

Bt maize and mycotoxins: the current state of research

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Received 28 July 2005 / Accepted 13 June 2006

Abstract - The commercialisation of Bt (*Bacillus thuringiensis*) maize expressing the Cry endotoxin specific for some types of Lepidopteran and Coleopteran pests has lead researchers to study the reduction in mycotoxin concentrations in Bt hybrids compared to the correspondent isogenic plants. Indeed, insect damage is one of the main inoculation pathways of mycotoxinogenic moulds. The present study aims to evaluate, according to the scientific literature published to date, the real efficiency of Bt maize hybrids in reducing the mycotoxin problem. The results obtained from the analysis of the literature do not show significant variations in the content of aflatoxins, zearalenone and trichothecenes, between Bt hybrids and corresponding isogenic control plants. The only mycotoxins where Bt hybrids have any effect are the fumonisin group, but even in this case studies in commercially planted fields have shown that their effect is mitigated by many biotic and abiotic factors.

Key words: Bt maize, mycotoxins, moulds, *Ostrinia nubilalis*, food.

INTRODUCTION

Mycotoxins are products of the secondary metabolism of some filamentous fungi that, once absorbed by human and animal organisms, can be metabolised in the targeted tissues and display, in this way, their toxic effect. The toxic response is variable, depending on the affected tissue, the quantity digested, the animals involved, the type of mycotoxin and various other factors, and in the worst instance leads to cancer affecting the relevant organs (Coulombe, 1993; Creppy, 2002; Bennett and Klich, 2003). The presence of mycotoxins can considerably limit the commercial value of the products and cause major economic loss.

Mycotoxin producing moulds are ubiquitous and therefore can be found in almost every crop of agricultural interest and at any part of the food chain. The major mycotoxins found in cereals (aflatoxins, fumonisins, trichothecenes, ochratoxin A and zearalenone) are produced by three genera of fungi: *Aspergillus*, *Penicillium* and *Fusarium*.

For some groups of mycotoxins, European law does not establish precise limits but provides guidelines recommending that their concentration be minimized. In this case, prevention throughout the different levels of the food chain is of fundamental importance and calls for an integrated approach promoting the use of good agricultural and productive practices aimed at preventing the fungal attack (cor-

rect agronomic practices, suitable varieties, reaping time, correct drying, adequate hygiene and sanitary conditions during transportation, storage, silage and processing, analytic controls of in-coming raw materials, and so on). However, mould characteristics and their complex mechanisms of interaction with the environment, make crop defence from fungal disease extremely difficult. In fact, interactions between plants and pathogenic fungi are extremely varied, not only in relation to the fungal penetration modality, but also due to the complex diversification and fluctuations of the fungal population that occur in space and time, as a consequence of climatic and environmental variations and of the different crops present in the field.

Currently, European legislation regulates certain groups of mycotoxins on the basis of specific product typologies while for others it has not established precise limits (Table 1).

A key point in the debate between the different actors involved in the food chain is that relating to the possibility of resolving the problem of mycotoxins in foodstuff raw materials by using Genetically Modified Plants (GMPs). Thanks to the biotechnological integrated approach aiming at the production of plants resistant to insects, to pathogenic fungi, and the ability to detoxify mycotoxins, it should be possible to eliminate respectively the inoculation vector, the pathogen producers of mycotoxins and the same toxins, that is to say, to reduce the mycotoxin problem at least until harvesting time (Duvick, 2001; Munkvold and Desjardins, 1997; Munkvold and Hellmich, 1999; Munkvold, 2003).

