World Mycotoxin Journal, 2016; 9 (3): 475-486

Wageningen Academic Publishers

The impact of *Bacillus thuringiensis* technology on the occurrence of fumonisins and other mycotoxins in maize

J. Díaz-Gómez[1], S. Marín[1], T. Capell[2], V. Sanchis[1] and A.J. Ramos[1*]

[1]Food Technology Department, University of Lleida, UTPV-XaRTA, Agrotecnio Center, Av. Rovira Roure 191, 25198 Lleida, Spain; [2]Plant Production and Forestry Science Department, University of Lleida, Agrotecnio Center, Av. Rovira Roure 191, 25198 Lleida, Spain; ajramos@tecal.udl.cat

Received: 28 July 2015 / Accepted: 19 November 2015

REVIEW ARTICLE

Abstract

In many developing countries, maize is both a staple food crop and a widely-used animal feed. However, adventitious colonisation or damage caused by insect pests allows fungi to penetrate the vegetative parts of the plant and the kernels, the latter resulting in mycotoxin contamination. Maize seeds contaminated with fumonisins and other mycotoxins pose a serious threat to both humans and livestock. However, numerous studies have reported a significant reduction in pest damage, disease symptoms and fumonisin levels in maize hybrids expressing the *Bacillus thuringiensis* (Bt) gene *cry1Ab*, particularly in areas where the European corn borer is prevalent. When other pests are also present, the *cry1Ab* gene alone offers insufficient protection, and combinations of insecticidal genes are required to reduce damage to plants caused by insects. The combination of Cry1Ab protein with other Cry proteins (such as Cry1F) or Vip proteins has reduced the incidence of pests and, indirectly, mycotoxin levels. Maize hybrids expressing multiple Bt genes, such as SmartStax®, are less susceptible to damage by insects, but mycotoxin levels are not routinely and consistently compared in these crops. Bt maize has a greater economic impact on *Fusarium* toxins than aflatoxins. The main factors that determine the effectiveness of Bt hybrids are the type of pest and the environmental conditions, but the different fungal infection pathways must also be considered. An alternative strategy to reduce mycotoxin levels in crops is the development of transgenic plants expressing genes that protect against fungal infection or reduce mycotoxin levels by *in situ* detoxification. In this review article, we summarise what is known about the relationship between the cultivation of Bt maize hybrids and contamination levels with different types of mycotoxins.

Keywords: fumonisins, aflatoxins, deoxynivalenol, *Bacillus thuringiensis*, European corn borer

1. Introduction

Maize (*Zea mays* L.) is the most common source of fumonisins in human and animal diets. Maize seeds are often contaminated with fumonisins produced primarily by *Fusarium verticillioides* and *Fusarium proliferatum.* These fungi can infect the seeds, or the silks can be contaminated by airborne or waterborne conidia, systemic infections can be caused by contamination of the roots, or pest insects can injure the plants allowing fungal penetration (Munkvold and Desjardins, 1997). Other mycotoxins may be present alone or together with fumonisins, including aflatoxins, deoxynivalenol (DON), zearalenone (ZEA), and some recently-discovered *Fusarium* metabolites collectively known as emergent mycotoxins, such as moniliformin (MON), beauvericin, enniatins and fusaproliferin (Desjardins, 2006; Marín *et al.*, 2013). Aflatoxins are mainly produced by *Aspergillus flavus*, which synthesises type B aflatoxins as well as cyclopiazonic acid (CPA) depending on the strain, and by *Aspergillus parasiticus*, which synthesises both type B and type G aflatoxins, but not CPA. DON and ZEA are mainly produced by *Fusarium graminearum* and *Fusarium culmorum* and they are often found as co-contaminants. Among the emergent mycotoxins, MON is the most prevalent and can be produced by several *Fusarium* species, including *Fusarium avenaceum*, *Fusarium* *tricinctum*, *F. proliferatum*, *Fusarium subglutinans* and *F. verticillioides* (Marín *et al.*, 2013).

The consumption of mycotoxin-contaminated kernels is associated with a range of diseases and disorders in humans and domestic animals, including cancer, immune system dysfunction and metabolic disorders (Marasas, 2001; Marín *et al.*, 2013; Sobrova *et al.*, 2010; Williams *et al.*, 2004). Fumonisins and aflatoxins are carcinogens (aflatoxin B_1 (AFB_1) is the most carcinogenic natural compound known), and this creates a strong impetus to restrict the exposure of human and animal populations as far as possible (IARC, 1993, 2002, 2012). In 2001, several countries submitted information on the concentration of fumonisins in maize and maize-derived foods, and fumonisins were detected in more than 60% of all food products (JECFA, 2001). These data are supported by a European Union report on the exposure of the EU population to *Fusarium* toxins. Among samples of raw maize material, 67% were positive for fumonisin B_1 (FB_1) (total=801) and a 51% were positive for fumonisin B_2 (FB_2) (total=544) (SCOOP, 2003).

The European Commission has set maximum levels for mycotoxins in maize and maize products. When such products are intended for human consumption, these values are currently 200-1000 µg/kg for fumonisins, 750 µg/kg for DON, 100 µg/kg for ZEA, 3 µg/kg for ochratoxin A (OTA), 5 µg/kg for AFB_1, and 10 µg/kg for total aflatoxins. Recently, indicative levels of 100 µg/kg in maize were also established for the total content of the trichothecene mycotoxins T-2 and HT-2 toxin (EC, 2006b, 2007, 2010, 2012, 2013a). In animal feed, only the level of AFB_1 is currently regulated in the EU (maximum 0.005-0.02 mg/kg, depending on the type of feed). Other important mycotoxins in feed are limited by guidance values that vary with the species of livestock: 0.9-12 mg/kg for DON, 0.1-3.0 mg/kg for ZEA, 0.05-0.25 mg/kg for OTA and 5-60 mg/kg for FB_1+FB_2. Recently, indicative levels of 250-2000 µg/kg in cereal products for feed and compound feed have been specified for the total content of T-2 and HT-2 toxin, with the exception of feed for cats, for which the guidance value is 50 µg/kg (EC, 2002, 2003b, 2006a, 2013a,b).

