Influence of Growing Bt maize on *Fusarium* Infection and Mycotoxins Content – a Review

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Abstract

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The literature linking Bt maize versus non-Bt maize and the changes in the fungal microflora spectrum and in the mycotoxins content have been summarised. 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). Mycotoxins in field maize lead annually economic losses of hundreds of millions of dollars in all regions of the world. The insecticidal proteins in genetically modified hybrid Bt maize reduce insect damage caused by certain Lepidopteran larvae, which in turn can reduce the infection of the grain by the mycotoxigenic fungi. Where such insect damage is a major factor in mycotoxin contamination, Bt maize can lower mycotoxin levels in many cases. The protection of maize plants against insect damage (European corn borer) through the use of Bt technology seems to be one of the ways to reduce the contamination of maize by *Fusarium* species and mycotoxins.

Keywords: *Zea mays* L.; GMO; non-GMO; *Fusarium* spp.

Maize (*Zea mays* L.) is an agricultural crop of worldwide importance grown both for the food industry and for other purposes. While in the Czech Republic it is mainly raised for the production of animal feed, interest has been growing recently in corn as a raw material for the production of biogas and bioethanol. In 2011, 109 600 ha were planted with maize for grain in the Czech Republic. Ten years earlier, by comparison, 54 300 ha were planted with maize for grain. Conventional hybrids are predominantly grown, and since recent years the so-called transgenic Bt hybrids (MON 810) also are raised. The latter are resistant to attack by caterpillars of the European corn borer (ECB) (*Ostrinia nubilalis* Hübner). This genetic modification essentially consists in the plants' ability to create Bt toxin in their tissues, which specifically binds to the receptors inside the insect intestines where it creates pores causing the insect to die (Kocourek *et al.* 2008).

The main virtue of Bt-maize is its 100% effectiveness against ECB. In the Czech Republic the first Bt hybrids of the MON 810 event were released into agricultural practice in 2005. In that year, 150 ha of Bt maize were planted for animal feed purposes. In the following years up to 2008 the planted area markedly increased, and in that year the Czech Republic, with an area of 8380 ha, was second only to Spain in Europe. In 2009, this decreased to approximately 6500 ha and the decline continued also in subsequent years. We can only speculate about the reasons for this, but they are mainly political and economic, and only minimally on the scientific grounds.

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In all European maize-growing areas, large losses are caused due to the infestation by fungi of the *Fusarium* genus, which cause red and pink rot (Bottalico 1998; Logrieco *et al*. 2002). Red rot is caused by *F. graminearum* Schwabe [teleomorph *Gibberella zeae* Schwein (Petch)], *F. culmorum* (W. G. Smith) Saccardo, and *F. avenaceum* (Fries) Saccardo (t. *G. avenacea* Cook), while pink rot is caused by the species *F. verticillioides* (Saccardo) Nirenberg (t. *G. moniliformis* Wineland), *F. proliferatum* (Matsushima) Nirenberg [t. *G. intermedia* (Kuhlman) Samuels], and *F. subglutinans* (Wollenweber & Reinking) Nelson, Toussoun & Marasas (t. *G. subglutinans* Nelson, Toussoun & Marasas). The species causing both types of rot include *F. equiseti* (Corda) Saccardo (t. *G. intricans* Wollenweber), *F. poae* (Peck) Wollenweber, *F. sporotrichioides* Sherbakoff, *F. solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen[t. *Nectria haematococca* (Berkeley & Broome) Samuels & Nirenberg], and *F. oxysporum* Schlechtendahl emend. Snyder & Hansen (Logrieco *et al*. 2002).

Fusarium spp. can infect maize during emergence and after the plant has been damaged by birds (Reid 1999), and in some other cases during the vegetation period. Maize kernels can be infected by conidia growing through stigmata with styles which are very sensitive during the first six days after their emergence (Reid & Hamilton 1996; Munkvold *et al*. 1997b). The infection via the stigmata is important for *F. verticillioides* and probably also for *F. proliferatum* and *F. subglutinans* (Munkvold *et al.* 1997a). Another pre-disposing factor for the infection of maize by *Fusarium* spp. is stress (such as due to drought and waterlogging). Nevertheless, the main factor influencing the injury to maize by *Fusarium* spp. is the damage to tissues due to feeding by caterpillars of European corn borer (Munkvold *et al*. 1997a; Munkvold & Hellmich 1999).

