

FORUM

The Environmental Effects of Genetically Modified Crops Resistant to Insects

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Efeitos Ambientais de Culturas Geneticamente Modificadas Resistentes a Insetos

RESUMO - Cultivares transgênicas de várias culturas estão sendo utilizadas em escala comercial em muitos países. A área dedicada ao cultivo com plantas transgênicas resistentes às pragas em todo o mundo alcançou 13 milhões de hectares em 2001. As cultivares transgênicas proporcionam benefícios, mas também apresentam riscos potenciais. As avaliações do seu impacto no ambiente são conduzidas antes da sua aprovação para uso comercial, como requerido pelas normas de biossegurança. Nesta revisão, serão discutidas as consequências ecológicas potenciais do uso comercial na agricultura de cultivares geneticamente modificadas que apresentam resistência aos insetos-pragas. Também serão discutidos os impactos ambientais causados pelas mudanças nas práticas agrícolas, identificando-se falhas e oportunidades de pesquisa, considerando-se essa nova ferramenta tecnológica. Os comentários e análises serão baseados no conhecimento atual que se tem dos riscos e benefícios do uso de cultivares resistentes a insetos, geneticamente modificadas, dentro do contexto dos programas de manejo integrado de pragas tradicionais.

PALAVRAS-CHAVE: Planta transgênica, *Bacillus thuringiensis*, organismo não-alvo, fluxo gênico, biossegurança

ABSTRACT - Transgenic crops are currently being cultivated on a commercial scale in many countries. The area devoted to transgenic pest resistant varieties worldwide reached 13 million hectares in 2001. These varieties offer valuable benefits but also pose potential risks. Assessments of their impact on the environment are conducted before they are approved for commercial use, as required by the regulatory biosafety frameworks. In this review, we discuss the potential ecological consequences of the commercial use in agriculture of genetically modified insect resistant crops. We also discuss the impacts caused by the change in agricultural practices, and attempt to identify gaps and possible opportunities for research, considering this new technological tool. We based our analysis and comments on the current knowledge of the risks and benefits of these genetically modified insect resistant crops, within the context of traditional insect management strategies.

KEY WORDS: Transgenic crop, *Bt* gene, non-target organism, gene flow, biosafety

In recent years, the introduction of genetically modified (GM) crops through modern biotechnology is been considered a new technological breakthrough in agriculture, only comparable to the green revolution of the early 70's. The potential impact of this technology has also been compared with the introduction in the market of synthetic insecticides and many believed that this would solve pest problems around the world. Parallel to this great expectation, the molecular breeding of cultivated plants through genetic engineering has raised a worldwide debate about their potential impact on the environment.

Since the introduction of genetically modified (GM) crops to the market in 1996 (James 2001), there has been an increasing interest on how changes in agriculture practices might indirectly

affect the environment (Dale *et al.* 2002). The discussions on GM pest resistant crops in many parts of the world has led to questions about their potential impacts on biodiversity, particularly on their effects on non-target organisms, including insect herbivores and natural enemies, and soil microbiota. Further concern arises from the possibility of resistance development in insect pests, which could endanger important natural resources such as the bacterium *Bacillus thuringiensis* Berliner, a natural microbial insecticide. Another issue frequently cited as a potential risk of pest resistant GM crops is the possible movement of the inserted gene from crops to wild or weedy relatives, and the consequences of such movement.

These questions have been the subject of research for the

past 15 years. The information raised is being used for the environmental risk assessments of GM crops, which is a fundamental procedure in the biosafety regulatory process internationally.

The objective of this article is to discuss the ecological principles and questions that have emerged from the environmental impact of this technology, and to consider the present state of knowledge about the different kinds of environmental effects of GM insect resistant crops available so far. Our review was based on diverse and consistent peer-reviewed literature, which we have tried to reproduce in a style easily understood by entomologists; we have tried to avoid much of the specific details of the diverse and complex issues involved in this subject.

The Market of GM Plants in the World

The global area of genetically modified plants reached 52.6 million hectares in 2001 (James 2001). Transgenic plants expressing *Bt* toxins conferring resistance against insects were tested in the field in at least 18 crops. However, only corn, cotton and potato are commercially available (Shelton *et al.* 2002). In the United States, pest resistant transgenic crops have been cultivated in large areas since 1996. In 2001, approximately 13 million hectares were cultivated with *Bt* corn and *Bt* cotton, mainly in the USA and Canada (James 2001). *Bt* crops are also being cultivated in China, India, South Africa and Argentina, with a great expansion in China (Carpenter *et al.* 2002). Consequently, some experience is being accumulated on the commercial use of insect resistant transgenic crops.

Protecting Plants from Pests - Risks and Benefits

Plant breeders attempt to minimize the impacts of crop pests by breeding plants resistant to the attack of pests. These breeders use the natural plant genetic variation and induced mutations to select desirable traits and introduce them into cultivated plants, using a combination of classical and modern plant breeding methods.

To make crop plants resistant to the attack of insects and diseases, breeders have relied on the chemical defenses of plants. Plants produce a variety of antimicrobial or insecticidal or defensive substances that provide protection against pests. These are secondary plant metabolites that include phenolics, terpenoids and steroids (Kogan 1986).

Traditional breeding to alter the plant's chemical composition to intoxicate target herbivores is achieved through the transfer or exchange of the desired traits among sexually compatible, i.e., related plant species which share genomes that are largely homologous. On the other hand, molecular breeding through genetic engineering can use genes and gene constructs derived from completely unrelated organisms, and add them to the existing genome in an entirely novel genetic context (Regal 1994). The added novel gene constructs contain not only the desired trait, but also marker genes, promoters and terminators.

The greater diversity of genes that can be transferred by genetic engineering methods, their enhanced effectiveness, and the ability to insert the same gene into many cultivated

species have led to concerns about transgenic crops. Some transgenic breeding result in pest-protective traits that are phenotypically indistinguishable from those conferred by traditional breeding methods. In addition, transgenic methods are based on a more complete knowledge of the genes that are being transferred into cultivated plants. There are cases, however, when transgenic pest-protection traits may result in plants having new phenotypes that could affect human or animal health, non-target organisms, or the weediness of crop relatives. Transgenic methods can also introduce extraneous traits when they involve marker genes, such as antibiotic resistance genes. These are concerns that are taken into consideration by the biosafety regulatory process before transgenic varieties are commercialized.

Some of the investigated potential benefits of transgenic insect resistant crops include reduced applications of broad-spectrum insecticides (Carpenter *et al.* 2002), increased or protected yields due to season-long control of the target insect pest (Rice & Pilcher 1998); protection of stored corn from Lepidopteran insect pests (Giles *et al.* 2000); and, lower mycotoxin levels due to a reduction in fungal plant pathogens associated with *Ostrinia nubilalis* (Hübner) feeding on corn (Munkvold *et al.* 1999).