In contrast, the United States Food and Drug Administration (FDA) has proposed a guideline for total fumonisin levels in food of 2-4 mg/kg (depending on the product), and total aflatoxin levels of 20 µg/kg in maize and maize products for human consumption. For animal feed, the levels vary from 5 to 100 mg/kg for fumonisins and from 20 to 300 µg/kg for aflatoxins, depending on the animal species (FDA, 2000, 2001).

2. Transgenic maize and mycotoxins

Factors that affect mycotoxin occurrence

The presence of mycotoxins in maize results from the interaction of several factors, including temperature and humidity, nutrient availability, the presence of other fungi, stress, and physical damage caused by pest insects. Before harvest, important factors include the weather (temperature, humidity and rainfall), exposure to insect pests, fungi and other pathogens, planting dates, the maize genotype and cropping system. Fumonisin contamination in maize is directly associated with *Fusarium* pink ear rot (mainly produced by *F. verticilliodes*) and its incidence depends on both environmental conditions and pest damage. Kernel damage caused by insects exposes the kernels to fungal spores, although there are several additional infection pathways. Fungal growth and mycotoxin accumulation can also be stimulated post-harvest by poor storage conditions such as high humidity and the presence of other pests (Marín *et al.*, 2004; Miller, 2001).

Fungal growth and mycotoxin production are affected by multiple ecophysiological factors. The main factors that control fumonisin production in grain are temperature and water activity (a_w). *F. verticillioides* and *F. proliferatum* germinate at 5-37 °C when a_w exceeds 0.88, although the growth range fluctuates in the range 7-37 °C when a_w exceeds 0.90. The optimum conditions for fumonisin production by *F. verticillioides* are 30 °C at 0.97 a_w and for *F. proliferatum* the corresponding values are 15 °C at 0.97 a_w. Physicochemical and nutritional factors such as pH and carbon/nitrogen ratio can also affect fumonisin production. The presence of other fungi, such as *A. flavus* and *Aspergillus niger*, can affect the growth of *Fusarium* species, which is most competitive at 15 °C and 0.98 a_w. At high a_w values, fumonisin production can be stimulated by *A. niger* and other species (Marín *et al.*, 1999; Picot *et al.*, 2010; Sanchis *et al.*, 2006).

Suppression of insect pests using Bt technology

Injuries caused by insects are common sites of fungal infection on maize ears and stalks. The fungi can be airborne or may be suspended in water droplets that splash the wound, but insects can also act as vectors. One of the most prevalent examples is the European corn borer (ECB) (*Ostrinia nubilalis* Hübner), a maize pest that not only injures plants and exposes them to infection, but also vectors fungal spores, particularly *F. verticillioides* and *F. proliferatum*. ECB therefore promotes *Fusarium* infection of maize kernels and stalks, and may reduce yields by increasing the incidence of stalk rot (Munkvold and Desjardins, 1997; Munkvold *et al.*, 1997; Sobek and Munkvold, 1999). *F. verticillioides* is the most prevalent fungal pathogen of maize but fungicides are only partially effective, with efficacy depending on the pathogen strain and the fungicide mechanism of action (Falcão *et al.,* 2011). Therefore, pest insects are more appropriate targets than fungi for the development of strategies to reduce mycotoxin levels in maize.

In many parts of the world, the management of ECB now relies on transgenic hybrid maize lines expressing the *cry1Ab* gene from the Gram-positive soil bacterium *Bacillus thuringiensis* (Bt). This gene encodes a potent pro-toxin that is activated in the alkaline environment of the insect gut and is highly specific towards particular insect species. These insecticidal crystal proteins are also named δ-endotoxins or Cry proteins. *B. thuringiensis* has been used since 1938 to produce an insecticidal spray, but Bt transgenic plants resistant to ECB larvae were first made available in the USA in 1996 and in the EU in 1998. Different strains of the bacterium express different *cry* genes producing different pro-toxins that can protect plants against many different pests, including the corn rootworm complex (*Diabotrica virgifera*) (EPA, 2011; Höfte and Whiteley, 1989; Koziel *et al.*, 1993; Schnepf *et al.*, 1998). Therefore, alternative Bt genes (such as *cry1F*) have also been expressed in maize to protect against further lepidopteran pests (Abbas *et al.*, 2013; Bowers *et al.*, 2013; Koziel *et al.*, 1993). Economically-important maize pests that can be partially controlled using Bt hybrids include the corn earworm (CEW; *Helicoverpa zea*), common stalk borer (*Papiapema nebris*), southwestern corn borer (SWCB; *Diatraea grandiosella*) and western bean cutworm (WBC; *Striacosta albicosta*), whereas this strategy has proven less efficient against the fall armyworm (FAW; *Spodoptera frugiperda*) and black cutworm (*Agrotis ipsilon*) (Bowers *et al.*, 2013, 2014; Dowd, 2000; Munkvold and Hellmich, 1999; Williams *et al.*, 2002, 2005, 2006). Table 1 lists the Bt events targeting lepidopteran pests and the corn rootworm complex that are currently commercially available in the USA.

Since its adoption in the USA, Bt maize has become the second most widely cultivated genetically modified (GM) crop worldwide, after herbicide-tolerant soybean. About 30% of global maize production in 2014 (184 million ha) was represented by GM varieties (55.2 million ha) (James, 2014). However, the EU has a strict, complex and contradictory legislative framework for GM crops, with only the Mon810 maize event currently authorised for cultivation (EC, 1998, 2003a, 2008).

Fumonisin contamination in Bt and non-Bt maize

The ability of Bt genes to protect maize against ECB and other lepidopteran pests means that Bt maize tends to suffer a lower frequency of fungal infections and the infections that occur are often less severe or even symptomless. In the case of fumonisins, there is a large body of evidence to support the benefits of Bt maize, as shown by the comparison of fumonisin concentrations in Bt and non-Bt maize hybrids in different field locations (Supplementary Table S1).