The commercialisation of Bt (*Bacillus thuringiensis*) hybrids expressing the Cry endotoxin specific to some types of lepidopteran pests, such as the European Corn Borer

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TABLE 1 – Characteristics of maize pathogenic fungi and EC legal limits for mycotoxin content

Species	Growing conditions (t, RH, KH)*	Mycotoxin	Contaminated products	Health effects	Legal limits
<i>Aspergillus flavus</i> <i>Aspergillus parasiticus</i>	t: 10-42 °C t Opt.: 32 °C RH: 82% KH: 16-30%	Aflatoxin B1 (M1), B2, G1, G2	Maize, cereals, peanuts, cotton seeds, nuts, soybean, coffee, figs, milk and milk-derived products	Hepatotoxic Reduce body weight Reduce growth efficiency Hemorrhagic necrosis Carcinogenics Genotoxic	Food: - Reg. 466/01/EC of 08-03-2001 - Reg. 2174/03/EC of 12-12-2003 - Reg. 683/04/EC of 13/04/2004 Feed: - Dir 29/1999/EC of 22-04-1999
<i>Aspergillus ochraceus</i> <i>Penicillium sp.</i>	t: 5-35 °C t Opt.: 28 °C RH: >80% KH: 16-20%	Ochratoxin A	Maize, cereals, peanuts, coffee, cocoa, grape, raisin, dried fruit, wine, spices, cheese, conserved pork meat	Hepatotoxic and kidneys toxicity	Food: - Reg. 472/02/EC of 12-03-02 - Reg. 683/04/EC of 13-04-2004
<i>Fusarium graminearum</i> <i>Fusarium culmorum</i> <i>Fusarium sporotrichioides</i>	t: 4-35 °C t Opt.: 25 °C RH: 94% KH: 20-21%	Deoxynivalenol (DON)	Maize, cereals	Gastrointestinal disturbance (vomiting, diarrhoea, inflammation) Decrease food intake Reproductive alteration Hemorrhagic necrosis Reduce growth Alimentary toxic aleukia	Food: - Reg. 856/2005/EC of 06-06-2005
<i>Fusarium verticillioides</i> (<i>moniliforme</i>) <i>Fusarium proliferatum</i>	t: 4-36 °C t Opt.: 25 °C RH: 91% KH: 18-20%	Zearalenone Trichothecenes (T2 - HT-2) Fumonisin (B1, B2)	Maize, cereals	Suspected human oesophageal cancer Hepatic toxicity ELEM syndrome Pulmonary oedema in swine Reduce growth	Food: - Reg. 856/2005/EC of 06-06-2005

* t: temperature, °C; RH: relative humidity, %; KH: kernel humidity, %.

(*Ostrinia nubilalis*) and Corn Earworm (*Helicoverpa zea* Bodie), has given researchers the opportunity to study the real reduction of mycotoxins obtainable through the use of Bt plants compared to the correspondent isogenic ones.

In fact, damage to the ear or stalk resulting from insect feeding provides sites for the penetration of the fungi, and some insect pests also serve as vectors for mycotoxigenic fungi (Sobek and Munkvold, 1999). Experimental studies show that insect resistant transgenic plants have lower fumonisin content than the correspondent isogenic lines. Other studies have shown instead that several abiotic and biotic factors could mitigate such efficiency.

The debate is divided between those who hold that Bt technology can be a good strategy to considerably reduce this problem, and those who state that the best way is to use good agricultural and productive practices.

THE CHARACTERISTICS AND SPREAD OF BT MAIZE

The Gram-positive bacterium *Bacillus thuringiensis* is found in the soil and during the stationary phase of its life cycle forms a crystalline paraspore known as Cry which is toxic to some insect species and nematodes (Griffiths *et al.*, 2005). By means of genetic engineering, the genes that codify the insecticide protein, when isolated from the bacterium can be modified in the laboratory and inserted into the genome of the maize plant. Thanks to this modification, the Bt plants

are able to produce a toxin in a form which is directly active and which does not need to be subjected to a biotransformation to render it toxic to other organisms that possess the specific receptors for the toxin. Indeed, in nature the protein expressed by the *cry* genes is a protoxin that, to carry out its toxic activity, needs to be activated by the digestive enzymes found in the midgut of insects which feed on them. Once ingested, the Cry protein attaches itself to the intestinal lining of the insect and destroys its cells. Within two hours of ingesting the tissue of Bt maize, the receptive pests stop feeding and die within two or three days. To date, numerous genetic sequences have been identified which produce toxic proteins which can act on different insect orders such as: *Lepidoptera*, *Diptera*, *Coleoptera*, *Orthoptera*, *Mallophaga* and *Imenottera* (Schnepf *et al.*, 1998).

