Impact of Insect-resistant Transgenic Crops on Above-ground Non-target Arthropods

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Abstract
Genetically modified (GM) maize and cotton varieties that express insecticidal proteins derived from Bacillus thuringiensis (Bt) have become an important component in integrated pest management programmes worldwide. A number of other crops producing Bt toxins, or more broad-spectrum insecticidal proteins, are likely to enter commercial production in the near future. Because insecticidal GM crops target insect pests, an important part of the environmental risk assessment is their potential impact on nontarget arthropods. Those include protected species and organisms providing important ecological services such as biological control of herbivores. Non-target arthropods can be exposed to the plant-produced insecticidal proteins through various routes, but mainly by feeding on GM plant material or herbivores that have consumed GM plant material. The Bt proteins produced in today’s GM plants appear to have no direct effects on natural enemies due to their narrow spectrum of activity. Furthermore, it has become clear that in crop systems where the deployment of Bt varieties has led to a decline in insecticide use, biological control organisms have benefited significantly. Future GM plants that produce broader-spectrum insecticidal proteins will need to be assessed for their potential non-target effects case by case and compared to the impact of the conventional pest control methods that they replace.

Keywords
biological control, Bt crops, exposure assessment, Lepidoptera, predators, parasitoids, risk assessment

Disciplines
Agronomy and Crop Sciences | Entomology | Plant Breeding and Genetics | Systems Biology

Comments

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Keywords: Biological control, Bt crops, exposure assessment, Lepidoptera, predators, parasitoids, risk assessment

Summary
Genetically modified (GM) maize and cotton varieties that express insecticidal proteins derived from Bacillus thuringiensis (Bt) have become an important component in integrated pest management programmes worldwide. A number of other crops producing Bt toxins, or more broad-spectrum insecticidal proteins, are likely to enter commercial production in the near future. Because insecticidal GM crops target insect pests, an important part of the environmental risk assessment is their potential impact on non-target arthropods. Those include protected species and organisms providing important ecological services such as biological control of herbivores. Non-target arthropods can be exposed to the plant-produced insecticidal proteins through various routes, but mainly by feeding on GM plant material or herbivores that have consumed GM plant material. The Bt proteins produced in today’s GM plants appear to have no direct effects on natural enemies due to their narrow spectrum of activity. Furthermore, it has become clear that in crop systems where the deployment of Bt varieties has led to a decline in insecticide use, biological control organisms have benefited significantly. Future GM plants that produce broader-spectrum insecticidal proteins will need to be assessed for their potential non-target effects case by case and compared to the impact of the conventional pest control methods that they replace.

Introduction

Growers use various methods to control insect pests, which generally include host-plant resistance, cultural control methods (e.g. crop rotation), biological control and chemical insecticides. Genetic modification, however, has produced a new type of control, which can be considered host-plant resistance or a type
of chemical insecticide. Genetically modified (GM) plants are produced by transferring specific genes into the genome of a crop plant. For example, bacterial genes that encode insecticidal proteins have been introduced into cotton and maize. Such insect-resistant, genetically modified (IRGM) plants are commonly used today and have proven to be effective against devastating insect pests worldwide (Hellmich et al., 2008; Naranjo et al., 2008; Qaim et al., 2008).

Crystal (Cry) proteins derived from the soil bacterium Bacillus thuringiensis (Bt) are known for their narrow spectrum of activity and long history of safe use as microbial Bt products (Glare and O’Callaghan, 2000). Use of IRGM maize and cotton that express cry genes has grown steadily worldwide since their introduction in 1996, reaching 42.1 million ha in 2007 (James, 2007). The highest commercial adoption rates of Bt maize varieties (percentage of maize acreage) are in Argentina (63%) and in the USA (50%); James, 2007). Commercial Bt maize varieties produce a Lepidoptera-specific toxin (Cry1 or Cry2) that targets stem borers such as the European corn borer (Ostrinia nubilalis; Lepidoptera: Crambidae), a Coleoptera-specific toxin (Cry3 or Cry34/35) for the control of corn rootworms (Diabrotica spp.; Coleoptera: Chrysomelidae) or both (Hellmich et al., 2008). The latter are called stacked traits since the two Cry proteins target different insect pests. Potato plants that produce Cry3Aa to control the Colorado potato beetle, Leptinotarsa decemlineata (Coleoptera: Chrysomelidae), were introduced commercially in 1996 but withdrawn in 2001 due to marketing issues, consumer concerns and the introduction of a novel insecticide that controls beetles as well as aphids (Kaniewski and Thomas, 2004; Grafius and Douches, 2008).

Several countries have adopted Bt cotton varieties. The largest proportional adoption is in Australia, where in 2007 95% of the cotton acreage was planted to Bt varieties, followed by the USA (72%), China (69%) and India (66%); James, 2007). Current Bt cotton varieties produce Lepidoptera-specific Cry proteins targeting the budworm-bollworm complex, i.e. Heliolphis virescens, Helicoverpa spp. (Lepidoptera: Noctuidae) and Pectinophora gossypiella (Lepidoptera: Gelechidae; Naranjo et al., 2008). Cotton plants that produce Cry1Aa and cowpea trypsin inhibitor (CpTI) are commercially grown in China (Wu and Guo, 2005; see also Chapter 16, this volume). There is published evidence that the insecticidal activity of Bt Cry proteins is enhanced when they are used in combination with protease inhibitors (PIs; Macintosh et al., 1990). This claim, however, has not been observed in cotton producing both CpTI and Cry1A infested by the main target pest, Helicoverpa armigera (Lepidoptera: Noctuidae; Wu and Guo, 2005). Besides single-gene cotton (expressing cry1Ac), plants that express two Bt genes (cry1Ac and cry2Ab, called pyramids because they target the same pest complex) have recently been released to provide an even more efficient and reliable control of the Lepidoptera pest complex and to delay the evolution of resistance in the target pest populations (Stewart et al., 2001; Greenplate et al., 2003; Naranjo et al., 2008).

New GM plants producing other Cry proteins, vegetative insecticidal proteins (Vips) or combinations of Cry proteins with non-Cry toxins are likely to be released soon (Bates et al., 2005; Malone et al., 2008; see also Chapter 6, this volume). Genes expressing insecticidal proteins like PIs, α-amylase inhibitors,
biotin-binding proteins or lectins have also been introduced into crop plants for controlling pests that are not susceptible to known Cry proteins [Jouanin et al., 1998; Ferry et al., 2004; Malone et al., 2008]. With the exception of the Bt-CpTI cotton plants grown in China, these new types of GM plants have not been commercialized. Some of these novel proteins have a broader spectrum of activity than the Bt Cry proteins and Vips, and consequently they have a higher potential to affect non-target organisms (O’Callaghan et al., 2005; Romeis et al., 2008a).

IRGM crops can be used in integrated pest management (IPM) programmes to control pest populations economically and in ways that promote sustainability [Romeis et al., 2008b]. Of particular interest in this respect is the impact of IRGM crops on non-target organisms. Agronomically, a non-target organism is any organism associated with the crop that does not cause economical damage. Because IRGM crops target insect pests, an important part of the environmental risk assessment is their potential impact on non-target arthropods. In particular, arthropods that provide ecosystem services like biological control, pollination or decomposition should not be harmed. Also non-target arthropods not directly associated with the IRGM crop, such as lepidopteran larvae that feed on non-crop host plants in or near the field, should be considered. Many Lepidoptera are Red List (i.e. threatened) species or species of aesthetic or cultural value, like the Monarch butterfly, Danaus plexippus (Lepidoptera: Danaidae), in the USA.