Munkvold *et al.* (1999) published a fundamental study concerning the effect of Bt maize on disease management and concluded that transgenic hybrids expressing *cry1Ab* were less susceptible to ECB, suffered less from *Fusarium* ear rot and had lower fumonisin levels than their non-transgenic counterparts. However, if other insect pests were present alone or concurrent with ECB then the levels of fumonisins remained high (Clements *et al.*, 2003; Dowd, 2000; Hammond *et al.*, 2004; Papst *et al.*, 2005).

Many studies of natural infestations confirm the significant reduction in fumonisin levels associated with Bt hybrids (Abbas *et al.*, 2013; Ostry *et al.*, 2010; Pazzi *et al.*, 2006). These studies were carried out at different times in different countries, including Italy (Masoero *et al.*, 1999; Pietri and Piva, 2000), France (Bakan *et al.*, 2002; Folcher *et al.*, 2010; Pinson *et al.*, 2002), Spain (Bakan *et al.*, 2002), Argentina

Table 1. Current Bt maize varieties against lepidopteran pests and corn rootworm complex (EPA, 2011).

Pest	Bt event	Protein(s) expressed	Target pests[1]	Registrant
Lepidopteran pests	Bt11	Cry1Ab	ECB	Syngenta
	Mon810	Cry1Ab	ECB	Monsanto
	TC1507	Cry1F	ECB, BCW, FAW, SWCB	Dow/Mycogen Pioneer/Dupont
	Mon89034	Cry1A.105 + CryAb2	ECB, SWCB, CEW, FAW	Monsanto
	MIR162	Vip3Aa20	CEW, FAW, BCW, WBC	Syngenta
Coleopteran pests	DAS-59122-7	Cry34Ab1 + Cry35Ab1	WCRW, NCRW, MCRW	Pioneer/Dupont
	Mon88017	Cry3Bb1	WCRW, NCRW, MCRW	Monsanto
	MIR604	Cry3A	CRW	Syngenta

[1] ECB = European corn borer; CEW = corn earworm; WBC = western bean cutworm; BCW = black cutworm; FAW = fall armyworm; SWBC = southwestern corn borer; CRW = corn rootworm; WCRW = western corn rootworm; NCRW = northern corn rootworm; MCRW = Mexican corn rootworm.

(Barros *et al.*, 2009; De la Campa *et al.*, 2005) and the USA (Abbas *et al.*, 2006, 2007, 2013; Bruns and Abbas, 2006; Dowd, 2001).

Importantly, Bowers *et al.* (2013) confirmed that lower levels of fumonisins were present in Bt hybrids exposed to ECB, but found that a *cry1Ab* × *vip3Aa* hybrid was more resistant to ECB, CEW and WBC than the *cry1Ab* hybrid and the non-Bt hybrid in all of the years covered by the study. The vegetative insecticidal protein Vip3Aa can therefore be combined with other toxins, such as Cry1Ab to target additional lepidopteran pests. Unlike the *cry* genes, which are expressed during sporulation, *vip* genes are expressed during the *B. thuringiensis* vegetative growth phase and they do not share sequence homology with *cry* genes (Lee *et al.*, 2003; Schnepf *et al.*, 1998). The *cry1Ab* × *vip3Aa* hybrid also showed a lower level of pest damage, a lower incidence of *Fusarium* ear rot and lower levels of fumonisins when infested with ECB, whereas *cry1F* hybrids were better protected against WCB because *cry1F* specifically targets this pest (Bowers *et al.*, 2014). A comparison of eight commercially available Bt hybrids expressing multiple genes found no significant differences in the content of fumonisins among the hybrids (Abbas *et al.*, 2013). Recent studies of SmartStax® maize, which produces Cry1F, Cry1A.105+Cry2Ab2, Cry34Ab1/Cry35Ab1 and Cry3Bb1 to protect against common lepidopteran pests and the corn rootworm complex, reported less pest damage compared with single Bt hybrids and non-Bt hybrids, but did not consider mycotoxin levels (Head *et al.*, 2014; Rule *et al.*, 2014).

Aflatoxin contamination in Bt and non-Bt maize

Whereas the link between Bt maize and lower fumonisin levels is clearly established, the data for aflatoxin contamination are more contentious (Ostry *et al.*, 2015). Windham *et al.* (1999) showed a significant correlation between fungal and insect exposure, inoculation or infestation dates and aflatoxin contamination. They found that Bt hybrids suffered less damage from insects and had lower aflatoxin levels than the other hybrids studied (*A. flavus* resistant and *A. flavus* susceptible hybrids and a non-Bt isogenic hybrid) when manually infested with SWCB. More recent studies have focused on the inoculation technique, showing that inoculation with *A. flavus* by kernel wounding, which facilitates fungal penetration, results in high-level aflatoxin contamination regardless of the hybrids used. There were no differences among the hybrids when infected with fungi alone because lower aflatoxin levels in the Bt hybrids reflected the reduction in insect damage, which indirectly reduced fungal contamination. In contrast, a non-wounding inoculation technique combined with SWCB infestation resulted in significantly lower levels of aflatoxin in the Bt hybrids (Williams *et al.*, 2002, 2005; 2006). A testcross involving aflatoxin-resistant and aflatoxin-susceptible lines crossed with Bt and non-Bt maize revealed lower aflatoxin levels in the Bt testcrosses, but the difference for individual lines was significant in only two of 10 lines investigated. The low insect pressure during the experiment could explain these results, because the higher the insect pressure, the greater the differences between hybrids (Williams *et al.*, 2010).

In a 3-year study, Wiatrak *et al.* (2005) observed significantly lower aflatoxin levels in Bt compared to non-Bt hybrids during the first year of the experiment, but there was no difference with a tropical non-Bt hybrid. In the second year, significantly lower levels of aflatoxins were detected in the Bt hybrids than the tropical non-Bt hybrid, but there were no differences compared to the non-Bt hybrids. In the final year, there were no differences in aflatoxin contamination among the hybrids. Another 3-year study reported lower levels of aflatoxins in Bt than non-Bt hybrids, but only in one of the years (Abbas *et al.*, 2006; 2007; Bruns and Abbas, 2006). Nevertheless, a subsequent study showed that aflatoxin contamination was significantly reduced in the Bt hybrid compared to its non-Bt isoline (Abbas *et al.*, 2008). The authors continued these field trials until 2009, reporting lower mycotoxin levels in Bt maize, but the difference was not significant, perhaps due to the continuous cultivation (Abbas *et al.*, 2013).

