

A review on comparative data concerning *Fusarium* mycotoxins in Bt maize and non-Bt isogenic maize

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Abstract The European corn borer reportedly promotes the infection of maize by *Fusarium* spp. Stalk and ear rots caused by *Fusarium* spp. are often related to mycotoxin accumulation in maize kernels. As a result, food and animal feed from maize are more severely contaminated with *Fusarium* mycotoxins: e.g. fumonisins (FUM), deoxynivalenol (DON) and zearalenone (ZEA). Bt maize is primarily an important potential tool for insect pest protection, both in the European Union and in other countries. Bt maize carrying the Bt genes is highly resistant to European corn borer larval feeding due to Bt toxin (δ toxin) production. Effective measures to combat pests therefore often have a positive side-effect in that they also reduce mycotoxin levels. Comparative analysis was used to the evaluation of the studies dealing with the reduction of *Fusarium* mycotoxins in Bt maize. Nineteen out of 23 studies on Bt maize came to the conclusion that Bt maize is less contaminated with mycotoxins (FUM, DON, ZEA) than the conventional control variety in each case.

Keywords Bt-maize · *Fusarium* · Deoxynivalenol · Fumonisin · Zearalenone

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Introduction

Maize (*Zea mays* L.) is one of the world's major cereal grains, with a world annual average production (2004–2006) of 678 million metric tons (Mt), representing 32% of the 2.15 billion Mt of world cereal grain production (Chung et al. 2007). Maize is cultivated on large areas world-wide and serves as a staple food for millions of inhabitants. Maize is, however, a particularly problematic commodity in the context of mycotoxins and looks to become more so given the nature of the predicted changes in climate. It has a relatively high water requirement and under drought stress conditions that could be predicted to occur more frequently in a climate change scenario, its *Fusarium* symbionts seem to produce more of their respective mycotoxins. Under these conditions a person's meal could contain significant quantities of all of fumonisins (FUM), deoxynivalenol (DON), other trichothecenes and zearalenone (ZEA). This combination of factors, which global climate change is predicted to make more commonplace, already occurs in the tropics and there is no doubt at all that many people are exposed to cocktails of mycotoxins from maize, with adverse health consequences that we probably underestimate (Strosnider et al. 2006).

Heavy pest infestation makes maize plants more susceptible to fungal infections. The European corn borer (*Ostrinia nubilalis* Hübner) and other stalk-boring pests reportedly promote the infection by *Fusarium* spp. Stalk and ear rots caused by *Fusarium* spp. are often related to mycotoxin accumulation in maize kernels. As a result, food and animal feed from maize are more severely contaminated with *Fusarium* mycotoxins: e.g. FUM, DON and ZEA (Munkvold et al. 1997; Munkvold and Desjardins 1997).

Bt maize is one of the most commonly grown genetically modified crops in the world today. It contains a gene from

the soil bacterium *Bacillus thuringiensis* Berl., which encodes for the formation of a crystal (Cry) protein that is toxic to certain members of the orders *Lepidoptera* and/or *Coleoptera* (Table 1). It is one of the most commonly grown transgenic crops in the world today. Bt maize production (including Bt maize with herbicide tolerance) has expanded to more than 35 million hectares worldwide. Now, about 80% of the maize produced in the US is genetically modified. The share of insect-resistant varieties in the total acreage decreased from 21 to 17%. Even varieties were less sold. The trend moves clearly towards varieties with combined insect resistance and herbicide tolerance (stacked genes). Here the proportion has risen from 28 to 40% of the maize land. Meanwhile, there are varieties on the market which produce two different variants of the Bt protein: one resists the corn borer, the other resists the corn root worm. While overall maize cultivation has dropped, the area of GM maize remains almost the same (increase from 27.4 to 27.7 million hectares).

Many countries in North and South America, Africa, and Asia grow GM maize. Maize is the only GM crop that is grown commercially in the European Union (EU). It is planted in four countries: Spain, Portugal, the Czech Republic and Slovakia. For the most part, maize is used for feeding livestock and as raw material for the starch industry. Starch, however, forms the basis of many foods and food additives (GMO Compass 2009).

