Review

Bt corn and impact on mycotoxins

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Received: 28 June 2007
Accepted: 8 August 2007

doi: 10.1079/PAVSNNR20072060

The electronic version of this article is the definitive one. It is located here: http://www.cababstractsplus.org/cabreviews

Abstract

This review summarizes the literature linking Bt corn and the reduction of the mycotoxins fumonisin, aflatoxin, deoxynivalenol (DON) and zearalenone. Mycotoxins in field corn cause hundreds of millions of dollars in economic losses annually in the USA, and substantially greater losses in other regions of the world. Most of the losses are from aflatoxin contamination, while significant but smaller losses are due to *Fusarium* mycotoxins, fumonisins and DON. The insecticidal proteins in genetically modified hybrid Bt corn (*Zea mays* spp.) reduce insect damage from certain *Lepidopteran* larvae, which in turn can reduce infection of the grain by mycotoxigenic fungi. Where such insect damage is a major factor in mycotoxin contamination, Bt corn can lower mycotoxin levels. Since such damage is not always the most important factor, experimental results have been mixed, especially with aflatoxin levels. Bt corn appears to have a greater impact on the *Fusarium* mycotoxins than on aflatoxin levels. Studies on economic impacts of Bt corn's mycotoxin reduction are briefly summarized. The benefits in developing countries from mycotoxin reduction could be more significant, particularly in regions where unprocessed corn is a staple in the human diet.

Keywords: Mycotoxins, Aflatoxin, Fumonisin, Deoxynivalenol, Bt corn

Review Methodology: The Agricola and Medline databases were searched for the most up-to-date information about the link between Bt corn and mycotoxin reduction. The key words "Bt corn," "Bt maize," "mycotoxin," "fumonisin," "aflatoxin," "deoxynivalenol" and "zearalenone" were used in various combinations of searches.

Introduction

Genetically modified Bt corn contains a gene from the soil bacterium *Bacillus thuringiensis*, which encodes for formation of a crystal (Cry) protein that is toxic to common lepidopteran corn pests. It is one of the most commonly grown transgenic crops in the world today. In 2006, Bt corn, including that combined with herbicide tolerance made up 40% of total field corn acreage in the USA [1]. Around the world in 2006, Bt corn was planted on 20.1 million ha (including Bt corn with herbicide tolerance), making up 20% of all corn planted globally. It is planted in 13 countries: the USA, Argentina, Canada, South Africa, the Philippines, Spain, Uruguay, Honduras, Portugal, Germany, France, the Czech Republic and Slovakia [2]. However, the USA has by far the largest acreage of Bt corn planting.

One indirect benefit that has emerged from Bt corn adoption is lower levels of mycotoxin contamination. Mycotoxins are secondary metabolites of fungi that colonize crops. They are considered unavoidable contaminants in foods, as best-available technologies cannot completely eliminate their presence in crops [3]. Insect damage is one factor that predisposes corn to mycotoxin contamination, because insect herbivory creates kernel wounds that encourage fungal colonization, and insects themselves serve as vectors of fungal spores [4–6]. Thus, any method that reduces insect damage in corn also reduces risk of fungal contamination. Indeed, in a variety of field studies, Bt corn has been shown to have significantly lower levels of common mycotoxins, the subject of which is reviewed in this article.

Mycotoxins are an important regulatory concern worldwide today because of their toxic and carcinogenic effects in humans and animals. Yet the benefit of Bt corn's reduction of mycotoxin damage has been virtually ignored in policy debates anywhere in the world [7]. As adoption of agricultural biotechnology continues to increase on a global scale, policy-makers worldwide should consider the economic and health impacts of this secondary benefit of transgenic pest-protected crops. Mycotoxin reduction has already had significant economic impacts in the USA at current levels of Bt crop planting [8]. In less developed countries (LDCs), the mycotoxin reduction that Bt crops can provide could have important economic as well as health impacts. This review summarizes the currently available research on the link between Bt corn and the reduction of important agricultural mycotoxins.

Four Common Mycotoxins in Corn

Four agriculturally important mycotoxins that contaminate corn are fumonisin, aflatoxin, deoxynivalenol (DON) and zearalenone. Fumonisins are produced by the fungi *Fusarium verticillioides* (formerly *Fusarium moniliforme*) and *Fusarium proliferatum*. They were first discovered in 1988 in connection with two events in two different parts of the world: high human oesophageal cancer rates in Transkei, South Africa; and unusually high horse and swine death rates in the USA [9]. Now more than 28 types of fumonisins have been isolated and characterized worldwide, of which fumonisin B_1 (FB_1) is the most common in corn.