In the USA, Odvody *et al.* (2000) observed less insect damage in Bt hybrids but aflatoxin levels were not consistent. In a subsequent study of different Bt hybrids, the lowest level of insect damage was observed in the Mon840 event (*cry2Ab*) correlating with significantly lower aflatoxin levels compared to non-Bt and *cry1Ab* hybrids in 2000, but only compared to the non-Bt hybrid in 2001 (Odvody and Chilcutt, 2002). Different *cryAb* events were also evaluated, revealing less insect damage in the Bt hybrids Mon810 and Bt11 compared to non-Bt hybrids, but aflatoxin levels were also inconsistent in this experiment (Odvody and Chilcutt, 2003). Similarly, Maupin *et al.* (2001) did not find significant differences in the levels of ear rot and aflatoxin accumulation when comparing Bt and non-Bt hybrids inoculated with *A. flavus*. Buntin *et al.* (2001) found no significant differences in aflatoxin levels between Bt and non-Bt maize, but the Bt hybrids suffered less severe FAW infestations. These data indicate that insect damage is strongly correlated with fumonisin levels but not aflatoxin levels, suggesting that other factors such as drought stress and individual hybrid vulnerability may play a more dominant role than insect damage in the determination of aflatoxin levels. The field experiments concerning aflatoxin levels in Bt and non-Bt hybrids are summarised in Supplementary Table S2.

Contamination with other mycotoxins in Bt and non-Bt maize

The results obtained with other mycotoxins are also controversial. Significantly lower levels of DON were observed in Bt compared to non-Bt hybrids in some studies (Magg *et al.*, 2002; Schaafsma *et al.*, 2002; Selwet, 2011; Valenta *et al.*, 2001), whereas in other cases the mycotoxin levels appeared to be location-dependent (Bakan *et al.*, 2002; Papst *et al.*, 2005; Pinson *et al.*, 2002) or there was no difference between Bt and non-Bt hybrids (Barros *et al.*, 2009). A few studies have even found evidence for slightly higher DON levels in Bt hybrids, although the location was an important confounding effect (Folcher *et al.*, 2010; Bakan *et al.* 2002). Schaafsma *et al.* (2002) analysed 102 commercial maize fields in Canada, reporting a reduction in DON levels in Bt hybrids depending on the severity of ECB infestation in each field. This was supported by a study carried out in Germany showing a reduction in DON levels in Bt compared to non-Bt hybrids (Valenta *et al.*, 2001). In another study, also in Germany, Magg *et al.* (2002) found significantly lower concentrations of DON in Bt maize in one of the two years of the experiment. Pinson *et al.* (2002) described differences in DON and ZEA levels between plots in two different fields in south and central France. Lower levels were observed in two Bt hybrids, but two others contained significantly higher levels of both DON and ZEA compared to the corresponding non-Bt cultivars. Bakan *et al.* (2002) reported low ZEA levels in their study, but significantly higher concentrations were observed in a traditional cultivar in France. In all the studies, the Bt hybrids expressed *cry1Ab* (Supplementary Table S3).

Only one study has investigated the impact of Bt on MON levels, and the Bt hybrids showed significantly lower levels of MON than non-Bt hybrids (isogenic and commercial hybrids) when infested with ECB. MON levels were significantly higher following the manual infestation of unprotected plants (296 μg/kg) compared to those treated with insecticide (66.2 μg/kg). In the infested plots, the MON concentrations were 153.5, 336.7 and 266.1 μg/kg for the transgenic, isogenic and commercial hybrids, respectively. In contrast, in the protected plots, the MON concentrations were 49.1, 99.3 and 42.9 μg/kg for the transgenic, isogenic and commercial hybrids, respectively (Magg *et al.*, 2003).

Economic impact of mycotoxin reduction in Bt maize

The main goal of Bt technology is to reduce pest damage and promote higher yields. However, indirect benefits, such as the reduction of fumonisin levels, also increase the percentage of maize grain that meets US and/or EU regulatory limits, which can have a significant economic impact and may also reduce the prevalence and severity of human and animal diseases (Bowers *et al.*, 2013; Folcher *et al.*, 2010; Hammond *et al.*, 2004; Magg *et al.*, 2002, 2003; Munkvold *et al.*, 1997, 1999; Pinson *et al.*, 2002).

Estimations for the cost of crop losses due to mycotoxin contamination in the USA range from US$ 500,000 to US$ 1.5 billion, reflecting variations in contamination levels, regulatory limits, price variations and production outputs (CAST, 2003). However, in the USA most losses are regulatory in nature (i.e. based on the rejection of grain based on quality) rather than actual harvest losses, and maize grains rejected for food and feed use may still be suitable for industrial processes, such as biofuel production. On the other hand, the economic benefit of Bt maize in the USA has been specifically valued at US$ 8.8 million in terms of preventing losses caused by fumonisins, and similarly US$ 8.1 million for DON and US$ 14 million for aflatoxins, even though the aflatoxin levels depend on the predominant pest species (Wu, 2006, 2007; 2014; Wu *et al.*, 2004). These data based on studies carried out over a decade ago do not take into account the increased adoption of Bt maize, which has risen from 30% in 2005 to more than 80% in 2015 (USDA-ERS, 2015). A recent study of the Thailand maize market estimated the economic losses due to aflatoxins. A loss of US$ ~6.9 million per annum was estimated assuming low levels of aflatoxin contamination (data from harvest and dried maize supplied by a pet company) but this increased to US$ ~100 million per annum assuming higher aflatoxin levels (data from retail markets). The rejection of aflatoxin-contaminated maize by the livestock sector is the most influential factor contributing to economic losses. Thus, the selection of high-quality maize (by the pet company) reflects lower levels of aflatoxin contamination, and lower economic losses (Lubulwa *et al.*, 2015). Bt technology can improve the quality of maize by reducing mycotoxin levels as an indirect consequence of preventing infestations with insect pests. This technology also reduces the need for chemical insecticides, resulting in lower levels of pesticide residues in food and water and less environmental impact (Brookes and Barfoot, 2013; Qaim, 2009).