Perceived disadvantages of genetically modified crops may be grouped into five categories: 1) potential impact on non-target species; 2) potential for increased weediness; 3) increase in toxin levels in the soil; 4) exchange of genetic material between the transgenic crop and related plant species; and 5) selection for resistance among populations of the target pests.

Transgenic Strategies for Plant Protection

Characteristics. The transgenic breeding method or the DNA recombinant technology allows the transference of genes from one species to another without sexual hybridization. Plant breeding by biotechnological processes (Brasileiro & Carneiro 1998) starts with prospecting desirable traits expressed under control of a single gene, e.g., production of a protein that could be toxic to herbivorous insects. Microorganisms and plants that produce toxins against pests have been the main source of genes for this technology. The next step in the process is the identification of the gene in charge of expressing the selected trait and then its isolation using restriction enzymes. This gene is then cloned and linked to other gene fragments to form the cassette of expression. This cassette is a piece of DNA that will be inserted into the plant to promote the expression of the desired characteristic.

Besides the gene of interest the cassette of expression is commonly comprised of a marker gene, a promoter gene and a terminator gene. The promoter gene has a central role in the gene transfer. Its function is to promote the expression of the gene of interest since many foreign genes (mainly those isolated from prokaryotes) will not express in plants. The 35S promoter gene isolated from the cauliflower mosaic virus is largely used due to its strength in inducing the expression of selected genes, and because it has a constitutive character, i.e., it is expressed in all tissues of the plant. Another important gene usually present in the cassette of expression is the marker gene. It allows the selection of the transformed cells in the

cell culture media. The most commonly used marker is the antibiotic resistance gene. In this case, the selection is made by adding to the culture media a specific antibiotic. The transformed cells that became resistant to that antibiotic will survive and the other cells will die. Biosafety concerns about the use of antibiotic resistant genes has lead researchers and the industry to avoid these genes and use new markers such as herbicide resistance and the expression of special carbohydrate (mannose) degradation enzymes. The terminator gene contained in the cassette “informs” to the cells the end of the expression code.

The last step in the transformation process is to grow the selected cells to regenerate the plant that will express and transmit the new trait to other varieties through sexual hybridization or cloning.

Insecticidal Toxins from *B. thuringiensis* (*Bt*). During sporulation *B. thuringiensis* produces crystalline inclusions that are protoxins called δ -endotoxins. These structures called cry proteins, when ingested by the insect, are dissolved in the gut and cleft by digestive proteases activating the toxin. The toxin then binds to specific glycoprotein receptors on the surface of cells lining the gut, causing an imbalance in ion concentration, destroying the cells, and resulting in the death of the insect (Choma *et al.* 1990). In addition, some strains of *B. thuringiensis* produce “vegetative insecticidal proteins” (VIPs) before sporulation (Lacey & Kaya 2000).

Since 1942, when E. A. Steinhaus demonstrated the potential of this bacterium for pest control, the use of *Bt* formulations increased as bioinsecticides because of its safety, been harmless to vertebrates and other non-target fauna, and because of the specificity of each cry protein which infects only a narrow range of hosts (Tanada & Kaya 1993, Peferoen 1997). However, instability and degradation of cry proteins when exposed to ultra violet radiation and short persistence on the plant (easily washable by rain and irrigation), have constrained large adoption of *Bt* pesticides by farmers.

B. thuringiensis produces a broad diversity of cry proteins with a range of around 100 holotype toxins distributed in 40 groups (cry1, cry2, etc.) each one with several subgroups and a narrow range of host (Bravo *et al.* 1998). This variability is being used to develop transgenic plants resistant to pest attack.

Transgenic Plants Expressing *Bt* Toxins. Several *Bt* toxins have been inserted into crop plants to provide protection against different groups of pest insects. A brief list was presented by Jouanin *et al.* (1998), which we updated (Table 1). Many crops such as vegetables, forage crops, root crops, cereals, and trees are now being transformed to be protected against insects by *Bt* toxins (Shelton *et al.* 2002). New constructs of *Bt* toxin genes with promoters to be expressed in monocots or dicots, and in different tissues of the plant are been studied, including the integration of a native *Bt* gene into the chloroplast genome of tobacco (McBride *et al.* 1995). The chloroplast genome is bacterial in nature, avoiding the need of modifying the toxin gene for higher expression. Although it is still not commercially available, this technique opens new perspectives for the *Bt* plant breeding in the future.

Inhibitors of Insect Digestive Enzymes. Plants extensively produce proteins with antimetabolic activity against diverse digestive enzymes of herbivores. These proteins have either a role as part of defense strategy against herbivores or for preserving stored nutrients as proteins and carbohydrates. Genes encoding these proteins are a valuable source to enhance resistance of plants against insects and could be used in plant breeding programs.

Inhibitors of Digestive Proteases. There are four classes of protease inhibitors based on their specificity: serine, cysteine, metallo- and aspartyl-proteases. All are small proteins that bind to the insect's digestive enzymes producing an inactive complex that prevents the absorption of amino acids, leading to nutrient starvation. Consequently, these inhibitors induce death or reduction in larval growth. The protease inhibitors can also induce the over-expression of digestive enzymes affecting larval growth. Serine protein inhibitors have activity sites, which inhibit trypsin and chymotrypsin, and are effective antimetabolites against Lepidoptera and Diptera (Duan *et al.* 1986, Hilder *et al.* 1995, Xu *et al.* 1996, Gatehouse *et al.* 1997). A successful case is the cysteine protease inhibitor gene introduced into poplar to protect the plant against beetles (Leplé *et al.* 1995).

Inhibitor of Alfa-Amylase. The alfa-amylase inhibitor is a seed protein contained in the common bean, *Phaseolus vulgaris* L., and its linkage with certain amylases forming inactive complex provide seed protection against several bruchid beetles (Shade *et al.* 1994, Shroeder *et al.* 1995). However, other species of bruchids (*Zabrotes subfasciatus* Boh. and *Acanthoscelides obtectus* Say) have a protective mechanism to inactivate alpha-amylase (Ishimoto *et al.* 1996). The limited range of target insects makes it difficult to evaluate the real value of this gene in the protection of seeds against post-harvest pests.

Lectins. This is a heterogeneous group of proteins with carbohydrate-binding activity. They are found in many plants, in higher concentrations in storage tissues and seeds. Lectin expression genes have been inserted into several plant species which then demonstrated enhanced resistance against insects, although the way these proteins cause deleterious effects on insects is not well known (Gatehouse & Gatehouse 2000). The well-documented anti-nutrient action and toxicity of these proteins towards mammals and birds in nature, in spite of its inactivation by cooking (Shatters 2000), have served as a baseline of an intense discussion about the biosafety of transgenic plants.

Other Genes and Traits. Transgenic expression of other proteins is also an alternative to the use of *Bt* genes. Chitinase against the beetle *Orizaephilus mercator* Fauvel (Jouanin *et al.* 1998), and cholesterol oxidase against the cotton boll weevil *Anthonomus grandis* Boh., are some examples of enzymes expressed by transgenes with potential effects on insect, although protective effects on plants have still not been demonstrated (Gatehouse & Gatehouse 2000).