Consumption of fumonisin has been associated with elevated human oesophageal cancer incidence in various parts of Africa, Central America and Asia [10] and among the black population in Charleston, South Carolina [11]. Because FB_1 reduces uptake of folate in different cell lines, fumonisin consumption has been implicated in connection with neural tube defects in human babies [10, 12]. No confirmed cases of acute fumonisin toxicity in humans have been found. Fumonisins can be highly toxic to animals, causing diseases such as equine leukoencephalomalacia (ELEM) in horses and porcine pulmonary edema (PPE) in swine [13].

Aflatoxins are produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, and are the most potent naturally occurring liver carcinogens known. Acute aflatoxicosis, characterized by haemorrhage, acute liver damage, edema, and possibly death, can result from extremely high doses of aflatoxin. More common are health effects associated with chronic low to moderate levels of aflatoxin consumption. For people who are infected with hepatitis B and C (common in China and sub-Saharan Africa), aflatoxin consumption raises more than tenfold the risk of liver cancer compared with either exposure alone [14]. Aflatoxin consumption is also associated with stunting in children [15] and immune system disorders [16].

Aflatoxins cause a variety of illnesses in animals as well. In poultry, aflatoxin consumption results in liver damage, impaired productivity and reproductive efficiency, decreased egg production in hens, inferior egg-shell quality, inferior carcass quality and increased susceptibility to disease [17]. In cattle, the primary symptoms are reduced weight gain, liver and kidney damage and reduced milk production [18]. Unfortunately, loss of income from decreased animal production can lead to greater poverty among farmers, reinforcing conditions conducive to poor human health [19].

DON (or vomitoxin), the most common mycotoxin in cereals, is produced by the fungus *Fusarium graminearum* and the related species *Fusarium culmorum* in cooler climates. It is a significant contaminant of corn, wheat and barley in generally more temperate regions of the world, such as the USA, Canada and Europe [20]. It causes *Fusarium* head blight in wheat, and *Gibberella* or pink ear rot in corn. Epidemics of *F. graminearum* infection in crops can occur worldwide when relatively warm temperatures and rain coincide with corn silk emergence. In the 1990s, DON was a major problem in the northern USA (primarily in wheat). Because of a near-zero tolerance policy for DON, grain buyers and food processors refuse to purchase crops from highly contaminated regions. As a result, crop market losses around the Great Lakes due to DON contamination were significant [21]. DON is an inhibitor of protein biosynthesis and causes human and animal effects ranging from feed refusal, vomiting and nausea, to immunosuppression and loss of productivity [22].

Zearalenone, like DON, is produced by *F. graminearum*. Zearalenone is sometimes referred to as a mycoestrogen, as it causes estrogenic responses and vulvovaginitis in swine [23]. Also, the mycoestrogen can be transmitted to piglets in sows' milk, causing oestrogenism in the piglets [3]. At higher concentrations, zearalenone causes similar effects in poultry and cattle [24]. In humans, there has been limited evidence of an association between zearalenone consumption and premature puberty [25].

Many nations have established regulatory standards on maximum-tolerated levels of mycotoxins in food and feed. Thus, aside from the health risks described above, mycotoxin contamination can also reduce the price paid for food crops, or in extreme cases, can cause market rejection of entire food or feed shipments. In much of the industrial world, losses from mycotoxins are typically associated with these economic losses as opposed to illnesses or deaths from the toxins. However, exposures in LDCs to food-borne mycotoxins are often high enough to harm health. Globalization of food trade has further exacerbated mycotoxin-related losses in two important ways. First, strict mycotoxin standards imposed by importing nations mean that LDCs are likely to export their best-quality foods while keeping contaminated foods domestically, resulting in higher risk of mycotoxin exposure in those nations [26]. Secondly, even the best-quality foods produced in these nations may be rejected for export at more strict standards, meaning millions of dollars in losses [27].

Bt Corn

Bt Corn is one of the most commonly grown genetically modified crops in the world today. It contains a gene from the soil bacterium *B. thuringiensis*, which encodes for a protein that is toxic to certain members of the order *Lepidoptera*. These include the common corn pests European corn borer (ECB) *Ostrinia nubilalis*, Southwestern corn borer (SWCB) *Diatraea grandiosella*, corn earworm (CEW) *Helicoverpa zea*, and corn rootworm (CRW) *Diabrotica* spp. Bt corn is harmless to vertebrates and non-lepidopteran insects [28–32].