Alternative strategies to reduce mycotoxin levels in maize

Although targeting pest insects can help to reduce opportunistic fungal infections of maize, other transgenic approaches have emerged more recently in which the fungus itself is the target. For example, Kant *et al.* (2012) reported a field study of transgenic maize expressing a modified rice *Rp13* gene encoding ribosomal protein L3, a primary target of DON. They developed two transgenic maize lines expressing the *Rp13* gene, one using the constitutive CaMV 35S promoter and the other using the silk-specific ZmGRP5 promoter. Both plants were less susceptible to *F. graminearum* than wild-type plants, and those containing the silk-specific promoter were the most tolerant, with the mildest symptoms under field conditions (DON levels were not evaluated). The difference in efficacy may reflect the broader activity of the silk promoter in the seed pericarp tissue. Maize silks are the primary route used by *F. graminearum* to infect the kernels. Expression of the modified *Rpl3* gene in silk tissue may therefore help to reduce *Gibberella* ear rot and hence DON levels.

Maize plants expressing the α-amylase inhibitor protein from *Lablab purpurea* (AILP) can also be used to reduce fungal infection. Fungal amylases liberate fermentable sugars from kernel starch which are essential for mycotoxin production. Kernel screening assays in AILP-transgenic maize plants revealed aflatoxin levels 56% lower than controls. AILP expression therefore appears to reduce both fungal growth on the kernels and aflatoxin accumulation (Chen *et al.*, 2015).

Another promising approach to reduce mycotoxin contamination is enzymatic mycotoxin detoxification *in situ*, which converts the mycotoxins into less toxic compounds. The ZEA lactone ring is sensitive to hydrolysis by the fungus *Clonostachys rosea*, which synthesises an alkaline lactonohydrolase responsible for detoxification (Kimura *et al.*, 2006; Takahashi-Ando *et al.*, 2002). The corresponding gene (*zhd101*) was able to reduce ZEA levels *in vitro* and in field-grown plants compared to non-transgenic controls, even when infected with *F. graminearum.* The ability of transgenic seeds to degrade ZEA was evaluated by immersing the seeds in 50 μg/ml ZEA for 48 h. The kernel tissues were then analysed by HPLC, showing that wild-type seeds contained 24.6±1.7 μg ZEA/g, whereas the transgenic seeds contained only 1.6±0.4 μg ZEA/g. The detoxification of ZEA in *Fusarium*-infected transgenic kernels was evaluated after inoculation with *F. graminearum*. The wild-type seeds contained 15.4±3.7 ng ZEA/g, whereas the level of ZEA in non-inoculated seeds and inoculated transgenic seeds was below the detection threshold (Igawa *et al.*, 2007). Similarly, the yeasts *Exophiala spinifera* and *Rhinocladiella atrovirens*, and the Gram-negative bacterium ATCC 55552, can produce enzymes that metabolise fumonisins. Duvick *et al.* (2003) patented a fumonisin esterase produced by these yeasts which can hydrolyse the tricarballylate esters of FB_1, and this is active in transgenic maize. The esterase gene reduced fumonisin levels in *Fusarium*-infected grain from 1.522 mg/kg without the enzyme to 0.379 mg/kg in esterase positive plants. Aflatoxin detoxification in transgenic plants has yet to be reported (Duvick, 2001; Hartinger and Moll, 2011; Jard *et al.*, 2011).

3. Concluding remarks

Several transgenic strategies can be used to reduce mycotoxin contamination in food and feed but Bt maize hybrids are widely grown and several studies have confirmed the reduction in pest damage, disease symptoms and fumonisin levels, particularly when ECB is the predominant pest. This is because fumonisin levels are reduced in Bt hybrids if the *Fusarium* population is dominated by species whose colonisation of the plant is promoted by ECB damage (Miller, 2001).

Among the commercial Bt hybrids, Mon810 and Bt11 (which express *cry1Ab*) were associated with a reduction in the occurrence of *Fusarium* ear rot and fumonisins due to the lower level of kernel damage caused by susceptible lepidopteran pests (Dowd, 2000, 2001; Magg *et al.*, 2001; Munkvold and Hellmich, 1999; Papst *et al.*, 2005). Conversely, no difference has been reported between Bt 176 hybrids (Bt event 176 was withdrawn in 2001) and non-Bt maize (Magg *et al.*, 2002; Schaafsma *et al.*, 2002), and it has even been proposed that resistance against ECB and *Fusarium* ear rot may be inherited independently (Magg *et al.*, 2002; Miller, 2001).

The pest species and its abundance are key determinants of mycotoxin levels, particularly if the main pest is CEW which is unaffected by Cry1Ab (Clements *et al.*, 2003; Dowd, 2000; Hammond *et al.*, 2004). Lower levels of aflatoxins occur in Bt maize if the main pest is SWCB or CEW, but there is no difference between Bt and non-Bt maize if the pest is FAW (Buntin *et al.*, 2001; Williams *et al.*, 2002, 2005, 2006, 2010). When other pests are present, hybrids expressing Cry1Ab are inefficient and must be combined with further Cry proteins (such as Cry1F) or Vip proteins to increase the level of protection (Bowers *et al.*, 2013; 2014). Thus, if Bt events are not selected by taking into account prevalent insect pests and environmental conditions in each field, Bt technology will not be effective. Hence the importance of Bt hybrids expressing multiple genes, whose performance in the presence of different pests and climate conditions has yet to be studied in detail.

