas broad-spectrum insecticides generally drastically reduce natural enemy populations as well as the biological control function.

There is an urgent need to establish guidelines and protocols for non-target risk assessment of transgenic crops. This includes the determination of test endpoints (for example, mortality) with threshold-values to establish acceptable effect sizes below which the natural pest control function is not impaired. For example, in insecticide testing, mortalities of up to 50% in standardized studies under confined conditions are considered to be acceptable<sup>82</sup>. For biological control agents, it has been suggested that they have to cause more than 40% mortality on a non-target species in the field to lead to permanent, significant, population effects<sup>83</sup>. Although the methods and thresholds applied for risk evaluation of insecticides and biological control agents should not be adopted for transgenic plants without a review, they give a first indication of the magnitude of accepted effects with other pest control methods. Recent

data from *Bt* cotton suggest that an average decrease of about 20% in some predatory species do not seem to be ecologically meaningful in terms of the biological control function of the natural enemy community<sup>57,65</sup>.

However, even after the most thorough risk assessment, uncertainties will remain<sup>15</sup>. For this reason the European Union requires post-market monitoring of ecological effects caused by transgenic crops<sup>10,12</sup>. The published large-scale studies in commercial *Bt* cotton fields have not revealed any unexpected nontarget effects other than subtle shifts in the arthropod community caused by the effective control of the target pests<sup>60,61</sup>. These findings confirm the original conclusions drawn from the risk assessment that preceded the commercial release of *Bt* crops.

The data compiled in this study emphasize the importance of well-designed, ecologically relevant studies conducted under confined conditions and the use of a systematic tiered approach for nontarget risk assessment. They furthermore provide evidence that *Bt* crops grown today are more specific and have fewer side effects on parasitoids and predators than most insecticides currently used. For crops like cotton<sup>5</sup> and sweet corn<sup>66</sup>, it has been shown that insecticide applications can be reduced substantially by the adoption of *Bt*-transgenic varieties. In these and other systems, the *Bt* technology can contribute to natural enemy conservation and be a useful tool in integrated pest management systems.

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#### COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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