Although the technology of Bt corn was first envisioned in the 1980s, the seed was not commercially available until 1996 [8]. The toxins in currently registered Bt corn events (trade varieties) are nearly 100% effective against the ECB, SWCB and CRW; to a more limited extent also control CEW. This is a marked improvement over previous pest management strategies such as conventional insecticide sprays, which provide anywhere from 40 to 95% protection against ECB [33]. Although ECB can cause billions of dollars in damage in the USA [34, 35], the majority of field corn growers do not use any pest management strategy at all because of low infestation levels, the cost of conventional pesticides, or their mediocre performance against ECB. Food grade corn, however, may be treated with insecticides as needed.

Currently, as US regulations do not require segregation of genetically modified grains, Bt corn and traditional grain corn are treated as identical for almost all commercial uses in the USA; with the exception of a small number of food companies that will not use genetically modified food (such as Gerber in its baby food) [7]. The majority of harvested Bt corn is used as animal feed. A small percentage and specific varieties of corn are designated 'food grade' for human consumption. In most cases, however, corn intended for both food and feed are treated equally from the planting stage through to the grain elevator. Other uses include non-food items such as ethanol, paper, adhesives and pharmaceuticals.

How Bt Corn Reduces Mycotoxin Levels

Several different factors can predispose corn to fungal growth and subsequent mycotoxin accumulation. In pre-harvest corn, high or unusually fluctuating temperatures, drought stress, incompatibility of the corn hybrid for the region in which it is planted, and insect pest damage increase mycotoxin levels [4, 5]. As drought stress increases insect herbivory on corn, it is not really possible to separate these two factors.

Notably, insect damage is well recognized as a collateral factor in mycotoxin development. Insect pests create wounds on the corn kernels and act as vectors for certain types of fungal spores [4, 5]. In post-harvest corn, storage conditions such as high humidity, pre-harvest presence of fungi, and the presence of stored grain insects may contribute to further fungal development and accumulation of mycotoxins in corn [5]. Again, insects in storage create grain wounds and spread fungal spores to cause further post-harvest accumulation of mycotoxins.

Where insect pests are present, Bt corn has been shown to have lower levels of certain mycotoxins than non-Bt isolines. The insect pests ECB, SWCB and CEW have been shown in field trials to contribute to the concentration of mycotoxins in corn [36]. Insect-damaged corn is also prone to mycotoxin accumulation in storage [5]. Therefore, to the extent that Bt corn has lower levels of insect damage, it indirectly controls for one of the most important predisposing factors of mycotoxin accumulation.

Field Evidence of Bt Corn's Reduction, or Lack Thereof, of Mycotoxins

Different corn-planting regions of the US and the world are affected by different insect pests and different fungi. It is not surprising that Bt corn is relatively more effective at reducing those mycotoxins that are associated with the insect pests it can best control. On the field, this has meant that the currently available events of Bt corn have shown more consistent control of fumonisin, for example, than aflatoxin. This section describes field studies on comparing levels of the above-described four mycotoxins in Bt corn and non-Bt isolines, in the US and in other parts of the world. A cautionary note is that many of the field studies invoked conditions that may not often be encountered in actual field conditions, such as application of larvae or fungi to the corn, and harvesting methods that are not representative of actual harvesting practices.

In the Corn Belt region of the USA, field studies have demonstrated that when insect damage from ECB or SWCB is high, fumonisin concentrations are significantly lower in Bt corn compared with conventional corn. Under circumstances of both a natural ECB infestation and a manual ECB infestation, the amount of *Fusarium* kernel rot and concentration of B_1 were significantly lower in Bt corn events Bt11 and MON810 than in their near-isogenic, non-transgenic counterparts [6, 37, 38]. Specifically, depending on the control level of pest damage, a 1.8–15-fold reduction of fumonisins in Bt corn over conventional corn was achieved [37]. In this study, the greatest reductions in fumonisins in Bt corn occurred where ECB was the predominant insect pest; where other types of pests were predominant, fumonisin reductions in Bt corn were less significant. Importantly, in multiple locations across the US, Bt corn (compared with non-Bt isolines) had fumonisin levels that were below the Food and Drug Administration (FDA)'s 2-ppm guideline for fumonisin in food [39]. Yet another study showed that Bt hybrids can reduce fumonisin levels when ECB is favoured, but not in seasons when CEW is favoured [40].

In Europe and in other parts of the world, Bt corn has been shown in field trials to have significantly lower fumonisin levels than non-Bt isolines. Significantly lower levels of fumonisin were measured in Bt hybrids when compared with controls in 288 separate test sites in France, Italy, Turkey, Argentina and the USA [41, 42]. Fumonisin concentrations in Bt grain were often lower than 4 mg/kg, with a significant proportion of these below 2 mg/kg. In a study of three field plots in Germany, Bt corn was shown to have lower fumonisin, but not DON, levels [43]. Studies in Argentina and the Philippines have also shown lower total fumonisin levels in Bt versus non-Bt isolines [44].