The impact of Bt maize on the accumulation of aflatoxins, DON and ZEA is inconclusive because the extent of contamination depends on many interrelated factors. The different fungal infection pathways must be considered to understand the effect of Bt hybrids. The most common route used by *F. verticillioides* to infect the kernel is through the silks or through wounds caused by insect pests, whereas *F. graminearum* primarily reaches the kernels via the silks (Munkvold, 2003; Munkvold and Desjardins, 1997). *A. flavus* can infect maize kernels through the silks too. This may explain why Bt maize hybrids that are resistant to insect pests have less *Fusarium* ear rot and lower fumonisin levels, whereas the relationship with DON, ZEA and aflatoxin levels is not so clear cut. Further studies are necessary to determine the interaction between Bt maize and the different fungal species that produce these toxins.

Mycotoxin contamination is frequently linked with drought, heat stress and insects. Drought favours the accumulation of fumonisins more than heat stress (Miller, 2001). A 2-year study was carried out by Traore *et al.* (2000) to characterise the effect of drought stress on Bt maize. Water deficit during the vegetative period delayed leaf emergence, reduced the leaf area and caused stunted growth in both Bt and non-Bt hybrids. It also reduced grain and biomass yields and kernel number per ear during both years. However, the Bt hybrids had greater biomass in 1997 and greater grain yields in 1998 because they were not so severely infected by second-generation ECB. Therefore, Bt maize continues to play an important role in insect resistance under drought stress.

The first commercially available drought-tolerant GM maize variety (MON87460) expresses a bacterial cold shock protein B (CspB), a molecular chaperone derived from *Bacillus subtilis,* which may provide a yield advantage under limited water availability. A recent field study confirmed the higher grain yield of MON87460 under drought conditions compared to a conventional hybrid (Nemali *et al.*, 2015). The combination of Bt and drought-tolerant maize should therefore achieve even higher yields and lower mycotoxin levels because it will be protected against two major environmental stress factors.

Studies that considered aflatoxins and fumonisins simultaneously reported variable results, suggesting that diverse environmental conditions may prevent the control of both mycotoxins in the same crops (Abbas *et al.*, 2002, 2006, 2007, 2008; Bruns and Abbas, 2006). More studies are needed to determine whether aflatoxin resistance traits can be crossed into Bt hybrids. Aflatoxin-resistant germplasm tends to possess undesirable agronomic traits such as tight husk coverage and late maturity. Breeding programs aiming to achieve the introgression of aflatoxin resistance into Bt hybrids could remove these undesirable characteristics while reducing aflatoxin contamination (Williams *et al.*, 2008, 2010). Bt maize has a greater economic impact on *Fusarium* mycotoxins than aflatoxins (Wu, 2006, 2007; Wu *et al.*, 2004). Further studies are needed to evaluate the effect of both Bt hybrids expressing multiple genes and Bt hybrids combined with maize lines that are resistant to the accumulation of other mycotoxins, especially aflatoxins.

A potential risk that must be borne in mind comes in the form of mycotoxin derivatives (modified mycotoxins) that escape routine analytical techniques but may be digested by animals triggering toxic effects comparable to free mycotoxins. These derivatives should be included in the total mycotoxin allowances, and future legislation must consider their presence even though this would increase the stringency of testing and rejection, resulting in further economic loss. This highlights the benefits of Bt maize, which would reduce the levels of mycotoxins and potentially their derivatives, although the impact of Bt hybrids on the accumulation of modified mycotoxins needs to be addressed in more detail (De Boevre *et al.*, 2012, 2014; De Saeger and Van Egmond, 2012; Wu, 2006). Finally, the development of transgenic plants expressing genes that protect against fungal infection (e.g. the modified *Rp13* gene and the AILP transgene) or reduce mycotoxin levels by *in situ* detoxification (e.g. *zhd101* and fumonisin esterase) could provide an additional strategy to control mycotoxins (Chen *et al.*, 2015; Duvick, 2001; Duvick *et al.*, 2003; Igawa *et al.*, 2007; Kant *et al.*, 2012) and could be combined with Bt hybrids to provide additive or even synergistic protection against mycotoxin-producing fungal pathogens.

Supplementary material

Supplementary material can be found online at http://dx.doi.org/10.3920/WMJ2015.1960.

Table S1. Summary of studies comparing fumonisin levels in Bt and non-Bt hybrids.

Table S2. Summary of studies comparing aflatoxin levels in Bt and non-Bt hybrids.

Table S3. Summary of studies comparing deoxynivalenol levels in Bt and non-Bt hybrids.

Acknowledgements

The authors are grateful for financial support from La Caixa (Recercaixa project VitaMaize: High quality and safe food through antioxidant fortified maize) and to the Catalonian Government (XaRTA Reference Network on Food Technology). The authors are also grateful to their research groups: Vegetal Products Technology (2014 SGR 540) and Agricultural Biotechnology (2014 SGR 1296). J. Díaz-Gómez thanks the University of Lleida for the pre-doctoral grant.

References

Abbas, H.K., Accinelli, C., Zablotowicz, R.M., Abel, C.A., Bruns, H.A., Dong, Y. and Shier W.T., 2008. Dynamics of mycotoxin and *Aspergillus flavus* levels in aging Bt and non-Bt Corn residues under Mississippi no-till conditions. Journal of Agricultural and Food Chemistry 56: 7578-7585.

Abbas, H.K., Cartwright, R.D., Xie, W. and Shier, W.T., 2006. Aflatoxin and fumonisin contamination of corn (maize, *Zea mays*) hybrids in Arkansas. Crop Protection 25: 1-9.

Abbas, H.K., Shier, W.T. and Cartwright, R.D., 2007. Effect of temperature, rainfall and planting date on aflatoxin and fumonisin contamination in commercial Bt and non-Bt corn hybrids in Arkansas. Phytoprotection 88: 41-50.

Abbas, H.K., Williams W.P., Windham, G.L., Pringle, H.C., III, Xie, W. and Shier, W.T., 2002. Aflatoxin and fumonisin contamination of commercial corn (*Zea mays*) hybrids in Mississippi. Journal of Agricultural and Food Chemistry 50: 5246-5254.