Direct losses in the yield and quality caused by the caterpillars of this pest are estimated at 10–20%, whereby the corn borer constitutes a risk for the cultivation of maize not only for grain but also for silage. The YieldGard-brand (i.e. GMO-modified for ECB control) hybrids yielded on average 2.33 t/ha more fresh-cut material with 37.46% average dry matter, while the corn borer infestation rate was 0.58 larvae per plant for the conventional hybrids and its occurrence was zero for the GMO hybrids (Nedělník 2010).

In 2008, 2009, and 2010, *Fusarium* spp. infection levels were monitored in kernels of conventional and transgenic Bt hybrids harvested from naturally infested stands at sites with a regular occurrence of ECB. The analysed samples were taken from seven sites (Čejč, Hodonín, Jiřice u Miroslavi, Loštice, Medlov, Otrokovice, and Rostěnice) representing the Czech Republic main growing areas of maize for grain. A total of 76 samples of maize kernels were included into the experiment. Several pairs of hybrids were represented (conventional hybrid + derived transgenic Bt hybrid) at each site (randomly 5 × 10 noodle from each hybrid).

A total of 246 isolates of *Fusarium* spp. were acquired from the kernels of conventional hybrids and 192 isolates from those of transgenic Bt-versions of maize hybrids (thus 438 isolates in total). Using microbiological and polymerase chain reaction methods, 10 species of the *Fusarium* genus were determined in the kernels: *F. subglutinans* (40.4%), *F. graminearum* (19.8%), *F. verticillioides* (18.2%), *F. poae* (9.3%), *F. proliferatum* (4.0%), *F. avenaceum* (3.8%), *F. oxysporum* (1.7%), *F. sporotrichioides* (1.3%), *F. sambucinum* (1.3%), and *F. culmorum* (0.2%) (Table 1) (Kmoch *et al.* 2011).

During the monitored years, the species *F. subglutinans*, *F. graminearum*, and *F. verticillioides* were dominant on the kernels of the maize hybrids. According to Lew *et al*. (2001) and Logrieco *et al*. (2002), *F. subglutinans*, *F. verticillioides*, and *F. graminearum* are among the species most frequently isolated from the infected maize plants in Europe. The same species were reported in a study performed by Görtz *et al*. (2008), who examined biological diversity of *Fusarium* spp. on maize kernels in Germany. By contrast, the species *F. culmorum*, *F. sporotrichioides*, *F. oxysporum*, and *F. sambucinum* were sporadically isolated from the kernels. *F. culmorum*, which was detected only in 2008 and then on the kernels of conventional hybrids, is, according to Logrieco *et al*. (2002), among the dominant fungal pathogens of maize in Europe while *F. sambucinum* is seen on maize less frequently.

In all monitored years (2008–2010), lower infection levels on kernels of transgenic Bt hybrids were seen for *F. subglutinans* and *F. proliferatum,* while statistically significant difference was shown by *F. subglutinans* in 2010 only. In two years, lower infection levels were seen for the species *F. graminearum*, *F. verticillioides*, and *F. porotrichioides* with a significant difference for *F. graminearum* in 2008. By contrast, there was a higher infection of the kernels of transgenic hybrids in all years due to

Table 1. Average infection levels (% of kernels) in conventional and transgenic maize hybrids in 2008–2010

Species	2008		2009		2010	
	non-transgenic hybrids	Bt hybrids	non-transgenic hybrids	Bt hybrids	non-transgenic hybrids	Bt hybrids
F. avenaceum	1.49[b]	0.33[a]	0.43[a]	0.80[a]	2.03[a]	2.41[a]
F. culmorum	0.11	0.00	0.00	0.00	0.00	0.00
F. graminearum	2.91[b]	1.62[a]	2.86[a]	2.00[a]	4.24[a]	5.00[a]
F. oxysporum	0.12[a]	0.32[a]	0.29[a]	0.20[a]	0.00	0.00
F. poae	0.11[a]	0.32[a]	0.86[a]	1.71[a]	0.50[a]	1.72[b]
F. proliferatum	0.22[a]	0.11[a]	1.00[a]	0.43[a]	0.17	0.00
F. sambucinum	0.00	0.33	0.14[a]	0.10[a]	0.00	0.00
F. subglutinans	5.60[a]	2.82[a]	6.01[a]	4.81[a]	1.86[b]	0.34[a]
F. sporotrichioides	0.43[a]	0.11[a]	0.14[a]	0.10[a]	0.34[a]	0.17[a]
F. verticillioides	3.23[a]	1.16[a]	1.86[a]	2.43[a]	0.85[a]	0.34[a]