Bt maize MON810 is primarily an important potential tool for insect pest protection, both in the EU and in other countries. Bt maize carrying the Bt gene (CryIAb) is highly

resistant to European corn borer larval feeding according to Bt toxin (δ toxin) production (Munkvold et al. 1997). The CryIAb protein controls lepidopteran insect pests such as the European corn borer, *Ostrinia nubilalis* Hübner, the most important stalk-boring and ear-damaging insect pest of corn in the United States and in the Europe too. It also controls southwestern corn borer (*Diatraea grandiosella* Dyar) and other related corn-boring insects. The CaMV 35S gene promoter enables constitutive expression of the CryIAb protein in hybrids during the growing season, thus providing season-long protection against corn borers. The *B. thuringiensis*-based microbial pesticides that contain Cry proteins such as CryIAb have been used commercially in agriculture for over 40 years to control larval insect pests (Hammond et al. 2004).

Effective measures to combat pests therefore often have a positive side-effect in that they also reduce mycotoxin levels. Munkvold et al. (1997, 1999) were the first to report that, in Iowa, maize hybrids protected with the CryIAb protein had significantly lower FUM levels in the grain than in the grain from control non-Bt hybrids. Potential economic, health and regulatory impacts, benefits and risks of Bt maize and *Fusarium* mycotoxin reduction have been described (Wu et al. 2004; Wu 2004, 2006a, 2006b, 2007). A review on the relationship between Bt maize and *Fusarium* mycotoxin levels in harvest produce have been published by Wu (2007). This review summarizes the currently available research on the link between Bt maize and the reduction of important agricultural mycotoxins. The present review will focus on current information about Bt maize and the reduction of important agricultural *Fusarium* mycotoxins (FUM, DON, ZEA).

Experimental procedures

A comparative analysis was used for the evaluation of the studies dealing with the reduction of *Fusarium* mycotoxins (FUM, DON, ZEA) in Bt maize (MON 810, Bt 176, Bt 11).

The comparative analysis can be used as a guide to answer the question, “Is there a reduction of *Fusarium* mycotoxin concentrations in Bt maize hybrids compared with the corresponding isogenic plants?”. Meta-analysis was used for the evaluation of the studies dealing with the reduction of *Fusarium* mycotoxins (FUM, DON, ZEA) in Bt maize (MON 810, Bt 176, Bt 11). A large collection of analysis results from individual studies was analysed by the given approach for the purpose of integrating the findings. The quality criteria for the comparative analysis of individual studies were field trial design, validation of analytical methods and quality of analytical results of *Fusarium* mycotoxins. Thirty-four studies about *Fusarium* mycotoxin contamination in Bt maize and non-Bt isogenic maize grown in Europe, USA, South America and Asia were collected.

Table 1 Current Bt maize strain developments

Bt maize	Inserted gene
MON810	<i>CryIAb</i> ^a
MON863	<i>Cry3Bb1</i> ^b
MON863xMON810	<i>Cry3Bb1</i> ^b , <i>CryIAb</i> ^a
NK603xMON810	<i>CryIAb</i> ^a
1507x59122xNK603	<i>CryIF</i> ^a , <i>Cry34Ab1</i> ^a
MON88017	<i>Cry3Bb1</i> ^b
MIR162	<i>vip3Aa19</i> ^a
MIR604	<i>Cry3A(mcry3A)</i> ^b
Bt11xGA21	<i>CryIAb</i> ^a
Bt11xMIR604xGA21	<i>CryIAb</i> ^a , <i>Cry3A(mcry3A)</i> ^b
Bt11xMIR162xMIR604xGA21	<i>CryIAb</i> ^a , <i>vip3Aa19</i> ^a , <i>Cry3A(mcry3A)</i> ^b
MON89034xNK603	<i>Cry2Ab2</i> ^a , <i>CryIA.105</i> ^a
MON89034xMON810	<i>CryIA.105</i> ^a , <i>Cry2Ab2</i> ^a , <i>CryIAb</i> ^a

^a Against the order *Lepidoptera*

^b Against the order *Coleoptera*

Only 23 relevant studies met our requirements according to the quality criteria for comparative analysis.