Table 1. Insect-resistant transgenic plants expressing *B. thuringiensis* toxins.

Crop plant	Toxin	Target insect	Reference
Alfalfa	cry1Ca	<i>Spodoptera littoralis</i> (Boiusduval) (Lep.)	Sthrizhov <i>et al.</i> (1996)
Broccoli	cry1C	<i>Plutella xylostella</i> (L.) (Lep.)	Zhao <i>et al.</i> (2001)
Cabbage	cry1Ab	<i>P. xylostella</i>	Bhattacharya <i>et al.</i> (2002)
Canola	cry1Ac	<i>Thrichoplusia ni</i> (Hübner) (Lep.), <i>Spodoptera exigua</i> (Hübner), <i>Heliothis virescens</i> (Fabr.), <i>Helicoverpa zea</i> (Boddie) (Lep.)	Stewart <i>et al.</i> (1996b)
Cotton	cry1Ac	<i>P. xylostella</i>	Ramachandran <i>et al.</i> (1998)
	cry1Ab	<i>H. virescens</i> , <i>H. zea</i>	Perlak <i>et al.</i> (1990)
Eggplant	cry1Ac and cry2Ab	<i>S. exigua</i> , <i>Pseudoplusia includens</i> (Walker) (Lep.)	Adamczyk <i>et al.</i> (2001)
	cry1Ab	<i>Leucinodes orbonalis</i> Guenée (Lep.)	Kumar <i>et al.</i> (1998)
Corn	cry3A	<i>Leptinotarsa decemlineata</i> (Say) (Col.)	Jelenkovic <i>et al.</i> (1998)
	cry1Ab	<i>Ostrinia nubilalis</i> (Hübner) (Lep.)	Kozziel <i>et al.</i> (1993)
Poplar	cry9c	<i>O. nubilalis</i>	Janssem <i>et al.</i> (1997)
	cry1Aa	<i>Lymantria dispar</i> (L.) (Lep.)	McCown <i>et al.</i> (1991)
Potato	cry3Aa	<i>Chrysomela tremulae</i> F. (Col.)	Cornu <i>et al.</i> (1996)
	cry1Ab	<i>Phthorimaea operculella</i> (Zeller) (Lep.)	Peferoen <i>et al.</i> (1992), Rico <i>et al.</i> (1998)
	cry1Ab	<i>Heliothis armigera</i> (Hübner)	Chakrabarti <i>et al.</i> (2000)
	cry3Aa	<i>L. decemlineata</i>	Adang <i>et al.</i> (1993), Perlak <i>et al.</i> (1993)
Rice	cry1Ab	<i>Chilo suppressalis</i> Walker (Lep.)	Coombs <i>et al.</i> (2002)
	cry1B	<i>C. suppressalis</i>	Fujimoto <i>et al.</i> (1993)
	cry1Ac, cry2A and GNA	<i>Cnaphalocrosis medinalis</i> Guenée (Lep.)	Marfa <i>et al.</i> (2002)
		<i>C. medinalis</i> , <i>Scirpophaga incertulas</i> Walker (Hom.) <i>Nilaparvata lugens</i> Stål (Hom.)	Wunn <i>et al.</i> (1996)
cry1Ab and cry1Ac	<i>C. suppressalis</i>	Maqbool <i>et al.</i> (2001) Cheng <i>et al.</i> (1998)	
Soybean	cry1Ac	<i>H. virescens</i> , <i>H. zea</i> <i>P. includens</i>	Stewart <i>et al.</i> (1996a)
Tobacco	cry1Aa	<i>Manduca sexta</i> (L.) (Lep.)	Barton <i>et al.</i> (1987)
	cry1Ab	<i>M. sexta</i>	Vaeck <i>et al.</i> (1987)
	cry1Ab and cpTI	<i>M. sexta</i>	Perlak <i>et al.</i> (1991)
	cry1Ab	<i>M. sexta</i>	Williams <i>et al.</i> (1993)
	cry1Ac	<i>H. virescens</i> , <i>H. zea</i> , <i>S. littoralis</i>	McBride <i>et al.</i> (1995)
	cry1C	<i>S. littoralis</i>	Strizhov <i>et al.</i> (1996)
	cry2A	<i>H. armigera</i>	Selvapandiyam <i>et al.</i> (1998)
	cry2A	<i>H. virescens</i> , <i>H. zea</i> <i>S. exigua</i>	Kota <i>et al.</i> (1999)
Tomato	cry1Ab	<i>H. virescens</i>	Fischolff <i>et al.</i> (1987)
	cry1Ac	<i>H. armigera</i>	Mandaokar <i>et al.</i> (2000)

Lep = Lepidoptera; Col = Coleoptera; Hom = Homoptera

Non-target Effects of Insect Resistance

In the scope of this review, following the definition of Dale *et al.* (2002), non-target effects are any undesirable effects of insect resistance GM plants on friendly organisms (unintended targets) in the environment. Non-target organisms include natural enemies, pollinators, and other non-target herbivorous that feed preferentially on the surrounding vegetation and might be affected.

Effect on Natural Enemies. Despite their apparent simplicity, agricultural systems consist of organisms that interact in food webs (Price *et al.* 1980). In the last 20 years the effect of host plants on higher-trophic-level organisms, such as parasitoids and predators, has been studied (Groot & Dicke 2002). Most of the published papers have demonstrated that differences in plant traits may affect natural enemies directly. The plants are a source of water or nutrition for many species of parasitoids and predators that feed on floral or extrafloral nectar, pollen or plant sap. Consequently, changes

in plant quality may affect the food source of those species.

Variation in the quality and quantity of plant secondary compounds may indirectly affect the natural enemies by reducing the nutritional suitability and palatability of their prey (Price *et al.* 1980, 1997). Alternatively, plant resistance traits may indirectly affect the population of natural enemies by severely depleting their supply of prey or hosts (Hoy *et al.* 1998, Schuler 2000). Yet plants provide not only food but also play an important role in the host searching behavior of many species of natural enemies that commonly seek herbivores on plants. Consequently, differences in plant chemicals produced constitutively or in response to herbivory may alter the attractiveness of the plant to some species of entomophagous arthropods (Vinson 1976, Lewis & Takasu 1990).

Despite many examples that have shown that plant breeding affects the effectiveness of biological control agents (Groot & Dicke 2002), conventional plant breeding programs have largely ignored natural enemies of pests in their protocols. A change in this tendency has been observed in regard to GM insect resistant crops (Schuler 2000). The

potential effects of insect resistant GM plants on ecological disruption of food webs and consequent effects on natural biological control has been discussed by Hoy *et al.* (1998) and Groot & Dicke (2002). Genetic engineering capability to produce precise genetic alterations increases confidence that unintended changes in the genome have not occurred. However, precise genetic characterization does not ensure that all ecologically important aspects of the phenotype can be predicted for the environments into which an organism will be introduced (Tiedje *et al.* 1989).