In this chapter, we describe how non-target organisms can be exposed to insecticidal proteins expressed by IRGM plants. We provide an overview of direct and indirect effects of IRGM plants on non-target arthropods including changes in agricultural practice. The data are discussed in the context of environmental risk assessment. We focus on above-ground arthropods, especially biological control organisms (predators and parasitoids) and non-target Lepidoptera. Pollinators and soil arthropods are covered elsewhere (see Chapters 9 and 10, this volume).

### Non-target Effects Caused by the Insecticidal Proteins

#### Routes of exposure

Insecticidal proteins expressed by current IRGM plants target the insect midgut, and thus they need to be ingested to be effective. Non-target organisms are exposed to the insecticidal protein if they feed on the GM plant tissue or consume organisms that have eaten the toxin. Consequently, fewer non-target species are likely to be exposed to the active ingredient via GM plants than via spray insecticides. On the other hand, most IRGM crops express the active ingredient through most of the growing season, which may lengthen exposure compared with the applications of rapidly degraded chemical insecticides; potentially long exposures must be considered in environmental risk assessments for IRGM crops. The insecticidal proteins may be transferred from herbivores to predators and parasitoids; so this must be assessed when evaluating...
exposure of non-target arthropods. The principal routes of exposure for herbivores and natural enemies are outlined in Fig. 8.1.

**Exposure through plant feeding**
The most obvious way to ingest plant-expressed insecticidal protein is by feeding directly on the GM plant (Fig. 8.1, route 1). This route is particularly relevant for most non-target herbivores. However, exposure to the insecticidal protein depends on a great extent on both the herbivores’ mode of feeding and on the site and time of protein expression in the plant (Dutton et al., 2003). Chewing herbivores, such as caterpillars or beetles, generally ingest the insecticidal protein. Similarly, herbivores with piercing-sucking mouthparts that feed on epidermal or mesophyll cells, such as thrips, mirid bugs or spider mites, are exposed to the insecticidal protein (Dutton et al., 2002, 2004; Obrist et al., 2005, 2006a). This is in contrast to phloem-feeders such as aphids. Aphids have received much attention since they are common in most crop systems and provide food for many entomophagous arthropods. Different aphid species feeding on Bt maize, cotton and oilseed rape varieties were reported to ingest no, or at most, trace amounts of Bt protein. Thus, Cry proteins apparently are not transported in the plant’s phloem sap (Raps et al., 2001). Even though Bt protein has occasionally been detected in aphids (e.g. Zhang et al., 2006; Burgio et al., 2007), these incidences can be explained by Bt contamination, either with toxin-containing herbivores (e.g. spider mites), faeces of the same or tiny fractions of plant material. For example, the faeces of thrips feeding on cry1Ab-expressing maize plants were found to contain 10-100 times higher Bt toxin concentrations than the fresh plant material (Obrist et al., 2005). This

**Fig. 8.1.** Exposure pathways. Routes through which non-target arthropods can be exposed to insecticidal proteins expressed by IRGM plants.
demonstrates that very little contamination can easily produce false positives, especially for samples containing little or no Bt toxin, like aphids.

Many predators are facultative herbivores (mainly of pollen, nectar and plant juice), while parasitoids primarily feed from (extra-)floral nectaries (Dickey, 1999; Coll and Guershon, 2002; Wackers, 2005). Insecticidal proteins have not been found in the (extra-)floral nectar of current IRGM cotton varieties. Cry protein production in pollen, however, varies with the promoter of the cry gene and the transformation event. Commonly grown Cry1Ab maize events Br11 and MON810 produce very small amounts of toxin in the pollen (<1/100 of that of leaves), which contrasts with the earlier IRMA maize variety Event Br176 that was designed to produce high levels of Cry protein in pollen (similar to that of leaves; Dutton et al., 2003). Bt protein concentrations in pollen of current corn rootworm (Diabrotica spp.)-resistant Bt maize events MON863 and MON88017 are also close to the concentration measured in leaves (Monsanto Company, 2003, 2004); on the other hand, in rootworm-resistant MR604 maize, the concentration of Bt protein in pollen is at least 25 times lower than the concentration in leaves (Raybould et al., 2007).

In the case of wind-pollinated plants such as maize, pollen can expose non-target organisms to the insecticidal protein both within and beyond the crop (Fig. 8.1, route 2). Organisms exposed within the crop include many biological control agents that actively seek pollen as a food source. An example is the ladybird beetle, Coleomegilla maculata (Coleoptera: Coccinellidae), which is abundant during maize anthesis and is known to consume maize pollen (Cotrell and Yaegar, 1995; Lundgren et al., 2004, 2005). Other pollen feeders that were found to contain Cry1Ab when present in a Bt maize field during anthesis include Orius spp., (Hemiptera: Anthocoridae) and adult Chrysoperla carnea (Neuroptera: Chrysopidae; Obrist et al., 2006b; Li et al., 2008). Furthermore, organisms can passively ingest pollen deposited on their host plant. For example, larvae of butterfly species may be exposed to the Bt toxins both within the maize crop itself and in areas adjacent to the crop. The most prominent example is the Monarch butterfly where the larvae feed specifically on milkweed that commonly occurs in and near maize fields (Lesse and Obrzycki, 2000; Oberhauser et al., 2001). Passive pollen feeding has also been reported for web-building spiders that ingest pollen caught in their web when recycling the web (Ludy and Lang, 2006).

**Exposure through honeydew**

In contrast to current Bt crops, certain experimental plants expressing lecins or Pls are known to transport insecticidal proteins in the phloem. When sap-feeding Sternorrhyncha (Hemiptera), such as aphids, feed on such plants, the insecticidal proteins are likely to appear in their honeydew (Shi et al., 1994; Kanrar et al., 2002; Rahbe et al., 2003a). This is because these insects typically possess low proteolytic activity in the gut and thus the foreign proteins are unlikely to be proteolytically degraded (Srivastava and Auclair, 1963; Rahbe et al., 1995; Fig. 8.1, route 3). Similar observations have been reported for secondary plant compounds (Wink and Römer, 1986; Malcolm, 1990). Honeydew is an important food source for many arthropods including predators, parasitoids, pollinators and adult herbivores (Wackers, 2005) and could potentially expose many non-target
organisms to the insecticidal protein (Romeis et al., 2003; Hogervorst et al., 2009). Honeydew appears to be a minor route of exposure of non-target organisms to current Bt proteins, but it may become important for plants with enhanced resistance towards aphids, such as those expressing certain lectins.