Various maize hybrids currently on the market have been engineered with differing transformation events. In most cases, the codifying genes of the Cry protein have been placed under the control of a constitutive promoter able to activate the toxin in all parts of the plant, including the ear of the maize. In other cases, the genes are regulated by specific promoters, which are activated in specific tissues of the plant. Some of these hybrids moreover possess genes which are different from the *cry* genes, such as the *pat*, *bar* and *epsps* genes expressing tolerance to glufosinate and glyphosate based herbicides (Table 2).

TABLE 2 – Bt maize events authorised for cultivation. Source: Agbios, www.agbios.com

Event name	Original developer	Inserted gene	Approval for cultivation	
			Principal country	Year
176	Ciba-Geigy	<i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>bar</i> (phosphinothricin N-acetyltransferase) from <i>Streptomyces hygrosopicus</i> <i>bla</i> (beta-lactamase)	Argentina	1996
			Canada	1996
			Japan	1996
			United States	1995
			European Union	1997
BT11	Syngenta Seeds, Inc.	<i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>bar</i> (phosphinothricin N-acetyltransferase) from <i>Streptomyces hygrosopicus</i>	Argentina	2001
			Canada	1996
			Japan	1996
			United States	1996
CBH-351	Aventis CropScience	<i>cry9C</i> from <i>Bacillus thuringiensis</i> subsp. <i>tolworthi</i> <i>bar</i> (phosphinothricin acetyltransferase) from <i>Streptomyces hygrosopicus</i> <i>bla</i> (beta-lactamase)	United States	1998
DBT418	Dekalb Genetics Corporation	<i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>bar</i> (phosphinothricin N-acetyltransferase) from <i>Streptomyces hygrosopicus</i> <i>bla</i> (beta-lactamase)	Argentina	1998
			Canada	1997
			Japan	1999
			United States	1997
MON80100	Monsanto Company	<i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>epsps</i> (5-enolpyruvylshikimate-3-phosphate synthase) from CP4 strain of <i>Agrobacterium tumefaciens</i>	United States	1995
MON802	Monsanto Company	<i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>epsps</i> (5-enolpyruvylshikimate-3-phosphate synthase) from CP4 strain of <i>Agrobacterium tumefaciens</i>	Canada	1997
			Japan	1997
			United States	1997
MON809	Pioneer Hi-Bred International Inc.	<i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>epsps</i> (5-enolpyruvylshikimate-3-phosphate synthase) from CP4 strain of <i>Agrobacterium tumefaciens</i>	Canada	1996
			Japan	1997
			United States	1997
MON810	Monsanto Company	<i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>	Argentina	1998
			Canada	1997
			Japan	1996
			United States	1997
			South Africa	1997
			European Union	1998
MON863	Monsanto Company	<i>cry3Bb1</i> from <i>Bacillus thuringiensis</i> subsp. <i>kumamotoensis</i>	Canada	2003
			United States	2003
TC1507	Mycogen; Pioneer (Dupont)	<i>cry1Fa2</i> from <i>Bacillus thuringiensis</i> var. <i>aizawai</i> <i>pat</i> (phosphinothricin N-acetyltransferase) from <i>Streptomyces viridochromogenes</i>	Canada	2002
			Japan	2002
			United States	2001
MON 863 x MON 810	Monsanto Company	<i>cry3Bb1</i> from <i>Bacillus thuringiensis</i> subsp. <i>kumamotoensis</i> <i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>	Argentina	1999
			Canada	NA*
			Japan	NA*
			United States	2000
MON810 x NK603	Monsanto Company	<i>cry1Ab</i> from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>epsps</i> (5-enolpyruvylshikimate-3-phosphate synthase) from CP4 strain of <i>Agrobacterium tumefaciens</i>	Argentina	Single event authorised
			Australia	
			Canada	
			Japan	
			United States	
DAS-06275-8	DOW AgroSciences LLC	<i>cry1F</i> from <i>Bacillus thuringiensis</i> var. <i>aizawai</i> <i>pat</i> (phosphinothricin N-acetyltransferase) from <i>Streptomyces viridochromogenes</i>	United States	2004

* NA: data not available.