Abbas, H.K., Zablotowicz, R.M., Weaver, M.A., Shier, W.T., Bruns, H.A., Bellaloui, N., Accinelli, C. and Abel, C.A., 2013. Implications of Bt traits on mycotoxin contamination in maize: overview and recent experimental results in Southern United States. Journal of Agricultural and Food Chemistry 61: 11759-11770.

Bakan, B., Melcion, D., Richard-Molard, D. and Cahagnier, B., 2002. Fungal growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. Journal of Agricultural and Food Chemistry 50: 728-731.

Barros, G., Magnoli, C., Reynoso, M.M., Ramirez, M.L., Farnochi, M.C., Torres, A., Dalcero, M., Sequeira, J., Rubinstein, C. and Chulze, S., 2009. Fungal and mycotoxin contamination in Bt maize and non-Bt maize grown in Argentina. World Mycotoxin Journal 2: 53-60.

Bowers, E., Hellmich, R.L. and Munkvold, G.P., 2013. Vip3Aa and Cry1Ab proteins in maize reduce *Fusarium* ear rot and fumonisins by deterring kernel injury from multiple Lepidopteran pests. World Mycotoxin Journal 6: 127-135.

Bowers, E., Hellmich, R.L. and Munkvold, G.P., 2014. Comparison of fumonisin contamination using HPLC and ELISA methods in Bt and near-isogenic maize hybrids infested with European corn borer or Western bean cutworm. Journal of Agricultural and Food Chemistry 62: 6463-6472.

Brookes, G. and Barfoot, P., 2013. The global income and production effects of genetically modified (GM) crops 1996-2011. Landes Bioscience. GM Crops and Food: Biotechnology in Agriculture and the Food Chain 4: 74-83.

Bruns, H.A. and Abbas, H.K., 2006. Planting date effects on Bt and non-Bt corn in the Mid-South USA. Agronomy Journal 98: 100-106.

Buntin, G.D., Lee, R.D., Wilson, D.M. and McPherson, R.M., 2001. Evaluation of Yieldgard transgenic resistance for control of fall armyworm and corn earworm (Lepidoptera: *Noctuidae*) on corn. Florida Entomologist 84: 37-42.

Council for Agricultural Science and Technology (CAST), 2003. Mycotoxins: risks in plant, animal and human systems. Task force report No. 139. Ames, IA, USA.

Chen, Z.-Y., Rajasekaran, K., Brown, R.L., Sayler, R.J. and Bhatnagar, D., 2015. Discovery and confirmation of genes/proteins associated with maize aflatoxin resistance. World Mycotoxin Journal 8: 211-224.

Clements, M.J., Campbell, K.W., Maragos, C.M., Pilcher, C., Headrick, J.M., Pataky, J.K. and White, D.G., 2003. Influence of Cry1Ab protein and hybrid genotype on fumonisin contamination and *Fusarium* ear rot of corn. Crop Science 43: 1283-1293.

De Boevre, M., Di Mavungu, J.D., Landschoot, S., Audenaert, K., Eeckhout, M., Maene, P., Haesaert, G. and De Saeger, S., 2012. Natural occurrence of masked mycotoxins and their masked forms in food and feed products. World Mycotoxin Journal 5: 207-219.

De Boevre, M., Landschoot, S., Audenaert, K., Maene, P., Di Mavungu, J.D., Eeckhout, M., Haesaert, G. and De Saeger, S., 2014. Occurrence and within field variability of Fusarium mycotoxins and their masked forms in maize crops in Belgium. World Mycotoxin Journal 7: 91-102.

De Saeger, S. and Van Egmond, H.P., 2012. Special issue: masked mycotoxins. World Mycotoxin Journal 5: 203-206.

De la Campa, R., Hooker, D.C., Miller, J.D., Schaafsma, A.W. and Hammond, B.G., 2005. Modeling effects of environment, insect damage, and Bt genotypes on fumonisin accumulation in maize in Argentina and the Philippines. Mycopathologia 159: 539-552.

Desjardins, A.E., 2006. *Fusarium* mycotoxins: chemistry, genetics and biology. The American Phytopathological Society (APS) Press, St. Paul, MN, USA.

Dowd, P.F., 2000. Indirect reduction of ear molds and associated mycotoxins in *Bacillus thuringiensis* corn under controlled and open field conditions: utility and limitations. Journal of Economic Entomology 93: 1669-1679.

Dowd, P.F., 2001. Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial fields. Journal of Economic Entomology 94: 1067-1074.

Duvick, J., 2001. Prospects for reducing fumonisin contamination of maize through genetic modification. Environmental Health Perspectives 109: 337-342.

Duvick, J., Maddox, J.R., Rood, T.A., Wang, X., Bowen, B.A. and Gilliam, J.T., 2003. Fumonisin detoxification composition and methods. US Patent 6670189. Pioneer Hi-Bred International, Inc, Des Moines, IA, USA.

Environmental Protection Agency (EPA), 2011. Current & previously registered section 3 PIP (plant incorporated protectants) registrations. Available at: http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm.

European Commission (EC), 1998. Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON 810), pursuant to Council Directive 90/220/EEC (98/294/EC). Official Journal of the European Union L131: 32-33.

European Commission (EC), 2002. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Official Journal of the European Union L140: 10-21.

European Commission (EC), 2003a. Regulation (EC) No. 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Union L268: 1-23.

European Commission (EC), 2003b. Commission Directive 2003/100/EC of 31 October 2003 amending annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Official Journal of the European Union L285: 33-37.

European Commission (EC), 2006a. Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC). Official Journal of the European Union L229: 7-9.

European Commission (EC), 2006b. Commission Regulation (EC) No. 1881/2006, of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L364: 5-24.

European Commission (EC), 2007. Commission Regulation (EC) No. 1126/2007 of 28 September 2007 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Official Journal of the European Union L255: 14-17.

European Commission (EC), 2008. Directive 2008/27/EC of the European Parliament and of the Council of 11 March 2008 amending Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, as regards the implementing powers conferred on the Commission. Official Journal of the European Union L81: 45-47.

European Commission (EC), 2010. Commission Regulation (EU) No. 165/2010 of 26 February 2010 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Official Journal of the European Union L50: 8-12.