**Exposure through predation or parasitization**

The major route of exposure to entomophagous arthropods is through their prey or hosts (Fig. 8.1, route 4). Usually the prey or host is an herbivore that feeds on the IRGM plant, but it can also be another entomophagous species. In either case, exposure through prey or host organisms is highly variable and difficult to predict for a number of reasons. As described above, the level at which different herbivores ingest the insecticidal protein depends on the site and time of toxin expression in the plant, the mode of feeding of the herbivore and the amount of plant material they ingest. Furthermore, the amount of toxin in different herbivores depends on the rate of proteolytic degradation and excretion; consequently, the amount varies considerably among species, even when feeding on the same plant. For example, the following arthropods all feed on mesophyll cells of Bt maize but contain different amounts of Cry protein: spider mites (toxin level similar to Bt maize leaf, 1X), thrips (1/6X) and leafhoppers (1/30X; Dutton et al., 2004). For other examples on Bt maize, see Dutton et al. (2002) and Obrist et al. (2006a,b). Highly variable Cry protein concentrations among different herbivore groups also have been reported from Bt cotton (Head et al., 2001; Torres et al., 2006; Torres and Ruberson, 2008). Even within one species, toxin contents may vary considerably among life stages (Howaki et al., 2003; Obrist et al., 2005). For example, while feeding, larval and adult stages of *Frankliniella tenuicornis* (Thysanoptera: Thripidae) contained relatively high Bt protein concentrations; non-feeding prepupae, pupae and newly emerged adults showed very low toxin levels (Obrist et al., 2005). Immobile stages are easier to prey on by predators than moving larvae or adults, so exposure levels could be overestimated if only a mean toxin content of all stages is considered (Obrist et al., 2005). The amount of insecticidal protein contained within an insect also depends on the nature of the protein. Studies on larvae of *C. carnea*, a species that is unable to excrete faeces during the larval stage, have revealed that Cry1Ab is degraded within a few days (Romeis et al., 2004), while the lectin GNA remained undegraded (Hogervorst et al., 2006). Another example is provided by Christeller et al. (2005) who studied the fate of aprotinin and avidin expressed by GM tobacco plants after ingestion by *Spodoptera litura* (Lepidoptera: Noctuidae). While both proteins were detected in the frass of *S. litura*, the biological activity of avidin was retained while around 90% of the trypsin-binding activity of aprotinin was lost. The apparent complexity in calculating the exact level at which non-target organisms are exposed to insecticidal protein produced by an IRGM plant has lead to simplifications for risk assessment by making conservative assumptions (see below).

**How behaviour affects exposure**

Other factors that can influence the exposure of non-target organisms to insecticidal proteins expressed by GM plants are behavioural responses to the plant...
itself or (in the case of natural enemies) to herbivores that have fed on the plant. There is evidence that Bt crops and non-Bt crops are similarly attractive to arthropods. For example, egg deposition of pest Lepidoptera on maize and cotton does not appear to differ between Bt and non-transformed varieties (Orr and Landis, 1997; Hellmich et al., 1999; Pilcher and Rice, 2001; Liu et al., 2002; Mellet et al., 2004; Pilcher et al., 2005; Torres and Ruberson, 2006; van den Berg and van Wyk, 2007). Likewise, D. plexippus oviposition on milkwheat plants with surface-deposited pollen from Bt and non-Bt maize hybrids was similar (Tschenn et al., 2001). However, insect behaviour can influence exposure in other ways. For example, there is evidence from flight chamber experiments that female D. plexippus oviposit less often on milkwheat plants with maize pollen than those with no pollen (Tschenn et al., 2001). This potentially restricts larval exposure to high (Bt) maize pollen densities.

It is well established that entomophagous arthropods, and parasitic wasps in particular, use volatiles that are emitted by herbivore-damaged plants for host or prey location (Vet and Dicke, 1992). Different hymenopteran parasitoids were found to respond similarly to the odours emitted by Bt and non-Bt plants that were damaged equally by mechanical wounding, or by Bt-resistant Lepidoptera larvae (Schuler et al., 1999, 2003; Turlings et al., 2005; Dean and De Moraes, 2006). A similar study with GNA revealed that the presence of the toxin in sugarcane plants does not affect the host location behaviour of the parasitoid Cotesia flavipes (Hymenoptera: Braconidae; Sétamou et al., 2002). In contrast, sensitive Lepidoptera larvae caused less damage to Bt plants than to control plants, which decreased their attractiveness to parasitoids (Schuler et al., 1999, 2003; Dean and De Moraes, 2006). This behavioural response has two important implications. First, parasitoids may avoid Bt-fed sensitive Lepidoptera larvae and thus hosts in which their offspring is unlikely to develop. Second, Bt-resistant larvae that cause severe feeding damage to the GM plants are highly attractive and more likely to be located and attacked with potential positive consequences for managing insect resistance. Recently, it has been established that Bt maize producing Cry3Bb1 for the control of Diabrotica spp. and non-transformed maize plants have the same ability to emit β-caryophyllene, a volatile shown to attract entomopathogenic nematodes, after chemical induction (Meissle et al., 2008).

Herbivores feeding on Bt crops may behave, look or taste differently, which may influence predator behaviour and potentially affect prey consumption. Choice experiments with the parasitoid Campsotettis sonorensis (Hymenoptera: Ichneumonidae) have shown no discrimination of Bt maize-fed S. frugiperda (Lepidoptera: Noctuidae) larvae despite larvae fed non-transformed maize being substantially larger (Sanders et al., 2007). Choice experiments conducted with larvae of C. carnea and adult Pterostichus madidus (Coleoptera: Carabidae) revealed that the predators avoid sublethally affected Bt-fed Lepidoptera larvae as prey (Meier and Hilbeck, 2001; Ferry et al., 2006). When Bt-resistant larvae were offered to P. madidus, however, the beetles did not differentiate between prey larvae fed Bt or non-Bt leaf tissue. However, avoidance of suboptimal Bt-induced prey by another carabid beetle (Poecilus cupreus) was not found by Meissle et al. (2005). Further evidence that Bt content in the prey does not
affect predator behaviour is provided by Ferry et al. (2007): the predatory beetles *Harmonia axyridis* (Coleoptera: Coccinellidae) and *Nebria brevicollis* (Coleoptera: Carabidae) did not differentiate between *Lacanobia oleracea* (Lepidoptera: Noctuidae) larvae fed with Cry3A-expressing potato plants or non-transformed plants. These studies suggest that certain predators are able to avoid suboptimal prey when given a choice, which potentially mitigates negative indirect effects of reduced quality of prey caused by consumption of *Bt* plants.

Hazards to natural enemies

*Bt* proteins

There is no indication in the peer-reviewed literature or in the data submitted to regulatory agencies that the Cry proteins produced in today’s *Bt* crops have a direct toxic effect on non-target organisms that are not closely related to the target pests (US EPA, 2001; Romeis et al., 2006; Wolfenbarger et al., 2008). This high level of specificity is due to the mode of action of the Cry proteins, which need certain conditions in the insect gut to activate and bind to specific midgut receptors (Knowles, 1994; Schnepf et al., 1998). Consequently, organisms that are unable to properly process the Cry proteins and do not possess the right receptors remain unaffected. Recently, Rosi-Marshall et al. (2007) suggested that *Bt* maize affects caddisflies (Trichoptera). Unfortunately, the authors do not state which *Bt* maize they were using for their studies. The discussion of the results, however, indicates that they have worked with a Cry1Ab-producing *Bt* maize. Phylogenetically, Trichoptera are closely related to Lepidoptera (Morse, 1997), so Cry1Ab activity would not be surprising. However, previous testing of *Bt* microbial formulations detected no adverse effects of Cry1Ab on trichopterans (Kreutzweiser et al., 1992). There is more evidence arising that the effects reported by Rosi-Marshall et al. (2007) were most probably due to some other plant characteristics and not to the expression of the toxin. A new study using both Cry1Ab- and Cry1Ab-and-Cry3Bb1-stacked maize plants could not repeat the reported finding (G. Dively, San Diego, 2007, personal communication).

In the near future, it is expected that some IRGM crops will express novel cry genes from *B. thuringiensis* either individually, pyramided/stacked with other cry genes or stacked with other traits such as herbicide tolerance. In addition, IRGM plants that express VipS also derived from *B. thuringiensis* are close to commercialization. Currently, the US Environmental Protection Agency (EPA) is evaluating maize and cotton plants expressing Vip3A, which has been reported to be specific to the order of Lepidoptera (Estruch et al., 1996; Warren, 1997). In contrast to Cry proteins, VipS do not need to be solubilized in the insect gut before they can act, and they bind to different receptors from those of Cry proteins (Lee et al., 2003, 2006). Given their specificity, Vip-producing GM plants are likely to cause non-target effects similar to current Cry1-producing crops. This hypothesis has recently been corroborated in field studies with Vip3A maize (Dively, 2005; Fernandes et al., 2007) and Vip3A cotton (Whitehouse et al., 2007).