It is reasonable to point out that the potential impacts on natural enemies associated with the plants produced by conventional and transgenic methods fall into the same general categories. Hilbeck (2002) discusses extensively the genetic engineering and conventional breeding for host plant resistance and the possible non-target effects of both breeding methods. She emphasizes that the season-long and high-level expression of *Bt* toxins on crops may have more three-trophic effects than conventionally bred crops. It is important to remember that any human interference to protect crops from pests will have some negative impact on those arthropods that depend on those pests (Hoy *et al.* 1998, Schuler 2000), and on the overall biological community (Shelton *et al.* 2002).

Natural enemies might be affected by GM plants in several ways: directly through feeding on the plant tissues (e.g., pollen) that express the gene product (*Bt* protein, transgenic lectin or proteinase inhibitors); feeding on prey or host that feed on GM plants; and indirectly through the interference with the production of volatile chemicals responsible for the plant's attractiveness to natural enemies. Additionally, the most obvious way the GM plants can affect natural enemies is by deploying the population of their prey or hosts. This applies to all pest control methods and will be more severe for specialized natural enemies that feed exclusively on the target insect of the pest resistant GM plant. Compared to predators, parasitoids that are relatively host-specific will probably be more affected by host population depletion.

The impact of transgenic plants resistant to insects on the population dynamics of natural enemies will depend on several factors, including the expression level of the transgene, the specificity of the transgene product, and the tissue specificity of the transgene (Schuler 2000). The expression level that induces pest mortality rates close to 100%, observed on most *Bt* plants, results in host depletion for parasitoids specific to the target pest and in a reduction in prey or host for generalist natural enemies, when compared to populations in unsprayed non-*Bt* crops (Schuler 2000). In some cases, a partial plant resistance could be advantageous because synergistic interactions between partially resistant plants and natural enemies are possible (Hoy *et al.* 1998). The specificity of the protein expressed by the transgene and the plant tissue where the gene product will be expressed determines the possibilities of any direct negative effects on non-target organisms.

The impact of transgenic plants resistant to insect on the population dynamics of natural enemies will also depend on the plant species in question, on the geographical location in which the GM plant is cultivated, and on the management of the crop (Schuler 2000). For example, crop plants with

different life cycles (annual versus perennial) will differ in their associated arthropod communities. The impact of the removal of one target pest species on the non-target fauna will differ between the two crops. Additionally, different pest and non-target species occur in different parts of the world. Therefore, risk assessment results obtained in one country do not necessarily apply to other geographical regions.

In the last seven years, some studies have examined the safety of transgenic crops, especially the effect of these plants on the fitness of non-target organisms (Groot & Dicke 2002, Obriky *et al.* 2001). Most of the available risk assessment information relates to insect-resistant transgenic plants containing genes coding for *Bt* toxins (corn, cotton, and potato). However, the points discussed here also apply to other insect resistant GM plants.

We analyzed 21 research reports where results of 41 studies were presented (Table 2). Twenty of them were conducted in the laboratory and 21 reported field sampling of natural enemies. In some of the laboratory studies (14 cases), no adverse effects of *Bt* plants were observed. In six other studies, negative effects on fitness of certain natural enemies were reported. In 14 of 21 field studies, the authors found no differences in density of natural enemies between *Bt* and non-*Bt* crops, whereas in seven cases lower density of natural enemies was registered in the *Bt* fields. From the 21 reports, only two were related to resistant traits other than *Bt*-protein. Birch (1999), working with transgenic lectin (GNA) expressing plants, found that GNA exhibited sublethal effects on aphids, in turn affecting reproduction and longevity of beneficial coccinellid predators. Bell *et al.* (2001) evaluated the effect of trypsin inhibitor expressed in potato leaves on the gregarious ectoparasitoid *Eulophus pennicornis* (Nees) and found a decrease in parasitism rates on hosts fed on GM potato leaves. Only one study (Schuler *et al.* 1999b) evaluated the possible effects of GM plants on the searching behavior of parasitoids. In this study the authors found that *Bt*-oilseed rape leaves, in the presence of *Bt* non-resistant larvae of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), were less attractive to *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) due to the low leaf consumption rate.

The studies of Hilbeck *et al.* (1998a, b; 1999) and Bell *et al.* (2001), despite their non-conclusive results, demonstrated the importance of experiments where the three-way interaction among plants, *Bt*-protein, and herbivores can be evaluated. They found highest mortality of the natural enemy when the prey's food source was GM plants compared to protein-incorporated diets. They observed highest mortality of the predator *Crysoperla carnea* (Stephens) (Hilbeck *et al.* 1998a, b; 1999), and low parasitism rate of the ectoparasitoid *E. pennicornis* (Bell *et al.* 2001) when the prey's food source was the GM plant compared to protein-incorporated artificial diets. Hilbeck (2002) argued that this may be due to the fact that higher-trophic-level organisms might receive the compound in an altered form due to processing by the herbivores.

The data available in the literature regarding the impact of *Bt*-crops on natural enemies are inconclusive. Most laboratory studies have concentrated on some but not all aspects of the fitness of insect predators. These experiments generally did not cover the entire larval period, and were

Table 2. Effects of insect resistant GM plants on natural enemies.