According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA), 11.2 million hectares of the world's surface are used to cultivate Bt maize, which equates to 14.9% of all cultivated area given over to the growing of genetically modified crops (which, in 2004, was estimated at 81 million hectares). Worldwide, 14 countries cultivate Bt maize, the main producers being the United States, Argentina and Canada. In Europe, hybrids of Bt maize were approved for cultivation before the moratorium in 1998, which blocked new approvals of transgenic cultivation. Thanks to Directive 2001/18/CE, which reopened the argument concerning Genetically Modified Organisms (GMO), 17 maize hybrids (MON810) have been added to the European common catalogue of GM plant and seed.

Apart from Germany where approximately 100 hectares have been planted for research purposes, Bt maize cultivation in Europe is currently limited to 58000 hectares in Spain (James, 2004).

The following analyses the studies carried out to date to evaluate the possible positive effects of mycotoxin concentrations in kernels due to the use of Bt hybrids. The analysis seeks to clarify the study results to quantify the usefulness of Bt hybrids in limiting the problem of mycotoxins during the maize productive cycle.

EXPERIMENTAL DATA ABOUT MYCOTOXIN CONTENT IN BT CROPS

The studies we examined used experimental field trial data; indeed, to date only one piece of research (Dowd, 2001) has been carried out in commercially cultivated fields.

In some Bt hybrids (MON810, MON802 and Bt11) it was observed that the Cry protein was expressed in all the plant's

tissues (including the maize ear), whereas in others (Bt176), the Cry protein was expressed only in the green tissues. The majority of the hybrids analysed had been transformed with *cry1Ab* genes deriving from *Bacillus thuringiensis* subsp. *kurstaki* that make the plant resistant to some Lepidoptera, amongst which *Ostrinia nubilalis*.

The studies looked at the comparison between the Bt hybrids and the corresponding isogenic plants; in particular, resistance to infesting insects was analysed, as well as resistance to pathogenic fungi and the concentration of mycotoxins in the maize grains. None of the studies related data on mycotoxin concentration in the maize hybrid grains treated against European corn borer (ECB). If one excludes the study carried out in commercial fields, the studies can be classified into two groups: the first group consists of small scale experimental tests where the evidence was gathered by means of infestation or inoculation, both natural and artificial, by insects (*Ostrinia nubilalis*) and pathogenic fungi (*Fusarium verticillioides* and *Aspergillus flavus*); the second group concerns work undertaken under natural conditions, which in general has foreseen a wider surface (equal or greater than 0,4 ha for each hybrid pair tested) (Table 3). Artificial infestation was conducted by dispensing ECB larvae on the plant leaves to simulate first generation (plant growth stage V8-V10) and second generation (plant growth stage R1) of *Ostrinia nubilalis*. Artificial infestation of moulds was conducted in different ways. In some studies where artificial infestation of larvae had been foreseen, spore infection took place by inoculating ECB larvae that had previously been immersed in a fungal spore solution (Munkvold et al., 1999). In other cases, the infestation was carried out by placing the spores in the top-ear leaf (Dowd, 2000; Maupin et al., 2001; Williams et al., 2003) or by distributing the infected material between treatment rows (Odvody et al., 2000).