Experimental IRGM plants have been developed that produce broad-spectrum insecticidal proteins with a higher potential for direct toxicity to non-target organisms (Jouanin et al., 1998; Carlini and Grossi-de-Sá, 2002; Ferry
et al., 2004; O’Callaghan et al., 2005; Malone et al., 2008). The best-studied examples include plants that express lectins or PI.

**Lectins**

Plant lectins are carbohydrate-binding proteins known to be components of the plant’s defence system (Peumans and Van Damme, 1995). Relatively little is known about the mode of action of plant lectins (Czapla, 1997). The proteins are reported to bind to the insect’s midgut epithelial cells causing morphological changes that are thought to affect nutrient absorption (Powell et al., 1998; Bandyopadhyay et al., 2001). While lectin binding is considered to be a prerequisite for toxicity, binding does not necessarily result in toxicity (Harper et al., 1995). In *vivo* and in *planta*, several lectins have been demonstrated to affect important life-table parameters in insect species in many different orders, including Lepidoptera, Coleoptera, Diptera and Hemiptera (Czapla, 1997; Jouanin et al., 1998; Carlini and Grossi-de-Sá, 2002; Ferry et al., 2004; Malone et al., 2008). Activity against aphids (Aphididae), planthoppers (Delphacidae) and leafhoppers (Cicadellidae) makes lectins particularly interesting, since no *Bt* proteins are known to control these pests.

Snowdrop lectin (*Galanthus nivalis* agglutinin (GNA)) has received much attention because it was the first lectin found to deleteriously affect aphids (Down et al., 1996), and planthoppers and leafhoppers (Rao et al., 1998; Foissac et al., 2000) when expressed in GM plants. Due to concerns regarding the food safety of this compound, albeit somewhat unfounded, research has partly moved to closely related lectins derived from edible plant species, such as garlic (*Allium sativum*). The garlic leaf lectin (ASAL) in particular was shown to affect lepidopteran pests (Sadeghi et al., 2008), planthoppers, leafhoppers (Saha et al., 2006) and aphids (Dutta et al., 2005a, b; Sadeghi et al., 2007) when expressed in different GM plants.

Given their mode of action, it is not surprising that lectins are not as specific as Cry or Vip proteins. A number of studies have shown that certain lectins have direct effects on parasitoids and predators when provided in artificial diets. Romeis et al. (2003), Bell et al. (2004) and Hogervorst et al. (2009) reported direct effects on the longevity and fecundity of four different species of parasitic wasp fed with sugar solution containing purified GNA. Similarly, growth of the predatory bug *Podisus maculiventris* (Hemiptera: Pteromalidae) was significantly reduced when fed with prey larvae injected with GNA (Bell et al., 2003); and larvae of two ladybird species and the lacewing *C. carnea* had reduced longevity when exclusively fed with GNA dissolved in a sugar solution (Hogervorst et al., 2006). When *C. carnea* larvae were fed alternately with sucrose solution containing GNA and insect eggs, their developmental time was significantly prolonged (Lawo and Romeis, 2008). Compared to these direct feeding studies, less pronounced effects have been reported from predators and parasitoids exposed to GNA via their prey or hosts (Malone et al., 2008), which is probably due to reduced exposure levels.

**Protease inhibitors**

Plant PIs play a potent defensive role against insect herbivores and pathogens (Ryan, 1990). Serine PIs and cysteine PIs have received the most attention since
they affect Lepidoptera and Coleoptera (Jouanin et al., 1998; Carlini and Grossi-de-Sá, 2002; Malone et al., 2008). More recently, aphids also have been reported to be affected by PIs of serine and cysteine proteases (Ceci et al., 2003; Rabhé et al., 2003a,b; Azzouz et al., 2005a,b; Ribeiro et al., 2006).

Potential direct effects of PIs deployed in IRGM plants on natural enemies have rarely been investigated. Studies with the larval and adult stages of the aphid parasitoids, Aphidius ervi (Hymenoptera: Braconidae) and Aphiinus abdominalis (Hymenoptera: Aphelinidae), revealed that the digestive proteolytic activity predominantly relies on serine proteases, especially those with chymotrypsin-like activities (Azzouz et al., 2005a,b). This could partly explain the detrimental effects of the Soybean Bowman-Birk inhibitor (SBBI), a dual serine PI (inhibiting both chymotrypsin and trypsin), on these parasitoids (Azzouz et al., 2005a,b). Also, the cysteine PI, oryzacystatin-1 (OC-1), was shown to be detrimental to these parasitoids when host aphids were dosed with the inhibitor via artificial diet. Another case is Eulophus penicicornis (Hymenoptera: Braconidae), an ectoparasitoid of L. celerioidea larvae. In vitro studies with larvae of the parasitoid have revealed a strong activity of trypsin and chymotrypsin-like serine proteases (Down et al., 1999). Nevertheless, the serine-type cowpea trypsin inhibitor (CpTI) did not markedly inhibit the larval proteolytic enzymes (Down et al., 1999) and also did not cause an effect on adult longevity in direct feeding studies (Bell et al., 2004).

Studies on adults and larvae of the two ladybird beetles Adalia bipunctata (Walker et al., 1998) and H. axyridis (Ferry et al., 2003; both Coleoptera: Coccinellidae) and the stinkbug Perillus bioculatus (Hemiptera: Pentatomidae) revealed that their major digestive proteolytic activity is cysteine-based (Ashouri et al., 1998; Overney et al., 1998). In contrast, carabid beetles have been found to rely upon serine proteases (both trypsin-like and chymotrypsin-like) for protein digestion (Goody and Huang, 1969; Terra and Cristofoletti, 1996; Ferry et al., 2005) and N. brevicollis adults exhibited both serine and cysteine digestive protease activity (Burgess et al., 2002).

Similar to herbivorous insects (Jongsma and Boiter, 1997), several predators appear to adapt their proteolytic digestive metabolism to counteract the presence of PIs in their food. Examples include: the ladybird H. axyridis (Ferry et al., 2003), different carabid beetles (Burgess et al., 2002; Ferry et al., 2005; Mulligan, 2006) and the predatory stinkbug P. bioculatus (Bouchard et al., 2003a,b). Indirect evidence for such an adaptation is also reported for larvae of C. carneae. Despite serine proteases dominating the digestive tract (Mulligan, 2006), feeding a high dose of soybean trypsin inhibitor (SBTI) did not affect larval development and survival (Lawo and Romeis, 2008). This potential for adaptation should be taken into account when assessing the potential risks that PI-expressing GM plants pose to natural enemies.

Hazards to Lepidoptera larvae

Lepidoptera may passively ingest insecticidal protein from GM maize via pollen deposited on their larval host plants within or close to the crop (Fig. 8.1, p. 175).
exposure route 2). Consequently, several studies have focused on pollen from Bt maize varieties that produce Lepidoptera-active Cry proteins. Early instar Monarch (D. plexippus) butterflies, consuming diet with either Cry1Ab or Cry1Ac toxins showed delayed development and increased mortality; yet they were not affected by Cry1F toxins, even at high doses relative to worst-case exposure in the field (Hellmich et al., 2001). Results from laboratory and semi-field studies varied with type of Bt maize when D. plexippus larvae were fed milkweed leaves with surface-deposited pollen. Short-term (4–5 days) studies conducted with Event Bt176 maize (high concentrations of Cry1Ab in pollen) showed negative effects (delayed development and increased mortality) with as few as ten pollen grains per square centimetre, but Event Bt11 and MON810 plants, which produce much smaller amounts of Cry1Ab toxin in pollen, showed little if any effects even with pollen densities of 1000 grains cm⁻² (Wraight et al., 2000; Hellmich et al., 2001; Stanley-Horn et al., 2001). Effects on D. plexippus, however, were found when they were continuously exposed throughout larval development to natural deposits of Bt pollen from both Bt11 and MON810 hybrids (Dively et al., 2004).