Natural enemies	Host/food source/ Expressed protein	Location of study	Biological parameters and results	References
Coleoptera				
Carabidae				
<i>Lebia grandis</i> Hentz	Larvae/ <i>Bt</i> -potato (cry3A)	L ¹	Prey consumption (NA ^a)	Riddick & Barbosa (2000)
Carabidae fauna	<i>Bt</i> -corn [(cryIA(b))]	F ²	Diversity (NA) Density (NA)	Lozzia (1999)
	<i>Bt</i> -corn [(cryIA(b))]	F	Density < non-spray field ^b Density = spray field ^c	Brazil/CTNBio (1999a) ^a
	<i>Bt</i> -corn [(cryIA(b))]	F	Density = non-spray field	Brazil/CTNBio (1999b) ^β
Coccinellidae				
<i>Hippodamia convergens</i> Guérin-Ménéville	Aphids/ <i>Bt</i> -potato (cry3A)	L	Development (NA) Survivorship (NA) Reproduction (NA) Prey consumption (NA)	Dogan et al. (1996)
	<i>Bt</i> -corn [(cryIA(b))]	F	Density (NA)	Pilcher et al. (1997)
<i>Coleomegilla maculata</i> (De Geer)	<i>Bt</i> -corn [(cryIA(b))]	F	Density (NA)	Pilcher et al. (1997)
	<i>Bt</i> -corn pollen [(cryIA(b))]	L	Development (NA) Survivorship (NA)	Pilcher et al. (1997)
<i>Coccinella septempunctata</i> (L.)	<i>Bt</i> -corn [(cryIA(b))]	F	Density = non spray field	Bourguet et al. (2002)
<i>Adalia bipunctata</i> (L.)	Aphids/GNA-potato	L	Fecundity (A ^d) Eclosion rate (A) Female longevity (A)	Birch et al. (1999)
Coccinellidae fauna	<i>Bt</i> -corn [(cryIA(b))]	F	Density = non spray field	Brazil/CTNBio (1999b)
Dermaptera				
Forficulidae				
<i>Doru luteipes</i> (Scudder)	<i>Bt</i> -corn [(cryIA(b))]	F	Density > spray field Density > non-spray field	Brazil/CTNBio (1999a)
	<i>Bt</i> -corn [(cryIA(b))]	F	Density = non-spray field	Brazil/CTNBio (1999b)
Diptera				
Syrphidae				
<i>Syrphus corollae</i> (Meigen)	<i>Bt</i> -corn [(cryIA(b))]	F	Density = non-spray field	Bourguet et al. (2002)
Hemiptera				
Anthocoridae				
<i>Orius majusculus</i> (Reuter)	Thrips/ <i>Bt</i> -corn [(cryIA(b))]	L	Development (NA) Survivorship (NA)	Zwahlen et al. (2000)
<i>O. tristicolor</i> (White)	<i>Bt</i> -potato leaves (cry IIIA)	L	Longevity (NA)	Armer et al. (2000)
<i>O. insidiosus</i> Say	<i>Bt</i> -corn pollen [(cryIA(b))]	L	Development (NA) Survivorship (NA)	Pilcher et al. (1997)
	<i>Bt</i> -corn [(cryIA(b))]	F	Density = non-spray field	Bourguet et al. (2002)
	<i>Bt</i> -corn [(cryIA(b))]	F	Density (NA)	Pilcher et al. (1997)
Lygaeidae				
<i>Geocoris</i> spp.	<i>Bt</i> -potato leaves (cry IIIA)	L	Longevity (NA)	Armer et al. (2000)
Miridae				
<i>Lygus hesperus</i> Knight	<i>Bt</i> -potato leaves (cry IIIA)	L	Longevity (NA)	Armer et al. (2000)
Nabidae				
<i>Nabis</i> spp.	<i>Bt</i> -potato leaves (cry IIIA)	L	Longevity (NA)	Armer et al. (2000)
Predator Hemiptera fauna	<i>Bt</i> -corn [(cryIA(b))]	F	Density = non-spray field	Brazil/CTNBio (1999b)

continues...

Table 2. Continued...

Hymenoptera				
Braconidae				
<i>Cotesia plutellae</i> (Kurdjumov)	<i>Bt</i> -susceptible larvae/ <i>Bt</i> -oil seed rape (cry 1Ac)	L	Emergence rate (A)	Schuler <i>et al.</i> (1999a)
	<i>Bt</i> -resistant larvae/ <i>Bt</i> -oil seed rape (cry 1Ac)	L	Emergence rate (NA)	
	<i>Bt</i> -susceptible larvae/ <i>Bt</i> -oil seed rape leaves (cry 1Ac)	L	Attraction (A)	
	<i>Bt</i> -resistant larvae/ <i>Bt</i> -oil seed rape leaves (cry 1Ac)	L	Attraction (NA)	
<i>Diaeretiella rapae</i> (McIntosh)	Aphids/ <i>Bt</i> -oil seed rape (cry 1Ac)	L	Parasitism rate (NA)	Schuler <i>et al.</i> (1999b)
<i>Macrocentrus grandii</i> Goid	<i>Bt</i> -corn (cry 1Ab)	F	Parasitism rate (NA)	Orr & Landis (1997)
Eulophidae				
<i>Eulophus pennicornis</i> (Nees)	Larvae/trypsin inhibitor-potato (CpTI)	L	Parasitism rate (A) Development (NA)	Bell <i>et al.</i> (2001)
	Larvae/CpTI-artificial diet	L	Parasitism rate (NA) Development (NA)	Bell <i>et al.</i> (2001)
Ichneumonidae				
<i>Erioborus terebrans</i> (Grav.)	<i>Bt</i> -corn (cry 1Ab)	F	Parasitism (NA)	Orr & Landis (1997)
Sphecidae				
<i>Stictia</i> sp.	<i>Bt</i> -corn [(cry1A(b))]	F	Density < non spray field Density = spray field	Brazil/CTNBio (1999a)
Tachinidae				
<i>Lydella thompsoni</i> (Herting)	<i>Bt</i> -corn [(cry1A(b))]	F	Parasitism rate (A)	Bourguet <i>et al.</i> (2002)
<i>Pseudoperichaeta nigrolineata</i> (Walker)	<i>Bt</i> -corn [(cry1A(b))]	F	Parasitism rate (A)	Bourguet <i>et al.</i> (2002)
Neuroptera				
Chrysopidae				
Chrysopidae fauna	<i>Bt</i> -corn [(cry1A(b))]	F	Density (NA)	Pilcher <i>et al.</i> (1997)
<i>Chrysoperla carnea</i> (Stephens)	Larvae/ <i>Bt</i> -corn [(cry1A(b))]	L	Survivorship (A) Development (A)	Hilbeck <i>et al.</i> (1998a)
	<i>Bt</i> -artificial diet [(cry1A(b))]	L	Survivorship (A) Development (A)	Hilbeck <i>et al.</i> (1998b)
	Larvae/ <i>Bt</i> -artificial diet [(cry1A(b))/ cry2A]	L	Survivorship (A) Development (A)	Hilbeck <i>et al.</i> (1999)
	Aphids/Br-corn [(cry1A(b))]	L	Development (NA) Survivorship (NA)	Lozzia <i>et al.</i> (1998)
	<i>Bt</i> -corn pollen [(cry1A(b))]	L	Development (NA)	Pilcher <i>et al.</i> (1997)
	<i>Bt</i> -corn [(cry1A(b))]	F	Density = non-spray field	Bourguet <i>et al.</i> (2002)
<i>Hemerobius</i> sp.	<i>Bt</i> -corn [(cry1A(b))]	F	Density < spray field	Brazil/CTNBio (1999a)
Beneficial arthropofauna	<i>Bt</i> -cotton	F	Density < spray field Density < non spray field	Greenpeace Report (2002)
Predator fauna	<i>Bt</i> -cotton	F	Density (NA)	Greenpeace Report (2002)
Beneficial entomofauna	<i>Bt</i> -cotton	F	Density (NA)	Cui & Xia (2000)
Parasitoid fauna	<i>Bt</i> -cotton	F	Density (A)	Cui & Xia (2000)
Predator fauna	<i>Bt</i> -potato cry3A	F	Density (NA)	Riddick <i>et al.</i> (2000)