F. poae with a statistical difference in 2008. The differences in the infection of kernels of conventional and transgenic hybrids were inconclusive for other identified *Fusarium* spp. (*F. culmorum*, *F. oxysporum*, and *F. sambucinum*). The differences between the infections of conventional and transgenic kernels depended on the species of fungus (Table 1) (Kmoch *et al.*, 2011).

In the Czech Republic, apart from the facilities of Mendel University in Brno and the Research Institute for Fodder Crops in Troubsko, this topic was also been studied by the Crop Research Institute in Prague-Ruzyně. During 2002–2004, studies were performed determining the micromycetes on maize kernels from transgenic (MON 810) and non-transgenic hybrids. In total, 84 taxa of microscopic fungi were isolated, 8 of which were of genus *Aspergillus*, 18 of genus *Fusarium*, and 25 of genus *Penicillium*. In both types of maize, similar sets of genera and species of micromycetes were recorded, but the frequency of the toxigenic species was significantly lower on Bt maize. Similarly, lower levels of selected mycotoxins were recorded on these hybrids (Slezáková 2005).

At the same institution during 2002–2007, samples were collected of maize grown using various means of protection against European corn borer (transgenic maize, chemical protection, biological protection). From four sites in the Czech Republic, a total of 17 species of *Fusarium* were identified, which supports the results described herein above. The most frequently isolated species included *F. subglutinans*, *F. verticillioides*, *F. oxysporum*, *F. avenaceum*, *F. sporotrichioides*, and *F. graminearum*. Again, it was confirmed that Bt maize had a quantitatively lower occurrence of these fungi (Slezáková *et al.* 2006).

While Munkvold and Desjardins (1997), Munkvold *et al.* (1997a,b), Munkvold and Hellmich (1999), and Clements *et al.* (2003) state that the ears of Bt maize are markedly less infected by the fungi of the *Fusarium* spp. than are those of non-transgenic maize, Naef and Défago (2006), who examined the fungi in maize stems, detected no differences between the conventional and Bt hybrids.

Gatch and Munkvold (2002) determined lower infection levels from *F. subglutinans* and *F. verticillioides* in Bt maize than in non-transgenic hybrids. By contrast, for the species *F. graminearum* they demonstrated a higher infection level in Bt hybrids.

Lower infection rates of transgenic hybrids determined for certain fungal species of the *Fusarium* genus can probably be explained by lower levels of infestation by European corn borer caterpillars, as the other infection ways (e.g. via stigmata, roots, and damage by birds) are identical fboth or the conventional and transgenic maize hybrids.

Apart from decreasing the maize yield (Ding *et al.* 2008), *Fusarium* spp. produce secondary metabolites in the form of mycotoxins. Especially those from the groups trichothecene, zearalenone, and fumonisin (Logrieco *et al.* 2003), may cause serious acute and chronic diseases in human beings and farm animals (D'Mello *et al.* 1999). The effects caused by the actions of mycotoxins on an animal organism are varied depending upon the type of toxin, dose and exposure duration, as well as upon the species, age, sex, and current health state of the individual. Mycotoxins can cause, for example, diminished immunity, allergic reactions, reproductive disorders, disorders of the nervous and respiratory systems, decrease of feed conversion and utilisation, and increased mortality in livestock. Mycotoxins damage the intestinal mucosa and thereby limit the absorption of nutrients, and also impair the functions of the liver, kidneys, reproductive organs, and immune system. Gastrointestinal absorption causes the toxins to enter the blood circulation and thereby other body tissues. Currently, a number of expert publications and studies exist describing the influence of these toxins on the productivity and health of farm animals (Miller & Wilson 1994; Marasas *et al.* 2001; Rotter *et al.* 1996; Eriksen & Alexander 1998). It has been demonstrated that the toxins spread further into the food chain via meat and milk (Gimeno & Martins 2002; Bertuzzi *et al.* 2003). All current knowledge on this topic unambiguously confirms that the most economical means of addressing this problem is to focus on the prevention of and averting the occurrence of mycotoxins in feedstuffs, including maize silage. Soon it may become reality that a certain portion of a farming enterprise maize silage will not be permitted to be fed to animals due to an excessive amount of these toxins.