Monarch larvae have also been tested with two other types of maize pollen: one from an experimental hybrid that produces pyramided proteins Cry1Ab and Cry2Ab2 and another from a hybrid that produces the Coleoptera-active Cry3Bb1 protein (Mattila et al., 2005). Delayed development and increased mortality effects were found with the pyramided proteins but no effects were found with the Coleoptera-active protein. Also, Lee et al. (2003) detected no adverse effects of Vip3A on Monarch larvae. Studies that tested larvae from other Lepidoptera species that were fed host plants with surface-applied Bt pollen also found negative effects with pollen from Event Bt176 hybrids (Zangerl et al., 2001; Felke et al., 2002; Shirai and Takahashi, 2005; Lang and Vojtech, 2006) and, as with D. plexippus, little if any effects with pollen from MON810 hybrids (Wraight et al., 2000; Li et al., 2005). Interestingly, larvae of the milkweed tiger moth, Euchaetes egle (Lepidoptera: Arctiidae), were not affected when they consumed milkweed leaves with deposits of pollen from Event Bt176 hybrids (Jesse and Obyrski, 2002). One general conclusion that can be made from the data available is that susceptibility to Cry proteins differs among Lepidoptera.

Indirect effects

Entomophagous arthropods can suffer indirectly as a consequence of toxin effects on the target herbivores. This includes the absence of herbivores as prey or hosts and the presence of sublethally compromised (sick) prey or host herbivores. A significant reduction of the target pest population in the crop is the aim of all pest control programmes, including those that use IRGM plants. High adoption rates of Bt varieties can lead to region-wide pest suppression as has been reported for P. gossypiella and O. nubilalis (Carrière et al., 2003; Chu et al., 2006; Storer et al., 2008). Similar area-wide suppression has been reached by conventional pest control methods including insecticides. A prime example is the boll weevil eradication programme in the USA.
(http://www.cotton.org/tech/pest/boliweevil/index.cfm; accessed 15 April 2008). This reduction in prey or host abundance will have a large impact on the population dynamics of natural enemies that attack these herbivores. The decline in abundance or activity of specialist natural enemies that depend on the target pests as prey or hosts has been observed in a number of field studies with Bt crops (Romeis et al., 2006, 2008a; Wolfenbarger et al., 2008). Most predators, however, are generalists consuming a broader range of prey, which allows them to shift between prey species (Symondson et al., 2002). Consequently, generalist predators are less affected by suppression of a particular prey species. Only one study has reported a consistent and significant reduction of generalist predators when lepidopteran prey was reduced in Bt cotton (Naranjo, 2005a). Interestingly, this reduction in abundance by about 20% had no effect on the biological control function in the crop (Naranjo, 2005b).

Under laboratory or glasshouse conditions, prey- or host-quality-mediated effects are commonly observed. When herbivores are exposed to insecticidal proteins, mortality and sublethal effects (e.g. extended development or decreased body size) increase. Sublethal effects are likely to be accompanied by changes in herbivore physiology, which also can affect higher trophic levels; while in the field, predators may compensate for reduced prey quality by feeding on more prey or by shifting to alternative prey species. Parasitoids, however, usually complete their development in a single host and thus are very sensitive to changes in host quality. Such indirect effects on natural enemies are expected if susceptible herbivores ingest a toxin at sublethal levels (Romeis et al., 2006). A prominent example is discussed in detail in Box 8.1 and Fig. 8.2. Many studies conducted under contained conditions (i.e. in the laboratory, climate chamber or glasshouse) that have investigated the impact of Bt plants on natural enemies through a herbivore have used susceptible species as prey or host. Negative effects on the natural enemies, as expected, became evident in some of the studies (Romeis et al., 2006). Recently, Chen et al. (2008) were able to separate direct from indirect, host-quality effects to a parasitoid. Using populations of the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae), that were resistant to Cry1C, the authors showed that GM broccoli plants and purified Cry1C had no direct toxicity to the larval parasitoid Diadegma insulare (Hymenoptera: Ichneumonidae) when it fed inside its host after the host had consumed either Bt plants or leaves dipped in Cry1C solution.

Prey- or host-quality-mediated effects also have been reported for IRGM plants other than Bt plants. Studies on the interactions of GNA-fed aphids and aphid parasitoids suggested that indirect host-size-mediated effects caused subsequent deleterious effects on the parasitoids, rather than the GNA itself (Coutry et al., 2001a,b). Similarly, studies assessing the impact of GNA-fed aphids on the predator A. bipunctata suggested that negative effects on the predator were due to feeding on sublethally affected aphids or due to unintended effects of transformation in those particular experimental plants rather than the GNA itself (Birch et al., 1999; Down et al., 2000). Indirect effects have also been reported for honeydew-feeding arthropods, such as parasitic wasps (Hogervorst
et al., 2009). Aphids that were sublethally impaired by GNA ingestion produced inferior honeydew with consequences on honeydew-feeding aphid parasitoids.

Tritrophic studies including L. oleracea larvae fed with CpTI-expressing potatoes revealed a detrimental effect on different parasitoid life-table parameters (Bell et al., 2001a). The study indicated that the observed effects on the

**Box 8.1. The case of the green lacewing**

Tritrophic effects of Cry1Ab-expressing Bt maize on larvae of the green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), were assessed by Dutton et al. (2002). They reared different prey organisms on maize leaves and fed them to newly emerged lacewing larvae. Lacewings feeding on aphids (*Rhopalosiphum padi*; Hemiptera: Aphididae) and on spider mites (*Tetranychus urticae*, Acari: Tetranychidae) had a low mortality and did not show differences between prey reared on Bt maize and on control plants (near-isolines; Fig. 8.2). In contrast, when *Spodoptera littoralis* (Lepidoptera: Noctuidae) caterpillars were given as prey, the mortality in the control treatment was relatively high and significantly increased in the Bt treatment (Fig. 8.2). Also the development time for lacewings fed caterpillars from Bt maize was longer than in the other treatments. In order to explain the mechanisms behind those effects, a series of additional experiments was conducted.

First, Dutton et al. (2002) asked whether the different prey items ingested the Cry1Ab protein (Fig. 8.2). Measurements revealed that aphids contained little if any Cry1Ab, consequently no protein-related effects on aphids and lacewings were observed. In contrast, caterpillars contained 0.72 µg Cry1Ab g⁻¹ (fresh weight) and spider mites an even higher concentration of 2.5 µg g⁻¹ (fresh weight).

Next, the researchers asked whether those herbivores that ingested Cry protein when feeding on Bt maize were susceptible to the toxin (Fig. 8.2). Feeding experiments showed that the

**Fig. 8.2. Survival of Chrysoperla carnea larvae fed with different prey species (adapted from Dutton et al., 2002). To interpret the results, a set of questions was answered for each of the prey species (according to Romeis et al., 2006).**
growth rate was similar for *T. urticae* that consumed *Bt* maize or control maize. In contrast, when *S. littoralis* larvae consumed *Bt* maize, mortality was significantly higher and development was delayed compared to those consuming control maize (Dutton et al., 2002).