¹L = laboratory, ²F = field, ³NA = non affected, ⁴non-spray field = conventional crop field without insecticide control, ⁵spray field = conventional crop with insecticide control, ⁶A = affected, ^aReport submitted to the National Technical Biosafety Commission (CTNBio) by Monsanto do Brazil - File number: 01200.002995/99-54, ^bReport submitted to the National Technical Biosafety Commission (CTNBio) by Syngenta Seeds Ltd. - File number: 01200.002109/2000-04.

conducted with few individuals. In many cases, the protein doses used in the experiments were much higher than those found in the plant tissue in the field. In addition, most of

the field trials were conducted on a small scale, and over a relatively short period of time. One exception is the work by Hoy *et al.* (1998) that reported a series of results of field

sampling in *Bt* potato systems, and showed that the diversity of arthropod species and densities of predators and parasitic Hymenoptera increased with the use of transgenic potato varieties. Their overall conclusion was that transgenic resistance, by controlling primary pests without insecticides, could preserve biological control agents that suppress secondary pests (Riddick & Barbosa 2000, Riddick *et al.* 2000). Nevertheless, long-term field studies are generally limited by funding as well as the interest in moving products into the market (Shelton *et al.* 2002).

Effect on Non-Target Lepidoptera. Many species of Lepidoptera, both target and non-target, are likely to be directly susceptible to the *Bt* toxins. Studies on the ecological interactions of *Bt* insecticide sprays have documented some effects on non-target organisms. For example, as summarized by Obrycki *et al.* (2001), *Tyria jacobaeae* (L.), a beneficial lepidopteran introduced into North America for biological control of the weed tansy ragwort, increased mortality of 4th and 5th instars after feeding on tansy ragwort leaves dipped in *Bt*, in laboratory bioassays (James *et al.* 1993). *Bt* sprays can affect non-target Lepidoptera from up to 30 days after spraying (Johnson *et al.* 1995). Furthermore, a reduction in lepidopteran species richness was found two years after forest plots were sprayed with *Bt* (Miller 1990).

Likewise, many species of Lepidoptera or Coleoptera (depending on the type of *Bt* toxin expressed), both target and non-target, are likely to be susceptible to the *Bt* toxins produced by transgenic crops. Losey *et al.* (1999) showed that the larvae of the monarch butterfly, *Danaus plexippus* (L.), living in weeds near corn fields, could be affected adversely by *Bt* corn pollen drifting onto the foliage of plant species explored by the butterfly. These results have been questioned on the basis that they came from small-scale laboratory assays with high levels of toxin expressed in no-choice tests. Indeed, recent studies suggest that risks posed by current corn crops incorporating the *Bt* toxin genes to monarch butterflies are not likely to be significant (Sears *et al.* 2001). These studies show that, while *Bt* pollen does have some toxic effects when fed to butterfly larvae, the pollen densities likely to be encountered in the field are too low to pose a risk to monarch larvae. However, another study showed that low concentrations of pollen from event 176*Bt* corn, dramatically reduced growth rates among black swallowtail caterpillars, *Papilio polyxenes* F., in field tests (Zangerl *et al.* 2001). An earlier study (Wraight *et al.* 2000) noted that a widely used *Bt* corn-containing event 810, had no adverse effect on black swallowtails living on weeds near cornfields. From these results, it is reasonable to infer that a careful event selection is advisable in the development of pest-protected crops, and that research is needed on the impact of *Bt* varieties on non-target species.

Effects on Bees. The proteins present on commercial formulation of *B. thuringiensis* have been considered non-toxic against bees (Atkins *et al.* 1981, Arpaia 1996, Malone *et al.* 2001). However, different proteinase inhibitors have shown different effects on performance and behavior of worker honeybees, *Apis mellifera* L. (Picard-Nizou *et al.* 1997, Girard *et al.* 1998, Malone *et al.* 2001). The proteins

inducing resistance to pest cowpea trypsin inhibitor and β -1,3 glucanase negatively affect the behavior of honeybees (Picard-Nizou *et al.* 1997) but three proteinase inhibitors, suitable for incorporation into oilseed rape, did not affect bee behavior and caused no short-term mortality (Girard *et al.* 1998). These results showed that a case-by-case analysis is needed when evaluating the effect of proteinase inhibitors on learning performance of bees (Girard *et al.* 1998). In addition, it is necessary to test the different protein inducing resistances to insects on other bee species because, in a diverse group such as bees, it is possible that different species present different susceptibilities to these insecticidal molecules.

Caution is needed on the interpretation of results of small sets of laboratory studies of insect resistant crops, which are conducted under artificial condition, and have minor ecological relevance. Laboratory conditions do not always reproduce the complex mechanisms by which plants affect natural enemies and the detailed interactions of food webs. Furthermore, field surveys with a focus on diversity and abundance of species are needed to understand the effect of insect resistant GM crops on arthropod communities.

Fate and Consequences of Insecticidal Toxins in Soil

Bacillus thuringiensis (*Bt*) insecticides have been used for more than 30 years and they are generally considered safe for the environment. This is probably because *Bt* does not survive or grow well in natural habitats such as soil and its spores are rapidly inactivated by ultraviolet radiation (Stotzky 2002). However, when the genes that code for the production of insect toxins are genetically engineered into plants, the toxins continue to be synthesized during the growth of the plant and are present throughout the whole life cycle (Saxena & Stotzky 2000). There are other differences between *Bt*-insecticides and transgenic *Bt* plants, including the *Bt* toxin mode of action (Hilbeck 2002), that make it necessary to verify the possible impact of *Bt* crops on the soil environment, particularly on the soil microbiota. Deviations in the numbers and kinds of soil organisms may influence the fertility considerably, for example by decreasing the ability to retain water and nutrients (Christensen 1989).

Genetically modified plants that produce *Bt* toxins may release these proteins into the environment when the plants are incorporated into the soil. It has also been shown that some toxins will be released to the soil from root exudates during the entire growth of a *Bt* crop (Saxena & Stotzky 2000). In this case, a question is raised whether an increasing amount of *Bt* toxins in soils could result in novel exposure of soil organisms to these toxins, with potential negative non-target effects. A series of studies has investigated the fate of the *Bt* toxins in the soil environment and their effect on soil organisms (Stotzky 2000). The toxins released in root exudates and upon disintegration of transgenic crop residues are adsorbed rapidly, bound to elements of the soil (clay particles, humic acids) and stabilized. In this case, only briefly they will be in a free state, susceptible to rapid degradation (Saxena & Stotzky 2000, Stotzky 2002).

The cry1Ab toxin from transgenic corn released in root

exudates persists in the rhizosphere and can be active for hundreds of days (Saxena *et al.* 1999). These authors investigated the effect of cry1Ab toxin released in the root and from biomass of *Bt* corn in the total number of earthworms, nematodes, protozoa, bacteria and fungi. Results suggested that degradation of biomass of *Bt*-corn is non toxic to a variety of species used as models (US EPA 2000).