Results and discussion

The currently available varieties of Bt maize hybrids are very effective against the European corn borer, stalk borer and southwestern corn borer, and they can reduce damage by armyworm and corn earworm. The currently available varieties of Bt maize (MON 810, Bt 176, Bt 11) hybrids have shown strong evidence in field conditions worldwide of having significantly lower FUM levels than non-Bt isolines. There is also limited evidence for lower levels of DON and ZEA in Bt maize, although there are fewer field studies on these relationships. The results of the comparative analysis from the international studies can be seen in Table 2. Nineteen out of 23 studies on Bt maize came to the conclusion that Bt maize is less contaminated with *Fusarium* mycotoxins (FUM, DON, ZEA) than the conventional control variety in each case. It stands to reason

because insects also play an important role in infection of maize by *Fusarium* spp. They can act as wounding agents or as vectors spreading the fungus from origin of inoculum to plants. It is likely that the presence of *Fusarium* spp. promotes insect attacks and insect infestation favours fungal infection (Dowd 1998; Schulthess et al. 2002).

Although the results vary, depending on the site, variety, trial design and type of mycotoxin, the general trend is clear: there is a clear correlation between the severity of the corn borer infestation and the *Fusarium* mycotoxin level. Cultivation of the conventional maize variety without any measures to control the corn borer produced both the highest pest infestation and the highest *Fusarium* mycotoxin levels. This effect is more pronounced on sites with high corn borer infestation. Chemical and biological methods of controlling the corn borer, e.g. the use of insecticides or parasitic wasps (*Trichogramma*), can reduce both the number of corn borer larvae and the level of mycotoxin contamination. On all sites, the Bt maize varieties used showed the best results: only isolated corn borers were found in the crops.

Table 2 International studies demonstrating information on the link between Bt maize and the reduction of *Fusarium* mycotoxins (FUM, DON, ZEA) in field trials

Bt maize lower mycotoxins than non-Bt isolines	No. of studies (country)		
	FUM	DON	ZEA
YES	4 (USA) Munkvold et al. 1999 Dowd 2001 Hammond et al. 2002 Hammond et al. 2004	4 (Germany) Aulrich et al. 2001 Papst et al. 2005 Valenta et al. 2001 Magg et al. 2002	1 (Germany) Aulrich et al. 2001
	2 (Argentina) De la Campa et al. 2005 Barros et al. 2009	1 (Canada) Schaafsma et al. 2002	1 (France) Bakan et al. 2002
	1 (France) Bakan et al. 2002		1 (Spain) Bakan et al. 2002
	1 (Spain) Bakan et al. 2002		
	1 (Germany) Papst et al. 2005		
	1 (Italy) Masoero et al. 1999	-	-
	1 (Philippines) De la Campa et al. 2005	-	-
NO	1 (USA) Clements et al. 2003	1 (Germany) Magg et al. 2002 1 (Switzerland) Naef et al. 2006 1 (Argentina) Barros et al. 2009	- - -

Conclusion

The Bt maize had the lowest *Fusarium* mycotoxin levels according to the comparative analysis results from the international studies. *Fusarium* mycotoxin reduction has already had significant economic impacts in the Europe at current levels of Bt crop planting. In less-developed countries, the *Fusarium* mycotoxin reduction that Bt crops can provide could have important economic as well as health impacts. Bt maize may prove to be a useful tool to lower dietary intake of FUM, particularly in regions of the world where chronically high exposures persist. It can also increase the percentage of maize that would be suitable for consumption.

There is no available information on the link between Bt maize (NK603xMON810, MON863, MON863xMON810, 1507x59122xNK603, MON88017, MIR162, MIR604, Bt11xGA2, Bt11xMIR604xGA21, Bt11xMIR162xMIR604xGA21, MON89034xNK603 and MON89034xMON810) and the reduction of important agricultural *Fusarium* mycotoxins. The new varieties of Bt maize that may be commercialized soon are likely to have a more significant impact on *Fusarium* mycotoxin levels. Therefore, Bt maize is an important potential tool for *Fusarium* mycotoxin control, both in Europe and in other countries.

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