In a follow-up study, Obrist et al. (2006a) tested whether the protein is transferred from the prey to the predator (Fig. 8.2). Measurements showed that lacewing larvae feeding on caterpillars or spider mites contained about 1:2 to 1:3 of the prey Cry protein concentration, thus lacewings ingest Cry1Ab when feeding on those prey species. Furthermore, they confirmed biological activity of the protein contained in the herbivorous prey with a sensitive insect bioassay. Consequently, lacewing larvae are exposed to active Cry1Ab when feeding on *T. urticae* and when feeding on *S. littoralis* reared on *Bt* maize. However, detrimental effects on survival and development time were only seen in the caterpillar treatment, even though the concentration of Cry1Ab was much higher in the spider mite treatment.

These experiments led to the conclusion that lacewing larvae were not affected by the Cry1Ab protein itself and effects observed in the caterpillar treatment were mediated by prey quality. The high mortality of lacewing larvae, fed *S. littoralis* larvae from control maize, indicates these caterpillars were an inferior food source compared to aphids or spider mites. Thus, caterpillars compromised from ingesting *Bt* maize (‘sick prey’) were likely an even lower-quality food source for predators than healthy larvae from control maize. This example shows the importance of carefully formulating the study objectives and, accordingly, properly designing the study. Earlier work by Hilbeck et al. (1998) assessed the impact of *Bt* (Cry1Ab) maize on *C. carnea* larvae by only using caterpillars as the prey. Consequently, this study had caused some confusion since it appeared to prove that *Bt* maize causes a risk to this non-target organism.

Testing the hazard potential of a transgenic protein on a non-target species can be done with high-dose toxicity tests (e.g. 10× the dose expressed in a transgenic plant), by mixing purified protein into an artificial diet. Such tests have been performed with *C. carnea* by Romeis et al. (2004), Rodrigo-Simón et al. (2006) and Lawo and Romeis (2008), showing no indication of negative Cry1A impact on *C. carnea* larvae. Furthermore, Rodrigo-Simón et al. (2006) did not find binding of the Cry protein to lacewing gut membranes, a prerequisite of toxicity, in both histopathological and in vitro binding studies. A lack of effects has recently been confirmed also for the pollen-feeding adult stage of *C. carnea* (Li et al., 2008).

Parasitoid were likely to be indirect since *L. oleracea* larvae are susceptible to CpTI (Bell et al., 2001a), while the parasitoid was found not to be sensitive to this particular PI (Down et al., 1999; Bell et al., 2004). In contrast, studies that have investigated the performance of aphid parasitoids on aphids that were sublethally affected when feeding on artificial diet containing OC-1 or Soybean Bowman-Birk inhibitor (SBBI) are difficult to interpret, since the parasitoids also were sensitive to the PIs used (Azzouz et al., 2005a,b).

Sublethally affected herbivores may also lead to positive effects for natural enemies: host defence behaviour against attacking natural enemies may be altered, leading to a higher parasitization or predation frequency; longer development may result in longer availability of hosts or prey (i.e. a larger ‘window of opportunity’ for parasitoids and predators to attack); and a weaker immune system of a host may result in a lower encapsulation rate of endoparasitoid eggs and consequently a higher parasitization rate. Such positive indirect effects were reported by Johnson and Gould (1992) and Johnson (1997) who observed a significantly higher parasitization rate of *H. virescens* larvae by *C. sonorensis* on *Bt* tobacco plants compared with non-*Bt* controls. A similar effect was shown by Bell et al.
Non-target Effects Caused by Changes in Agricultural Practice

The deployment of IRGM varieties often has an impact on the overall pest control strategy with consequences for non-target organisms. A replacement of chemical spray insecticides in particular has a strong effect on the arthropod community (Romeis et al., 2006; Marvier et al., 2007; Wolfenbarger et al., 2008). This is an important consideration when the agricultural impacts of an IRGM variety are assessed. Whether or not the introduction of an IRGM variety influences the use of chemical insecticides varies with crop, region, target pest complex and commonly used conventional pest control (Head et al., 2005; Fitt, 2008).

The introduction of maize varieties that produce Cry proteins (e.g. Cry1Ab, Cry1F) for stem borer control has resulted in lower use of insecticides on many farms in the US Western Corn Belt (Nebraska, Kansas, Colorado; Hunt et al., 2007), but only modest reductions on farms in the rest of the Corn Belt (Carpenter and Gianessi, 2001; Phipps and Park, 2002) as many farmers did not use insecticides to control stem borers. In some areas of Spain, significant reductions in insecticide use have been reported. On average, conventional-maize farmers applied 0.86 sprays per year compared with 0.32 for Bt maize farmers; the overall percentage of farmers who applied no insecticides was 70% for Bt maize farmers and 42% for conventional farmers (Gómez-Babero et al., 2008). In contrast to field maize, insecticide applications for Lepidoptera control are common in sweetcorn and the use of Bt varieties can potentially reduce the number of insecticide sprays by 70–90% (Musser and Shelton, 2003; Rose and Dively, 2007). Bt varieties have been found to be a very effective control option with fewer side effects on natural enemies compared with commonly used broad-spectrum spray insecticides (Musser and Shelton, 2003; Hoheisel and Fleischer, 2007; Leslie et al., 2007; Rose and Dively, 2007). However, the advantages were less obvious when compared to insecticides with a more specific mode of action (e.g. indoxacarb, spinosad) or application (e.g. imidacloprid, a systemic insecticide; Musser and Shelton, 2003).

Maize varieties that produce Cry3 toxins to control larvae of Diabrotica spp. have the potential to drastically reduce the use of chemical insecticides (Rice, 2004; Ward et al., 2005). In the USA, corn rootworms are controlled largely by application of broad-spectrum soil insecticides including organophosphates, carbamates, pyrethroids and phenyl pyrazoles. Field studies have confirmed that non-target arthropods are more abundant in fields or plots planted with Bt varieties when compared to those treated with insecticides for corn rootworm control (Bhatti et al., 2005a, b).

Similarly, Bt potato cultivars supported greater and more diverse natural enemy communities (Hoy et al., 1998; Reed et al., 2001; Duan et al., 2004; Kalushkov and Nedvěd, 2005). As with maize, the advantages of the IRGM potato varieties were most pronounced when compared with sprays of broad-
spectrum insecticides than with soil applications or systemic insecticides (Reed et al., 2001; Duan et al., 2004).

The most prominent example of reduced environmental impacts due to IRGM crops is cotton, where chemical insecticides are heavily used mainly to control a complex of pest Lepidoptera. The introduction of Bt cotton varieties has resulted in substantial reductions in insecticide use in many countries, including the USA, China, Australia, India, Argentina and South Africa (Fitt, 2008; Naranjo et al., 2008). While insecticide reduction varied between 25% and 80% for single-gene (Cry1Ac) varieties, results from four cropping seasons in Australia indicated that due to increased efficacy, pyramid (Cry1Ac and Cry2Ab) cotton varieties can reduce the use of insecticidal active ingredients by 65–75% (with a 80–90% reduction in number of sprays; Fitt, 2008). A number of studies have revealed a significant increase in the abundance of non-target organisms in unsprayed Bt cotton fields when compared to insecticide-treated conventional cotton (e.g. Hagerty et al., 2005; Head et al., 2005; Naranjo, 2005a,b; Torres and Ruberson, 2005; Whitehouse et al., 2005; Cattaneo et al., 2006; Sisterson et al., 2007).

Recent studies from China indicate that significant reductions in insecticide use can also be expected when Bt rice for the control of stem borers is introduced (Huang et al., 2005, 2008).