TABLE 3 – Experimental works on insect infestation and moulds inoculation

Country	Year	Type of trials	Tested GM events (No. of locations)	Insect infestation*	Moulds inoculation [§]	Reference
Italy	1997	EF	2	NI	NP	Masoero et al., 1999
Italy	1997	EF	2 (3)	NI	NP	Pietri and Piva, 2000
	1998		2 (4)			
	1999		4 (30)			
France/Spain	1999	EF	2 (5)	NI	NP	Bakan et al., 2002
Germany	1999	EF	7 (9)	MI and NI	NP	Valenta et al., 2001
Germany	1999	EF	5 (4)	MI and NI	NP	Magg et al., 2002
	2000		5 (5)			
US	1995	EF	2	MI and NI	NP	Munkvold et al., 1999
	1996	EF	4	MI and NI	NP	
	1997	EF	6	MI and NI	MP and NP	
US	1996	EF	1	MI and NI	MP and NP	Dowd, 2000
	1997		3			
	1998		3			
US	1998	EF	NS	MI and NI	MP and NP	Munkvold et al., 2000
	1999					
US	1998	CF	5 (3)	NI	NP	Dowd, 2001
	1999		5 (3)			
US	1999	EF	9 (2)	NI	MP and NP	Odvody et al., 2000
	2000		9 (3)			
US	2000	EF	12 (2)	NI	MP and NP	Maupin et al., 2001
US	2000	EF	5	MI and NI	MP and NP	Williams et al., 2003
	2001		5			
US	2000	EF	NS	MI and NI	NP	Hammond et al., 2004
	2001					
	2002					

* *Ostrinia nubilalis*; [§] *Fusarium verticillioides* and *Aspergillus flavus*. US: United States; EF: Experimental fields; CF: Commercial fields; NS: Not specified; NI: Natural infestation; MI: Manual infestation; NP: Natural inoculation; MP: Manual inoculation.

Effects of Bt maize on mycotoxin

The analysis of the mycotoxin content of the Bt hybrid kernels reported in the studies allows us distinguish two groups of mycotoxins: those whose reduced concentration levels are correlated with the presence of Bt maize (fumonisins) and those (aflatoxins, trichothecenes and zearalenone) where there is no such correlation.

The effects of the Bt hybrids will therefore be dealt with separately according to each group.

Effects of Bt maize on aflatoxins, trichothecenes and zearalenone

The analysis of the studies does not show a reduction in aflatoxins, trichothecenes and zearalenone content in the Bt maize hybrid grain samples when compared to isogenic maize grain. Indeed, in addition, the values of these mycotoxins in the different hybrids analysed were found to be extremely variable and in the majority of cases no statistically significant variations were found between Bt hybrids and correspondent isogenics. Even when comparing the data obtained from studies undertaken in similar experimental conditions, the results obtained differed widely. For instance, one study carried out in artificial conditions in Germany by Valenta *et al.* (2001) regarding deoxynivalenol (a trichothecene), found concentrations in Bt maize which were inferior to the corresponding isogenics, but another study, also undertaken in Germany under the same conditions by Magg *et al.* (2002), found no such differences, although the mean DON concentration was significantly ($P < 0.05$) lower in Bt hybrids than isogenic ones due to the high concentration of "Transal", the isogenic counterpart of "Transal Bt". The only study conducted in natural conditions that revealed significant DON concentration differences is Bakan *et al.* (2002). In this study, three out of five trials done in France (3 trials) and Spain (2 trials), had Bt hybrids showing a DON mean which was lower than the corresponding isogenics, whereas the other two trials had inconsistent results: one of them showed no difference in DON mean levels, whereas the other showed a DON mean concentration which was significantly higher ($P < 0.01$) in the Bt hybrids than in the corresponding isogenic plants. The studies regarding the other trichothecenes (nivalenol) produced similar results to those obtain for DON levels (Bakan *et al.*, 2002; Magg *et al.*, 2002).

Only in Williams *et al.* (2003), inferior concentrations of aflatoxins were found in the transgenic hybrids compared to the isogenics. Other analysed studies did not find significant variations in the aflatoxin content amongst the various hybrids. In addition, Odvody (2000) shows a higher concentration of aflatoxins in the Bt hybrids compared to conventional hybrids in the two 1999 locations.