However, compared with the case of fumonisin, insect pest damage is less strongly correlated with aflatoxin concentrations in corn, as multiple factors predispose corn to accumulation of aflatoxin. The lepidopteran insects that are controlled by Bt corn are not as important in predisposing plants to infection by *A. flavus* as they are for *F. verticillioides* and *F. graminearum* (45); and *A. flavus* can infect corn not just through kernel wounds caused by insects, but through the silks. Indeed, field tests of aflatoxin reduction in Bt corn show a mixed record.

In different field-tested regions of the USA, Bt corn occasionally showed lower levels of aflatoxin than their non-Bt isogenic counterparts depending on the type of insect pest present and the conditions under which the corn was infected by fungi. Bt hybrids were shown to have lower aflatoxin levels than non-Bt isolines in the southern USA in years when aflatoxin levels would otherwise have been high, but there was no significant difference in one year in which aflatoxin levels were low in both Bt and non-Bt isolines [46]. Williams *et al.* [47] found that the relationship between Bt corn and aflatoxin reduction depends on the *A. flavus* inoculation technique. The non-wounding technique (spraying *A. flavus* inoculum on young ears) and control case resulted in significantly lower aflatoxin levels in Bt corn, while the wounding technique (damaging the kernels) resulted in no difference in aflatoxin levels. Other studies show no significant effect of Bt corn, or mixed results. Buntin *et al.* [48] observed that while Bt11 and MON810 had significantly lower pest damage than non-Bt corn, there was no significant difference in aflatoxin levels between the two groups. Odvody *et al.* [49] found significantly lower levels of insect damage in Bt corn in regions of Texas, but inconsistent comparative results on aflatoxin levels in Bt and non-Bt corn. The authors concluded that other factors, such as drought stress and individual hybrid vulnerability, are more important in determining aflatoxin contamination levels than insect damage. In another study, Bt corn did not seem to significantly impact *Aspergillus* ear rot [50]. In a Mississippi study, Bt corn had lower aflatoxin levels than non-Bt isolines in one year out of three [51].

Two field studies in Italy independently showed no impact of Bt corn in reducing aflatoxin levels [52, 53]. These studies on the impact of Bt corn on aflatoxin levels are described in more detail in Munkvold [54]. New events of Bt corn are being developed that provide better protection against CEW and fall armyworm, insects that are closely associated with aflatoxin accumulation in corn. Field trials have demonstrated that these Bt corn varieties do indeed have significantly lower aflatoxin levels than non-Bt isolines [55].

F. graminearum is similar to *A. flavus* in that it can infect corn without insect damage. Correspondingly, the evidence for lower levels of DON in Bt corn is also mixed. Schaafsma *et al.* [56] found that where ECB pressure was high, the use of Bt hybrids reduced the level of DON by 59% compared with non-Bt isolines. In these cases, Bt corn consistently had levels of DON that were acceptable by FDA standards (i.e. below 1 mg/kg). Where ECB pressure was low, however, there was no significant difference between DON levels in Bt versus non-Bt hybrids (which were below 1 mg/kg in either case). Aulrich *et al.* [57] found that in animal feed, the only nutritional difference between Bt and non-Bt corn feeds was that Bt corn had lower levels of DON and zearalenone. However, in a central European field study, the association between ECB damage and DON concentrations was not consistent across years [58]. Likewise, the German study that showed lower fumonisin levels in Bt corn did not show correspondingly lower DON levels [43].

Two studies have examined whether Bt corn has lower levels of zearalenone, also produced by *F. graminearum*. Bakan *et al.* [42] had found that though zearalenone levels were generally low in field tests in France and Spain, Bt hybrids did show significantly lower zearalenone at certain test sites. As described above, animal feed made from Bt corn was shown to have lower zearalenone levels [57].

Table 1 summarizes the available literature on currently commercially available Bt corn events and mycotoxin reduction, or lack thereof, evidenced in field trials around the world.