A reduction in insecticide use in Bt crops has important consequences for the management of insect populations beyond the target pests. The data available indicate that this reduction can contribute to natural enemy conservation and increase the biological control function they provide (Romeis et al., 2006, 2008a). These natural enemies can help to suppress secondary pest populations, as has been suggested for aphids in Bt cotton, maize and potato fields (Hoy et al., 1998; Reed et al., 2001; Wu and Guo, 2003; Bhatti et al., 2005b) and for Spodoptera spp. in Bt cotton (Head et al., 2005). In addition, the deployment of IRGM plants can reduce the insecticide-induced resurgence of secondary pests, as reported from aphids in Bt cotton (Wu and Guo, 2003) and planthoppers in Bt rice (Chen et al., 2006).

However, secondary pest outbreaks also have been reported from Bt crops. This includes in particular regional outbreaks of plant and stinkbugs (Miridae and Pentatomidae) in Bt cotton fields. Those pests had earlier been controlled by insecticides used to control the Lepidoptera pest complex (Greene et al., 2001; Wu et al., 2002; Men et al., 2005). Thus, replacing a broad-spectrum control method (i.e. insecticides) by a more specific method (i.e. a Bt variety) may lead to secondary pest outbreaks, which again may require the application of control measures. While these outbreaks are not unexpected, there is little evidence that secondary pests have emerged as major problems requiring significant increases in insecticide to the extent that the reduction in insecticide requirement from the use of Bt cotton has been nullified (Fitt, 2008). The occurrence of secondary pests shows that IRGM plants should not be seen as a stand-alone control measure but as one component of an IPM programme for sustainable pest control (Kennedy, 2008).

While the reduction in broad-spectrum insecticide use is likely the most important factor explaining the increase in secondary pests, other factors also
appear to play a role. Secondary pest species may benefit from reduced competition with the target pests. A possible example of this is the case of the increase in the frequency and severity of attacks by the western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae), which is little affected by *Cry1Ab* and appears to benefit from the control of other Lepidoptera (Catangu and Berg, 2006; Storer et al., 2008).

Another factor to consider is the overall improved health of the plant as a consequence of being protected from insect damage. An example has been reported from Vip cotton where a higher abundance of mirids could be explained by higher numbers of bolls and flowers when compared to the unprotected control (Whitehouse et al., 2007). Another interesting observation is that some species benefit from herbivore (e.g. *O. nubilalis*) damage in non- *Bt* maize fields when compared to *Bt* maize. Examples are certain species of Niptidulidae (Coleoptera; Daly and Buntin, 2005; Bruck et al., 2006) that are fungivores and frequently found in tunnels made by *O. nubilalis* and saprophagous beetles and flies (Candolfi et al., 2004; Dively, 2005).

Broad-spectrum spray insecticides not only affect pests in the crop, but potentially also herbivores (e.g. Lepidoptera) on non-crop plants in and around the treated fields. For example, Stanley-Horn et al. (2001) found that all Monarch larvae on milkweed plants in a sweet-corn field sprayed with a pyrethroid insecticide were killed and larvae on plants 3 m from the edge of the field had low survival due to spray drift. Gathmann et al. (2006) found reduced abundances of lepidopteran larvae that occurred in strips near maize fields when the maize was treated with a pyrethroid insecticide. On the other hand, in the same study they found no negative impacts on lepidopteran larvae due to MON810 maize hybrids. Thus, reducing insecticide spraying may also benefit arthropods not directly related to the crop plant.

These examples show how important it is to select the appropriate endpoints and comparator when assessing the non-target effects of IRGM crops. Various risk assessment frameworks, including Annex III of the Cartagena Protocol on Biosafety (CBD Secretariat, 2000), refer to the importance of assessing risks of GM crops in the context of the risks posed by the conventional agricultural practice (Conner et al., 2003; Nap et al., 2003; Sanvito et al., 2007). Insecticide treatments, the 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, but only if they are of practical relevance.

**Implications for Regulatory Risk Assessment**

The cultivation of GM crops is strictly regulated worldwide (see Chapter 4, this volume), and before their seeds can be sold and cultivated without restriction, a licence must be obtained from a regulatory authority. The decision to license a GM crop for commercial cultivation uses risk analysis, a general method for regulatory decision making. Risk analysis comprises two activities: risk
assessment – the determination of the probability of specified harmful effects following a proposed action; and decision making – the evaluation of whether the risk, and the uncertainty associated with its estimation, is acceptable. Acceptability depends on the objectives of public policy, which can include the beneficial effects of the action, along with the ability to manage and communicate the risk (Wolt and Peterson, 2000; Johnson et al., 2007; Raybould, 2007a).

Risk assessment should begin with the identification of a problem, not with the acquisition of data (Raybould, 2006). As with other regulated agricultural practices, for cultivation of IRGM crops the problem for environmental risk assessment is the protection of attributes of the environment that are valued and require protection. Once the valuable environmental attributes have been identified, the next step is the creation of risk hypotheses about how the IRGM crops may harm those valued attributes; and risk is characterized by testing these hypotheses. A single cycle of risk characterization may be sufficient for decision making, in which case testing can stop; or risk characterization may identify further problems and so be the beginning of another round of hypothesis formulation, testing and characterization (Romeis et al., 2008c). Thus, an environmental risk assessment for proposed cultivation of an IRGM crop is simple in concept: decide what needs protection; assess how cultivation may cause harm to the entities requiring protection; and collect data to predict the probability and magnitude of harm following cultivation of the IRGM crop.

The protection objectives are not deducible by science alone; they are derived from the objectives of public policy, which will be based on political, economic, social and ethical, as well as scientific criteria (Wolt and Peterson, 2000; Johnson et al., 2007; Raybould, 2007a). Policy related to regulation of GM crops is usually enacted in laws that have the objective of protecting the environment. In the USA, pesticidal proteins produced in IRGM plants are regarded as pesticides and therefore are regulated under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), which seeks to ‘protect the public health and environment from the misuse of pesticides by regulating the labelling and registration of pesticides and by considering the costs and benefits of their use’. In the European Union, GM crops for commercial cultivation are regulated under Directive 2001/18/EC, which requires that risk assessments ‘identify and evaluate potential adverse effects of the GMO, either direct or indirect, immediate or delayed, on human health and the environment which the deliberate release or placing on the market of GMOs may have’.

Both laws seek to protect the environment; however, ‘environmental protection’ is too vague a concept to be analysed scientifically. Specific targets for protection, called ‘assessment end points’, must be identified; for these targets to be analyzable scientifically, they must comprise an entity and some property of that entity (Newman, 1998). For example in the UK, the objective of protecting biodiversity is represented by an assessment end point of an index of the population sizes of bird species common
on farmland (Gregory et al., 2004). In regulatory risk assessments of IRGM plants, the abundance and diversity of non-target arthropods that provide biological control is a common assessment end point (Raybould, 2007a; Romeis et al., 2008c); however, this end point is often implicit rather than explicit.

A simple and effective conceptual model of how IRGM crops may cause harm is that the abundance of non-target organisms is reduced by exposure to toxic substances in the IRGM crop. The model makes two important assumptions. First, reductions in the abundance of predators and parasitoids that result from control of the target pest are not considered harmful. Such effects are common for all pest control methods, including insecticides, biological control and conventional host-plant resistance, and are generally not regarded as ‘adverse’ (Romeis et al., 2006). As a consequence, the Organization for Economic Cooperation and Development (OECD) has stated that ‘such secondary effects as a consequence of pest control achieved with a trait should be of minor concern where the trait serves primarily to bring the pest population down to an ecologically more natural level’ (OECD, 1993). Similarly, the European Food Safety Authority (EFSA) has stated that ‘it is important that food chain effects due to reductions in target prey species (e.g. declines in parasitoid populations) are differentiated from, for example, population declines due to the effects of GM toxin accumulation in food chains’ (EFSA, 2006). We argue that the same applies for non-target effects on the natural enemy due to reduced nutritional quality of the susceptible hosts or prey. Romeis et al. (2006) have proposed that direct and indirect effects should be addressed separately. Probably the best-studied model species is the green lacewing, C. cornea, for which a series of experiments have allowed us to clearly distinguish between effects conferred by the Bt toxin itself (direct) and those caused as a result of reduced prey quality (indirect; Box 8.1). Similar to Bt crops, a range of studies have investigated the impact of PI- or lectin-expressing IRGM plants on natural enemies attacking sublethally affected herbivores. In most cases, effects have been detected (O’Callaghan et al., 2005). However, a clear separation of direct and indirect (prey- or host-quality-mediated) effects is difficult, if not impossible, since both effects are likely to co-occur. It is thus important to investigate whether observed effects are more pronounced on the target pest species or on the associated natural enemies.