As regards zearalenone, Pietri and Piva (2000) and Valenta *et al.* (2001) showed no significant differences between the Bt and isogenic plants, whereas Bakan *et al.* (2002) showed significant differences in only one location whereas the other four locations displayed no such differences.

The inefficacy of Bt hybrids in reducing aflatoxins, trichothecenes, and zearalenone is due to the small correlation between the damage caused by ECB attack and the infection by fungal pathogens that produce these mycotoxins. These types of mould use, apart from the wound created by the insect, different penetration pathways. The resultant mycotoxins produced can be found in high concentrations even when there has been no infestation by *O. nubilalis*.

Therefore, favourable climatic and environmental conditions have been shown to significantly influence their production of mycotoxins (Magg *et al.*, 2002). For instance, the infection by *Fusarium graminearum*, one of the principal producers of zearalenone and trichothecenes such as deoxynivalenol, is favoured by relatively cold and damp climatic conditions. In addition, this fungus can also penetrate the plant via the silk ear of the maize. This explains why there can be high concentrations of mycotoxins even in the absence of damage caused by insects.

This factor, together with the marked fluctuations in the population of fungal pathogens, can be considered amongst the principle causes of the variability experienced in the studies.

The only studies, previously cited, which have noted differences in aflatoxin and trichothecene levels were those conducted by artificially inoculating the larva and/or the spores of fungal pathogens. This type of analysis, however, can not be considered representative of Bt maize efficiency in a real cultivation context where, as will be described later, numerous abiotic and biotic factors can influence fungal attack and the consequent genesis of mycotoxins.

Effects of Bt maize on fumonisins

The various studies carried out on an experimental level, both under artificial and natural conditions have shown a reduction in fumonisin content in the kernels of the transgenic hybrids compared to the corresponding isogenic control group (Masoero *et al.*, 1999; Munkvold *et al.* 1999; Pietri and Piva, 2000; Bakan *et al.*, 2002; Hammond *et al.*, 2004).

The decrease in fumonisin concentration in the maize ear of the GM plant compared to the corresponding isogenic one, as demonstrated in the data obtained from the experimental fields, is due to the more significant correlation that exists between the ECB infestation and the infection of *Fusarium verticillioides* (*moniliforme*) that is the principal producer of fumonisin. Indeed, the damage caused to the maize ear by the second generation of the ECB larvae is the preferred site of penetration of *F. verticillioides*, the infection of which is favoured by hot and damp climatic conditions during the phase from flowering to harvesting (the same conditions are also ideal for the infestation by ECB larvae). For this reason, when the damage caused by the infestation of *O. nubilalis* is the principal cause of penetration, the defence from such insects leads to a lowering in the concentration of fumonisin in the maize ear. The use of late hybrids and delays in harvesting are factors that favour this type of positive interaction. However, even though the data evidence a decreasing trend in the concentration of fumonisins, the majority of the studies have been carried out on an experimental level which does not represent real field conditions. Indeed, whereas differences in mycotoxin content between Bt hybrids and isogenics were often significant in the experiments where insect larvae were artificially inoculated, these differences were lower and in many cases not significant if one restricts the data to only non-inoculated hybrids.

For example, in Munkvold *et al.* (1999), during all three years of the experiments (1995, 1996, 1997), significant differences were found in the plot with artificially inoculated larvae in the total fumonisin content of Bt hybrids compared to the correspondent isogenic plants. On the other hand, if one looks only at the experiments, which did not use artificial inoculation, all but one of, the hybrids show no significant differences.