The above studies determining rates of degradation of cry proteins in soil have been of sufficient duration, and were performed under adequate conditions. However, they were essentially developed in soil microcosms. Head *et al.* (2002) investigated what happens to *Bt* toxins released into the soil from *Bt* crops under field conditions, in the state of Arizona (USA). They collected soil samples from within and outside fields where insect-resistant transgenic cotton encoding the cry1Ac gene had been grown and subsequently incorporated into the soil by post-harvest tillage for 3-6 consecutive years. These samples were analyzed by enzymatic and bioassay tests for the levels of cry1Ac protein. They found no detectable cry1Ac protein in any of the soil samples collected from within or outside the *Bt*-cotton fields.

Other studies including analyses of different types of soils under *Bt*-crops cultivation are necessary to clarify the contrasting differences observed in the persistence of *Bt* cry1Ac protein between laboratory assays and field observations. Moreover, more information on the effect on soil microbiota is needed, including possible interference with nutrient cycles and effects on ecosystem functions, although these kinds of data are still very difficult to obtain.

Change in Persistence or Invasiveness of the Crop

One of the questions most frequently raised is how the long-term performance of an insect-resistant GM crop can be predicted, as the addition of the insect resistance trait can make these plants highly competitive to the point that they can become weeds. This possibility has been investigated with a focus on how the fitness of a plant species may be altered by small changes in its genome. Ramachandran *et al.* (2000) compared the competitive ability of an insect-resistant transgenic oilseed rape variety with a non-transgenic oilseed rape variety in seed mixtures. The transgenic variety was competitively superior when the two varieties were subject to selection pressure in the form of herbivory by the diamondback moth, in greenhouse experiments and in field plots. In a similar study, Stewart *et al.* (1997) showed that, where suitable habitat is available, there is a likelihood of increased fitness in oilseed rape varieties expressing *Bt* transgenes.

However, this does not mean that the crop will necessarily become a weed, as weediness is a combination of traits such as persistence of overwintering seeds, the production of biomass, and offspring production and survival (Williamson 1993). To become a weed, the crop plants must acquire several traits that make them as competitive as the weeds in natural habitats, and these traits are controlled by a group of genes. Some authors argue that cultivated plants do not possess weed characteristics

and the addition of only one gene to their genome is unlikely to provide the aggressive properties that typify weedy plants (Baker 1974, Luby & McNichol 1995). Other authors think that small genetic changes can cause large ecological alterations (Fitter *et al.* 1990, Williamson *et al.* 1990). Besides, some cultivated plants are known to possess invasive characteristics and are even considered weeds in some circumstances, such as the grass *Brachiaria decumbens* Stapf, in Brazil. Experience with traditional plant breeding has shown that plant protection against herbivores may tremendously increase seed production. This experience is an indication that insect resistant traits could have significant effects on persistence and invasiveness, and that insect resistant crop varieties should continue to have a rigorous case-by-case risk assessment.

Studies on the comparison between transgenic and non-transgenic crops also consider that any additional genetic material, which provides a benefit, will also carry a metabolic cost (Hails *et al.* 1997). The genetic baggage hypothesis (Regal 1988) predicts that, in the absence of selection pressure, which causes the transgenic plant to be favored, the additional cost of metabolising the detoxifier could cause the transgenic to be less competitive than the wild type. Other considerations related to the cost of transgenes refer to the uncontrolled position in which the genes have been integrated into the genome, and the possibility that some traits may result in features, which will be of selective advantage in particular habitats (Hails *et al.* 1997).

Transfer of Insect Resistance to Weeds or Feral/Wild Plants

It is known that in nature there is a genetic flow among plants of the same species and among plants of related species. There is a body of scientific evidence that genetic material introduced into certain species of crops will recombine with related weed species (Ellstrand 1999, Dale *et al.* 2002). Gene flow from crops to wild relatives is often cited as a potential risk in the commercialization of transgenic crops, based on the possibility that the introduced genes may be transferred by pollen to wild or weedy relatives and may produce offspring that will become more weedy or invasive.

The exchange of genes between weeds and crops is a natural phenomenon, but genetic engineering raises additional concerns because it enables the introduction into the ecosystem of genes that confer novel fitness-related traits. Furthermore, it allows novel genes to be introduced into many diverse types of crops, each with its own specific potential to outcross. Snow (2002) reported the results of a recent workshop held at the Ohio State University¹. The studies showed that transgenes disperse and become incorporated into the genomes of other species in the same manner as do other crop genes and that gene flow can be very widespread. It is now evident that some crops can pass genes to a wild relative even when those genes are carried on unshared (non-homologous) chromosomes. Snow (2002) also reported that a range of possible fitness costs and benefits has been

¹The Scientific Methods Workshop on Ecological and Agronomic Consequences of Gene Flow from Transgenic Crops to Wild Relatives, March-5-6, 2002, Ohio State University, Columbus, OH.

associated with particular GM traits, and that under some conditions, single or multiple transgenes will not have any detectable effect on the survival or reproduction of wild or weedy species. If there are effects, they may be difficult to detect unless weed populations are released from strongly limiting factors such as drought stress, salinity or herbivores.

This means that newly introduced genes could disperse into nearby populations, bringing new phenotypic traits such as resistance to insects, diseases, herbicides or harsh growing conditions. Data presented at the OSU meeting, indicated that wild sunflowers containing a *B. thuringiensis* gene for lepidopteran resistance can result in a large production of seeds. As Snow (2002) puts it, "when novel genes spread to free-living plant population, they have the potential to create or exacerbate weed problems by providing novel traits that allow these plants to compete better, produce more seeds and become more abundant."

Enhanced understanding of this process and, more importantly, of the impact of crop gene introgression into populations growing on roadsides, field margins, or uncultivated areas is needed as GM crops continue to be adopted. In view of these results, it becomes necessary to raise information about gene flow from transgenic plants to wild and weedy species in Brazil, aiming at the elaboration of regulatory measures to reduce the probability of unexpected and undesirable events.

Development of Resistance to *Bt* Toxins in Pests

Historically, pests have rapidly adapted to the techniques used to control them. The experience with chemical pesticides has proven to be disappointing, as pests quickly evolved resistance to them (Raymond *et al.* 1991, Gould 1991). More recently, several studies have shown that pests can also adapt to toxins produced by the bacteria *B. thuringiensis* under field and laboratory conditions, including resistance to *Bt* transgenic crops (Tabashnik 1994, Obryki *et al.* 2001).

One of the greatest concerns is that the widespread use of *Bt* crops could lead to the evolution of a number of important pest insects that are resistant to the *Bt* biopesticides. That is of particular concern to organic farmers because they use *B. thuringiensis* as a natural pesticide. Development of resistance to *Bt* crops among population of serious pests also brings concerns to the long-term use of the technology itself, as it may lose its effectiveness as a tool to control these pests.