Bt Corn, Insect Damage and Mycotoxins Post-harvest

As of yet, there have been few studies examining the role of Bt corn in continued mycotoxin control in post-harvest conditions. During harvesting and post-harvest conditions, many factors such as humidity, temperature, reaping and threshing machinery, and drying speed and methods can influence the presence or absence of fungi and subsequent production of mycotoxins. Therefore, it is

Table 1 Studies demonstrating current events of Bt corn's control, or lack thereof, of fumonisin, aflatoxin, DON and zearalenone in field trials

Bt corn lower mycotoxins than non-Bt isolines?	Yes	No
Fumonisin	■ US Midwest [6, 37, 38] ■ Throughout USA [39, 42] ■ US Midwest when ECB favoured [40] ■ France, Italy, Turkey and Argentina [41, 42, 44] ■ Germany [43] ■ Philippines [44]	■ US Midwest when CEW favoured [40]
Aflatoxin	■ US South when aflatoxin levels high [46], or fungus applied through non-wounding technique [47] ■ US South, some years [49, 51] ■ US South, new varieties controlling corn earworm and fall armyworm [55]	■ US South when aflatoxin levels low [46], or fungus applied by wounding technique [47] ■ US South [48] ■ US South, some years [49, 51] ■ taly [52, 53]
DON	■ Canada [56] ■ Germany (animal feed) [57] ■ Eastern Europe, some years [58]	■ Eastern Europe, some years [58] ■ Germany [43]
Zearalenone	■ France and Spain [42] ■ Germany (animal feed) [57]	

important to ask whether Bt corn still has pest-protective properties in post-harvest conditions.

A variety of studies have shown that Bt corn's crystal proteins are still active for months after grinding and processing, that Bt corn confers pest protection in storage, and that animal feed made from Bt corn has lower mycotoxin levels than that made from non-Bt corn. MacKenzie *et al.* [32] found that the crystal proteins that are toxic to lepidopteran insect pests are still active in Bt corn that had been ground for at least 90 days. This suggests that in intact form, Bt corn kernels would still contain stable and active proteins for longer than 90 days. Three studies [59–61] show that in storage conditions, Bt corn confers pest protection against *B. thuringiensis*-susceptible lepidopteran pests; in particular, Indian meal moth (Lepidoptera: Pyralidae) and Angoumois grain moth (Lepidoptera: Gelechiidae). Finally, Aulrich *et al.* [57] found lower concentrations of mycotoxins in animal feed made from Bt corn compared with feed made from non-transgenic isolines.

Economic Analyses of Mycotoxins and Bt Corn's Beneficial Impact

To date, there have been few analyses of the costs mycotoxins pose to society. Lubulwa and Davis [62] calculated the total social costs of aflatoxin in three developing Asian nations, the Philippines, Thailand and Indonesia, to be on the order of nearly one billion 1994 US dollars. These costs included losses in market return and from compromised animal and human health. A World Bank study [63] found that compliance with an aflatoxin standard of 2 ppb could cost African nations $720 million through lost exports alone. Within the USA, Vardon *et al.* [64] estimated the costs of three mycotoxins in various crops, and found that total annual costs within the USA were close to $1 billion. Annual losses through aflatoxin contamination were estimated to be close to $300 million of this total cost. Wu [27] found the costs to the USA, China and Argentina to comply with the EU standard for fumonisin in corn would exceed $400 million annually. In addition, Robens and Cardwell [65] estimated that the costs to manage mycotoxins in the USA, including research and testing, are in the tens of millions of dollars.

Wu *et al.* [7, 8] estimated the economic benefit from Bt corn's reduction of fumonisin, aflatoxin and DON in the USA at about $30 million annually. These were primarily market-related benefits (lower rejection rates for excessively high mycotoxin levels), although there are also small benefits in terms of improved animal health from lower mycotoxin levels in feed. They had reported significant uncertainty bounds, depending on insect-related corn disease in any given year, and the variability of Bt corn's performance based on the field studies described in this paper.

Summary

Mycotoxins in corn pose a serious economic and health threat in food and feed supplies worldwide. Annually, the global losses associated with mycotoxin contamination are in the hundreds of millions US$. The market and health risks associated with mycotoxins are most serious in LDCs.

Bt corn is being planted at an ever-growing rate around the world. Aside from its primary benefit of insect pest protection, it has the important secondary benefit of reducing mycotoxin concentrations, because of the relationship between insect pest damage and fungal colonization. The currently available varieties of Bt corn have shown strong evidence in field conditions worldwide of having significantly lower fumonisin levels than non-Bt isolines. There is also limited evidence for lower levels of DON and zearalenone in Bt corn, although there are fewer field studies on these relationships. The more extensive work on aflatoxin reduction in Bt corn has yielded mixed results, but new varieties of Bt corn that may be commercialized soon are likely to have a more significant impact on aflatoxin levels. Hence, Bt corn is an important potential tool for mycotoxin control, both in the USA and in other nations.

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