Moreover, it was noted that transgenic hybrids showed a level of total fumonisin concentration which was above the proposed 2000 ppb limits for total fumonisin concentration in unprocessed maize (Commission Regulation (EC) n. 856/2005 of 6 June 2005), although, in some of these cases, there were significant differences between Bt hybrids and the corresponding isogenic plants (Pietri and Piva, 2000; Hammond *et al.*, 2004). On the latter point, we note that studies carried out in northern Italy (Masoero *et al.*, 1999; Pietri and Piva, 2000), where strong ECB attacks occurred, showed

that fumonisin concentrations were greater in the isogenic hybrids than in the transgenic ones, which would tend to confirm the high correlation between ECB infestations and infections by *Fusarium verticillioides* (Table 4). However, we also note that the correlation between ECB infestations and infection by fungi of the *Fusarium* genera, including *Fusarium verticillioides*, was not confirmed by the study in Magg *et al.* (2002), which shows mycotoxin concentrations with no significant differences between insecticide-protected plots and ECB-infested plots.

TABLE 4 – Differences in fumonisin levels in studies conducted in Italy

Reference	Year	Fumonisin content (ppb)		Maximum level (Reg. 856/2005/EC)	
		Bt maize	Isogenic	Product	Limit (ppb)*
Pietri and Piva, 2000	1997	2.021	19.759	Unprocessed maize	2.000
	1998	5.448	31.632	Maize grits, maize meal and maize flour	1000
	1999	1.394	3.902	Maize-based foods for direct consumption	400
Masoero <i>et al.</i> , 1999	1997	1.970	20.050	Processed maize-based foods for infants and young children and baby food	200

* If no specific level is fixed before 1 October 2007.

Factors limiting self-protection of Bt crops against fungal infections

The data emerging from the studies, including those carried out in fields of commercially grown maize (Dowd, 2001) and therefore more representative of a real growing context, show that there are factors which can mitigate the efficiency of Bt hybrids in the reduction in fumonisins and, more generally, in mycotoxins. These factors are:

- the type of insert and the transformed parental lineage;
- the predominant insect populations and pathogenic fungi and the interaction between them and the plant;
- climatic and environmental conditions;
- cultural practices;

The influence of the insert and the transformed parental lineage

Hybrids, which express the Cry protein in all parts of the plant's tissues, including the maize ear, were shown to be more effective in defending the plant from ECB infestation compared to those hybrids in which the protein was expressed only in certain tissue and, in general, show a smaller concentration of fumonisins. This argument, however, cannot be applied universally: indeed, in Munkvold *et al.* (1999), although hybrids MON810 and MON802 both express CryIA(b) in their kernels, the former exhibited reduced fumonisin concentrations whereas the latter did not.

Hybrids, which express the gene at a low level in the maize ear, show more variability in reducing damage and, in general, are more efficient when the infestation occurs later (Munkvold *et al.*, 1999; Dowd, 2001).

The level of control from the infestation of *Helicoverpa zea* was shown to be variable, ranging from an almost total control to almost nothing (Dowd, 2001).

The predominant insect populations and fungal pathogens and the interaction between them and the plant

The expression of the Cry protein only limits the presence of *Ostrinia nubilalis* and, in part, *Helicoverpa zea*, but can-

not interfere with other insects, which can aid the penetration of pathogenic fungi in the plant. In fact, the higher levels of fumonisin reduction in Bt plants are registered only in the presence of the insect *O. nubilalis*. Such effect is seriously reduced in the presence of *H. zea*, the larva showing a susceptibility to the Bt protein which varies from one population to another in differing geographical distributions (Dowd, 2000, 2001).

ECB larval feeding is of secondary importance for initiating a successful *Fusarium* inoculation, but environmental factors and plant morphology may play a more important role in favouring alternative pathways of inoculum dispersal and plant colonization (Magg *et al.*, 2002).

The fungal infection is related more to the moment and the position of the larval infestation rather than to larval numbers (Dowd, 2000, 2001; Magg *et al.*, 2002) and, in addition, the fungus can infect the maize ear in an asymptomatic way (Dowd, 2001).