A second assumption of the conceptual model of how IRGM crops may cause harm to non-target organisms is the fact that any direct effects of non-GM counterparts of IRGM crops (near-iso lines) are acceptable. These assumptions greatly simplify the risk assessment as only differences in the composition of the GM and non-GM crop need to be assessed for their effects on non-target organisms.

The next stage of the risk assessment is to formulate risk hypotheses. The purpose of these hypotheses is to assist decision making, and therefore risk hypotheses should be formulated such that corroboration under rigorous testing indicates low risk with high confidence (Raybould, 2006). Tests of three risk
hypotheses provide an effective method for evaluating risks to non-target organisms from IRGM crops (Raybould, 2007b):

1. There are no ecotoxicologically relevant differences between the IRGM crop and non-GM counterparts, apart from expression of the intended insecticidal protein.
2. There is no exposure to the insecticidal protein.
3. If there is exposure, the expected environmental concentration (EEC) of the insecticidal protein is below the no observable adverse effect concentration (NOAEC) for each exposed non-target organism.¹

While the hypotheses are not stated explicitly, in effect, they form the basis for most regulatory risk assessments of IRGM plants.

Hypothesis 1 is tested in two main ways. First, a detailed molecular characterization of the inserted DNA and the genomic regions flanking that DNA is undertaken to ensure that no new open-reading frames have been created that could produce unintended new proteins. Second, the composition of the IRGM crop and an appropriate non-GM counterpart is compared. If there are no significant differences in the concentrations of certain nutrients, anti-nutrients and toxins (e.g. Kuiper et al., 2002), the GM plant may be regarded as ‘substantially equivalent’ to the non-GM plant, and the risk assessment can consider the effect of the insecticidal protein only (Raybould, 2006; Romeis et al., 2008c).

Hypothesis 2 is tested by measurements of the concentration of the insecticidal protein in various tissues (i.e. spatial expression) and considerations of exposure pathways given above. Expression of the insecticidal protein in leaves of the IRGM plant may mean that non-target arthropods are exposed to the protein through consumption of herbivores that eat leaves; on the other hand, if the protein is not detected in nectar or pollen, one may conclude that pollinators will not be exposed to the protein. For regulatory risk assessments, it is not necessary to have precise estimates of the concentrations of the insecticidal protein in the diet of the non-target organisms because conservative assumptions can be made. For this purpose, the highest mean protein expression level in any plant tissue is often taken as the worst-case EEC in regulatory risk assessments (Raybould et al., 2007).

The highest mean concentration of the protein in the IRGM crop may be suitable for protecting individuals, such as those of endangered species, which could consume a diet comprising only plant material. In many cases, the objective of the risk assessment is protection of populations of non-target organisms that are omnivorous or predators; for these species, an EEC based on the highest expression in the IRGM plant may be adequately conservative. Many studies have revealed a dilution of Cry proteins along the food chain (Head et al., 2001; Harwood et al., 2005; Vojtech et al., 2005; Obrist et al., 2006a,b; Torres et al., 2006; Torres and Ruberson, 2008). This dilution also seems to apply to other insecticidal proteins (e.g. Bell et al., 2003; Christeller et al., 2005). An exception to this finding is spider mites, which appear to contain Cry protein

¹ Hypothesis 2 is a special case of hypothesis 3. If the organism is not exposed, the EEC is not greater than zero and hence must be lower than the NOAEC.
levels comparable to, or greater than, those in green plant tissue (Dutton et al., 2002; Obrist et al., 2006b,c; Torres and Ruberson, 2008). Because of the dilution of insecticidal proteins and the mixed diet of many predators, Raybould et al. (2007) suggested a realistic EEC could be 0.2x the highest mean concentration in plant tissue for populations of non-target arthropods.

More precise refinements of exposure are needed only if a potential problem is indicated by conservative assumptions about exposure. Long-term exposure to Bt maize pollen, for example, has been shown to affect Lepidoptera larvae such as those of the Monarch butterfly (Dively et al., 2004). Consequently, exposure assessment became critical to address a potential risk for Monarch populations. Considering the entire range of the US Corn Belt, the impact on Monarch populations was estimated as being negligible. This primarily is due to the limited overlap between maize anthesis and the presence of Monarch larvae (Dively et al., 2004). Other studies that have focused on exposure (temporal and spatial overlap, pollen dispersal) in general have found that the potential impact of Bt maize pollen on lepidopteran larvae is small to negligible; and where impacts are possible, they are limited to specific geographies (Oberhauser et al., 2001; Sears et al., 2001; Wolt et al., 2003; Gathmann et al., 2006; Li et al., 2005; Shirai and Takahashi, 2005; Peterson et al., 2006).

Hypothesis 3 is tested using laboratory methods in the first instance; species that are representative indicators of potentially exposed non-target arthropods are exposed to concentrations of insecticidal protein in excess of conservative estimates of exposure in the field (often 10x the EEC, Rose, 2007). If no harmful effect is observed, hypothesis 3 is corroborated and the risk to non-target arthropods can be considered minimal. If effects are seen at high concentrations, the risk can be characterized further in experiments that use more realistic exposures (García-Alonso et al., 2006; Romeis et al., 2008c).

Overall, the studies conducted to assess the direct impact of different insecticidal proteins on non-target organisms have revealed a high level of specificity of the deployed Cry proteins (US EPA, 2001; Romeis et al., 2006). In contrast, other more broad-spectrum insecticidal proteins, such as lectins or PIAs, are more likely to have direct effects on a wider range of non-target organisms (Malone et al., 2008). Thus, it is necessary that each protein is assessed separately, on a case-by-case basis.

The IRGM maize and cotton varieties currently grown express insecticidal Cry proteins that are well understood in respect to their mode of action and spectrum of activity. Due to their specificity, the deployment of Bt (Cry) varieties appears to be safe to biological control organisms (Romeis et al., 2006; Wolfenbarger et al., 2008) and other non-target species (O’Callaghan et al., 2005; Sanvido et al., 2007). The studies conducted in the public sector overall confirm the negligible non-target risk conclusion from regulatory risk assessment. In many cases, the deployment of IRGM varieties has resulted in a substantial decrease in the use of chemical insecticides with clear benefits for non-target arthropods and biological control. Secondary pest outbreaks that have for example been reported from Bt cotton appear to be largely because Lepidoptera-resistant Bt varieties have replaced broad-spectrum insecticide sprays that had kept secondary pests under control (Naranjo et al., 2008).
There is evidence that a healthy biocontrol community in Bt crops can help to control potential secondary pests such as aphids (Romeis et al., 2008a). Overall, the currently deployed IRGM plants appear to be well compatible with biological control and thus can form an important part of a sustainable IPM system.

References


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Impacts of Insect-resistant Crops on Non-target Arthropods


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