Worldwide, 25% of crops are contaminated by mycotoxins every year. Concerning Europe, for example, extensive studies conducted since 2008 in Spain, Belgium, and other countries have shown that proportions in tens of percentage points of all feed samples were contaminated; for certain mycotoxins, the extent of mycotoxin contamination was nearly 100%. In the Czech Republic, we may state that the level of positive samples is high and for most mycotoxins it exceeds 50%.

The current relevance of the mycotoxins issue is also reflected in European Community legislation. Maximum limits have been established for certain contaminating substances in food, including mycotoxins, according to Commission Regulation (ES) No. 1881/2006. The Commission also issued its Recommendation 2006/583/ES to prevent and decrease *Fusarium* toxins in cereals and cereal products, and especially in relation to feedstuffs there applies Commission Recommendation 2006/576/ES on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2, and HT-2, and fumonisins in the products intended for animal feeding. In September 2007, a new Regulation of the Commission, 1126/2007/ES, was published in the ES Newsletter, changing the maximum values for the content of *Fusarium* toxins in maize and in the products from maize (maximum concentrations of mycotoxins in maize and derivatives to 4000 ppb for fumonisins B_1 and B_2, 1750 ppb for deoxynivalenol, and 350 ppb for zearalenone).

Recent data about mycotoxins contamination coming from the Czech Republic referred that in the experiments carried out over several years their authors had compared the protection of maize against ECB using a genetically modified Bt hybrid, the traditional protection using insecticides, biological protection using wasps of the genus Trichogramma, and a control variant (isoline to Bt hybrid). These experiments have demonstrated a very low or no contamination of GMO maize by ECB. A 60–70% effectiveness was achieved using insecticides. The effectiveness of biological approaches was strongly dependent upon the weather conditions, but the average effectiveness was less than that using chemical protection. Subsequent analysis of *Fusarium* mycotoxin showed a correlation with the insect resistance, i.e., mycotoxin content in GMO material was the lowest compared to the highest content in the control untreated maize. It should be noted that the mycotoxin content in GMO material was not always zero. Even if this material was not attacked by *O. nubilalis*, the material could still be contaminated by fungi of the genus *Fusarium*, because the genetic modification is intended as a protection against damage done by insects and it does not increase the resistance against fungal pathogens (Nedělník *et al.* 2009).

The results of the studies in which mycotoxin contamination levels were measured are varied. Some authors report no significant differences in the contents of aflatoxins, zearalenone, and trichothecene; only the contents of toxins from the fumonisin group were decreased in Bt hybrids and the corresponding isolines. On the other hand, a marked positive effect of Bt hybrids on many biotic and abiotic factors has been stated (Pazzy *et al.* 2006). In another review from 2010 (Ostry *et al.* 2010), the authors state that 19 of 23 studies comparing the contents of mycotoxins in Bt hybrids determined that genetically modified materials were less contaminated by mycotoxins than the conventional control cultivars. A French study by Folcer *et al.* (2010) reports the results from years 2005 and 2006, showing that in Bt maize, the content of fumonisins was lower by as much as 90% and that of zearalenone by as much as 50%, while the concentration of deoxynivalenol was slightly increased. As those researchers themselves state, their results indicate that Bt maize can result in a greater safety in food production. The findings published at www.gmo-safety.eu contain, among other things, an evaluation of a large number of studies from various countries showing the relationship between Bt plants and mycotoxins contents in production. Ten out of the 13 studies indicate decreased levels in GMO materials of contamination with the mycotoxins deoxynivalenol, zearalenone, and fumonisins in comparison with the conventional control cultivars. In several studies, particularly from North America, decreased aflatoxins content was not proven, which, as the authors reason, reflects the fact that certain *Aspergillus* spp. are not carried by the insect species controlled by Bt transgenes. In a review from 2006, Wu (2006) summarises the published results again in relation to Bt maize and the mycotoxins content. Especially in the US, he states, mycotoxins annually cause losses in the hundreds of millions of dollars, and the contamination with aflatoxins is the most serious off. The contamination with other fusariotoxins also causes important but nevertheless lower losses. As that author states, the positive influence of Bt maize has been proven and its contribution to reducing mycotoxins is an important contribution to food safety especially in developing countries. That is especially true where unprocessed maize is consumed (Wu 2006).