Several strategies for resistance management have been proposed to delay the chances of pest population adaptations to *Bt* crops (Gould 1998). The most widely used is the high-dose-refuge strategy, which has been implemented in North America (Alstad & Andow 1995). In general, it is recommended that a 20-50% refuge area be planted with non-GM varieties. In the case of *Bt* corn, it is recommended that a minimum of 20% of the area be planted with conventional varieties; and in fields where *Bt* corn is planted where cotton has been previously cultivated, at least 50% of the area must be planted with conventional varieties of corn, to avoid the development of insect pest populations resistant to *Bt* (Cannon 2000).

The strategy favored by the industry of producing

transgenic varieties expressing high doses of different toxins, associated with the tactics of refuges, seemed at first to be a good idea. This strategy, however, was not prove to be efficient, due to factors such as polyphagy among insect pests that also feed on other plants including weeds, or to the movement among different cultivated fields by some insect pests. When the pests move to non-transgenic fields they are exposed to low to moderate doses of the toxin, which prevents the desirable effects of high-dose exposure (Gould 1998). Another approach to delay the evolution of resistance much more effectively is the use of additional pesticide gene, called transgene pyramiding. The new generation of GM pest resistance crops already contain two insecticidal genes. The cotton variety sGK commercially available in China contain the *cry1A* gene and the *CpTI*, the cowpea trypsin inhibitor gene (Shelton *et al.* 2002).

Implementing resistance management practices when a pest protection substance or its functional equivalent are providing effective pest control, or when there is a threat to the utility of existing uses of the pest protection substance (e.g., *Bt* proteins) is crucial for obtaining the greatest benefits from pest resistance transgenic crops, and for allowing the continued use of *B. thuringiensis* biopesticides.

Concluding Remarks and Future Perspectives

The use of toxin by plants for defense against pests is a common phenomenon in nature. There is good evidence from traditional breeding varieties that plant resistance can reduce the use of broad-spectrum pesticides (NRC 2000), as it allows naturally occurring pest predators and parasites to act and suppress secondary pest populations (Hoy *et al.* 1998). Science-based solutions to some difficulties have yet to be sought to achieve the ecological benefits of GM pest resistant crops. Among them is the incompatibility of the high concentration and presence of the pesticidal toxin in all parts of the plant during the entire season. This fails to comply with the very principle of integrated pest management (IPM), which predicts the application of control measurements only when pest populations reach the economic injury level. It is likely that in the future, greater attention will be given to aspects of resistance mechanism that are sharply targeted to inhibit the pest of interest and that is active only during the phase of plant growth when the protection is mostly needed. Tissue-specific gene promoters expressing the insecticidal proteins only in structures attacked by the pests are being developed to reduce risks to human health and to enhance the environmental safety of the new GM pest protected varieties.

The possible interference on food webs and on non-target organisms is another issue for careful consideration in tropical areas, especially if the varieties were developed aimed at the control of insects that are mainly pests in temperate regions. Different pest and non-target species occur in different parts of the world, and the management of the crop as a whole will also differ among different geographical locations. Thus, risk assessment results obtained in one country do not necessarily apply to another geographical region. Furthermore, a case-by-case approach is necessary and each GM plant and

ecosystem must be looked at separately until there is sufficient experience to allow some extrapolation of the data and a wider view and interpretation of the environmental impacts.

Although the data presented in the literature regarding the impact of *Bt*-crops on natural enemies and biodiversity are, in general, not conclusive, they tend to support the idea that transgenic resistance, by controlling primary pests without insecticides, could preserve natural biological controls acting upon secondary pests. However, more information is needed on three-trophic level interferences. Effort should be given to evaluate the utility of community diversity studies for risk assessment, particularly those broad studies that include all possible species without prior judgments of what will be the relevant data to approach key ecological questions. Furthermore, some impacts of GM crops will be scale dependent and this emphasizes the need to extend impact assessments beyond small-scale field experiments to farm scale, and eventually to commercial scale impact monitoring, to detect cumulative effects over time (Marvier 2001). This is consistent with the CTNBio² regulation on post-commercialization monitoring of the Roundup Ready[®] soybean.

Cross-pollination between sexually compatible crops and between GM crops and native/weedy species is a highly probable event. Hybridization between GM crops and weeds could produce more persistent weeds, especially through genetic modifications that confer selective advantage such as reduced herbivory. To fully assess these risks, studies are needed on the ecology of potential transgene recipient populations to assess the consequences of the transfer of the insect resistant trait. This issue will need to be examined carefully in the GM crop regulatory approval process.

Based on past experience with traditional plant breeding and on field and laboratory studies with molecular breeding, problems related to the development of resistance in the pest populations can be anticipated. Pests have historically overcome resistance introduced into crops by traditional breeding programmes, and adaptation to *Bt* toxins in some Lepidopteran species induced by the *Bt* biopesticide sprays has also been observed. Currently available data show that resistance to GM *Bt* crops will also occur. Furthermore, there are field observations showing that outbreaks of secondary pests will also occur. These two effects tend to be accentuated in tropical regions, as in Brazil, where more than one harvest of the same crop is often obtained in one year (for instance, for corn and soybean). Additionally, insect species usually have a larger number of generations in a year than in temperate regions. In this case, there will be strong selection pressure due to the presence of high toxin doses during the whole plant's life cycle. Thus, in tropical agriculture, the above indirect undesirable impacts become not only a threat but also highly probable events. The development and implementation of pest resistance management programs is imperative based on different strategies as crop management and improvement of biological control methods and the insertion of stacking or different resistance genes. The use of refuges planted with conventional cultivars to maintain susceptible genes in pest populations is one crop management

alternative that has been used in the United States.

In assessing the environmental impact of genetically modified pest resistant crops we should acknowledge the fact that agriculture inevitably has an impact on the environment. We should also deal with the question of what is a reasonable trade-off between crop production, wildlife and the non-agriculture demands of the human population. It is especially relevant to take a baseline for comparison in the case of pest resistant crops, as current practices in conventional agriculture involves an intensive use of chemical pesticides. In this case, we must take into account the environmental damage caused by the use of pesticides in agriculture. In the United States alone, millions of birds and billions of insects, both harmful and beneficial are killed each year as a result of pesticide use (Pimentel & Raven 2000).

Recent studies documenting negative impacts indicate that the risk assessment conducted internationally during the registration process for governmental approval of transgenic pest resistant crops may be overlooking some subtle and complex ecological effects on several trophic levels within and outside crop fields. In Brazil, the National Technical Biosafety Commission is evaluating two varieties of *Bt* corn for commercial release, and has approved applications for field tests of *Bt* cotton. Other varieties, including transgenic soybeans resistant to *Anticarsia gemmatilis* Hübner are in the pipeline. Little is known about the non-target species occurring in and around crop fields that might be affected by the use of this technology. Research in this area is urgently needed and should be encouraged by the governments through public research funding agencies.

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²National Technical Commission on Biosafety, the leading biosafety regulatory body in Brazil.

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