Climatic and environmental conditions

The data analysed has shown a high level of variability in the reactions of hybrids during the experimental studies confirming that the efficacy of Bt maize is strongly dependent on the interaction with the environment (Dowd, 2000, 2001; Magg *et al.*, 2002). Favourable climatic conditions for fungal infection (temperature, humidity, etc.) and stressful situations for the plant (nutritional imbalances, mechanical damage, etc.), can considerably affect the concentrations of mycotoxins in the field.

The varying climatic and environmental conditions and the consequent fluctuations in the fungus populations limit the efficacy of the Bt hybrids both spatially and temporally. This is particularly the case with pathogenic fungi whose infection is strictly correlated to the damage inflicted by attacks from the ECB larva.

Cultural practices

A further factor that could influence the real efficacy of Bt hybrids, and not taken into consideration in the studies car-

ried out to date, is the management system for preserving Bt technology imposed by the National Corn Growers Association (NCGA, www.ncga.com). This system calls for the institution of refuge zones of traditionally cultivated maize to avoid the advent of resistant insects caused by the strong selective pressures exerted by the Bt hybrid. Such areas should be equal to 20% of the total surface given over to maize cultivation and not more than 400 metres away. In those areas where Bt cotton is also grown, this area should be extended to 50% of the total area cultivated with Bt maize. One needs to ask the following questions:

What will be the influence on the total concentration of mycotoxins in the following phases of the product chain due to the maize present in the refuge zone?

How will ECB infestations and indirectly fungal infections in Bt maize be affected by the proximity of traditional maize?

How will the traditionally cultivated maize from the refuge zones be treated?

All these factors are likely to be extremely relevant in the European agricultural context characterized by small and medium sized farms, should it be decided to adopt guidelines, which are similar to those of the NCGA.

Moreover, one must also consider that in all the studies, the maize kernels that were analysed were hand-picked instead of combine harvested. Indeed, mechanical harvesting can cause fractures in the kernels that can act as inoculation sites for pathogenic fungi when the grains are subsequently stored. Calibrating the combine can minimize the damage and ensuring that the humidity of the kernels is appropriate when harvesting occurs. (Codex Alimentarius, 1997, 2001, 2003).

CONCLUSION

The presence of mycotoxins in foods is a very complex problem that does not concern only cultivation but also involves all the phases of the production chain. Moreover, despite the fact that insect resistance is a condition that can indirectly contribute to a reduction in mycotoxin levels, the different studies analysed, demonstrate that the fungal attack depends on numerous abiotic and biotic factors and the interaction between them and the plant. The presence of one monogenic resistance character, able to limit just one of these factors, cannot be considered an efficient system to limit attacks by pathogenic fungi. The problem cannot be managed by means of one specific action, but must be dealt with by an integrated approach that takes into consideration all the various aspects of the production chain, as well as the implementation of good agricultural and production practices. This is the only approach currently available for the prevention of mycotoxin risks and on which the principal organizations involved in this sector are working (see, in this regard, the guidelines put forward by Codex Alimentarius, 1997, 2001, 2003).

Indeed, no positive correlation has been shown between the use of Bt maize hybrids and concentrations of aflatoxins, tricothecenes and zearalenone, all mycotoxins that are highly toxic for both humans and animals. The risk deriving from these mycotoxins is particularly high considering the high level of consumer exposure to contaminated products (Final SCOOP Task 3.2.7, 2002; Final SCOOP Task 3.2.8, 2002; Final SCOOP Task 3.2.10, 2003).

As demonstrated in this paper, the cultivation of Bt

hybrids resistant only to certain *Lepidoptera* and *Coleoptera* species does not therefore result in a sufficient means for preventing contamination by mycotoxins. The only mycotoxin group where Bt maize might have a role to play are fumonisins, but studies would need to be conducted in order to determine whether significant reductions in fumonisin levels can be achieved in Bt maize kernels and derived products in real production conditions.

Acknowledgments

We wish to thank Sacha Sardo Infirri and Christopher Worfolk for help in translating and proof-reading.

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