Maize grains from Bt hybrids and near-isogenic traditional hybrids were collected in France and Spain from the crop which was grown in year 1999 under natural conditions. According to the ergosterol level, the fungal biomass formed on Bt maize grain was 4–18 times lower than that on the isogenic maize. Fumonisin B_1 grain concentrations ranged from 0.05 to 0.3 ppm for Bt maize and from 0.4 to 9 ppm for isogenic maize. Moderate to low concentrations of trichothecenes and zearalenone were measured on transgenic as well as on non-transgenic maize. Nevertheless, significant differences were obtained in certain regions (Bakan *et al.* 2002).

Late in 2009, a new directive of the European Council and Parliament No. 2009/128/ES was adopted. It defines a framework for the Community activity for the purpose of achieving sustainable use of plant protection preparations and recommends that the member countries provide from 2014 necessary conditions for applying integrated protection of plants as an essential part of integrated production based upon the prepared national action plans. One of the objectives of this regulation is to optimise the use of chemical substances for the plant protection while concurrently supporting non-chemical methods of plant the protection. The non-chemical methods in the plant protection range widely from using biological agents to agro-technical and nutritional principles of proper agricultural practice, and also to raising cultivars resistant to harmful agents. Genetically modified plants can be classified into the last group.

The principles of integrated field crops production are today starting to be developed not just as a future basis for the grant policy, but more particularly as a set of measures leading to the production of safe raw materials and food. In growing maize, the inclusion of GMO hybrids seems an appropriate plant protection measure both for preventing crop losses caused by European corn borer and for providing an efficient, indirect instrument for decreasing mycotoxin contamination. At the same time, we need to stress that the growing of these hybrids must fully conform to the rules on coexistence.

In recommending the growing interventions, the farmer must be supported with the widest range of knowledge possible so that his or her business decisions are based on objective facts. Therefore, the research team dealing with the topic of growing GMO crops and various protection strategies also monitored the nutritional quality of the silage manufactured from Bt and non-Bt hybrids. The published results demonstrate that those qualitative measures monitored were fully comparable. The content of nutrients in silages was unaffected by the maize hybrid, no effect of the maize hybrid on the fermentation process was observed either. The digestibility of crude fiber and nitrogen-free extractives was lower in conventional hybrid than in Bt (Křížová *et al.* 2009).

At comparable costs for the Bt and conventional hybrids, the Bt maize inclusion into the crop rotation also has further advantages. A publication of the Ministry of Agriculture of the Czech Republic (www.mze.cz) summarising the current knowledge with growing Bt maize in the Czech Republic. Based upon respondents' three years of experience, it is reported that in addition to increasing yields, Bt maize provides farming enterprises with feedstuffs free of mycotoxins, which is important both in producing silage and mixed feeds. Bt maize crops can be harvested for grain at a later stage, as the risk of losses due to ears breaking off is reduced. In addition to the economic effects, the farming with Bt-maize contributes to the sustainability of the environment by avoiding one spraying and generally reducing its passes over the field.

A separate chapter relates to the harvesting of maize, its quality, the subsequent speed and quality of its ensiling, and the quickest possible sealing of the silage against air and its covering. The recommendations for increasing the quality of silage are provided. There can be no exception of secondary contamination of the ensiled material by "storage fungi" in practice, which may be also connected with mycotoxin production. The authors of the article have analysed a wide range of samples collected during the ensiling processes from individual locations of trench silos, as well as samples that have been taken from the face of the silage when loading out the silage for feeding. The results confirmed that, if the ensiled material contains a greater amount of mycotoxins, these can be found across the entire profile of the final silage. If mycotoxins are present during the period of silage fermentation, they are also present at the final opening of the silo. The conclusion is drawn, that, if maize cannot be cultivated without the possibility of mycotoxins being present, then the ensiling process will not decrease the amount of those substances, because they are chemical compounds with a high thermal and chemical stability.

The majority of the study results have indicated that GMO maize yielding can be an effective part of the integrated crop protection system not only with an impact on reduction insect damage but also as a non-direct way for mycotoxins content decreasing.

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