European Commission (EC), 2012. Commission Regulation (EU) No. 594/2012 of 5 July 2012 amending Regulation (EC) No. 1881/2006 as regards the maximum levels of the contaminants ochratoxin A, non dioxin-like PCBs and melamine in foodstuffs. Official Journal of the European Union L176: 43-45.

European Commission (EC), 2013a. Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products (2013/165/EU). Official Journal of the European Union L91:12-15.

European Commission (EC), 2013b. Commission Recommendation of 4 November 2013 amending Recommendation 2006/576/EC as regards T-2 and HT-2 toxin in compound feed for cats (2013/637/EU). Official Journal of the European Union L294: 44.

Falcão V.C.A., Ono M.A., de Ávila Miguel, T., Vizoni E., Hirooka E.Y. and Ono E.Y.S., 2011. *Fusarium verticillioides*: evaluation of fumonisin production and effect of fungicides on *in vitro* inhibition of mycelial growth. Mycopathologia 171: 77-84.

Food and Drug Administration (FDA), 2000. Guidance for industry: action levels for poisonous or deleterious substances in human food and animal feed. US-FDA, Washington, DC, USA. Available at: http://tinyurl.com/q86m8ul.

Food and Drug Administration (FDA), 2001. Guidance for industry: fumonisin levels in human foods and animal feeds. Available at: http://tinyurl.com/ohk5akp.

Folcher, L., Delos, M., Marengue, E., Jarry, M., Weissenberger, A., Eychenne, N. and Regnault-Roger, C., 2010. Lower mycotoxin levels in Bt maize grain. Agronomy for Sustainable Development 30: 711-719.

Hammond, B.G., Campbell, K.W., Pilcher, C.D., Degooyer, T.A., Robinson, A.E., McMillen, B.L., Spangler, S.M., Riordan, S.G., Rice, L.G. and Richard, J.L., 2004. Lower fumonisin mycotoxin levels in the grain of Bt corn grown in the United States in 2000-2002. Journal of Agricultural and Food Chemistry 52: 1390-1397.

Hartinger, D. and Moll, W.-D., 2011. Fumonisin elimination and prospects for detoxification by enzymatic transformation. World Mycotoxin Journal 4: 271-283.

Head, G., Carroll, M., Clark, T., Galvan, T. and Huckaba, R.M., 2014. Efficacy of SmartStax® insect-protected corn hybrids against corn rootworm: the value of pyramiding the Cry3Bb1 and Cry34/35Ab1 proteins. Crop Protection 57: 38-47.

Höfte, H. and Whiteley, H.R., 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiological Reviews 53: 242-255.

International Agency for Research on Cancer (IARC), 1993. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monographs on the evaluation of carcinogenic risks to humans. Vol. 56. IARC, Lyon, France,

International Agency for Research on Cancer (IARC), 2002. Monograph on the evaluation of carcinogenic risks to humans. Vol. 82. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC, Lyon, France.

International Agency for Research on Cancer (IARC), 2012. Aflatoxins. In: Chemical agents and related occupations. IARC Monographs on the evaluation of carcinogenic risks to humans. Vol. 100F. IARC, Lyon, France, pp. 226-248. Available at: http://tinyurl.com/ogoj86t.

Igawa, T., Takahashi-Ando, N., Ochiai, N., Ohsato, S., Shimizu, T., Kudo. T., Yamaguchi, I. and Kimura, M., 2007. Reduced contamination by the *Fusarium* mycotoxin zearalenone in maize kernels through genetic modification with a detoxification gene. Applied and Environmental Microbiology 73: 1622-1629.

James, C., 2014. Global status of commercialized Biotech/GM crops: 2014. ISAAA Brief No. 49. ISAAA: Ithaca, NY.

Jard, G., Liboz, T., Mathieu, F., Guyonvarc'h, A. and Lebrihi, A., 2011. Review of mycotoxin reduction in food and feed: from prevention in fields to detoxification by adsorption or transformation. Food Additives and Contaminants Part A 28: 1590-1609.

Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2001. Safety evaluation of certain mycotoxins in food. 56th Meeting of the JECFA, Geneva, International Programme on Chemical Safety. WHO Food Additives Series No. 47. WHO, Geneva, Switzerland.

Kant, P., Gulati, A., Harris, L., Gleddie, S., Singh, J. and Pauls, K.P., 2012. Transgenic corn plants with modified ribosomal protein L3 show decreased ear rot disease after inoculation with *Fusarium graminearum*. Australian Journal of Crop Science 6: 1598-1605.

Kimura, M., Takahashi-Ando, N., Nishiuchi, T., Ohsato, S., Tokai, T., Ochiai, N., Fujimura, M., Kudo, T., Hamamoto, H. and Yamaguchi, I., 2006. Molecular biology and biotechnology for reduction of *Fusarium* mycotoxin contamination. Pesticide Biochemistry and Physiology 86: 117-123.

Koziel, M.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M.R., Merlin, E., Rhodes, R., Warren, G.W., Wright, M. and Evola, S.V., 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Nature Biotechnology 11: 194-200.

Lee, M.K., Walters, F.S., Hart, H., Palekar, N. and Chen, J-S., 2003. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab delta-endotoxin. Applied and Environmental Microbiology 69: 4648-4657.

Lubulwa, G.A.S., Siriacha, P., Markwell, P.J. and Pitt, J.I., 2015. Estimating the burden of market loss due to aflatoxins in maize: methods and estimates for Thailand. World Mycotoxin Journal 8: 459-464.

Magg, T., Bohn, M., Klein, D., Merditaj, V. and Melchinger, A.E., 2003. Concentration of moniliformin produced by *Fusarium* species in grains of transgenic Bt maize hybrids compared to their isogenic counterparts and commercial varieties under European corn borer pressure. Plant Breeding 122: 322-327.

Magg, T., Melchinger A.E., Klein, D. and Bohn, M., 2001. Comparison of Bt maize hybrids with their non-transgenic counterparts and commercial varieties for resistance to European corn borer and for agronomic traits. Plant Breeding 120: 397-403.

Magg, T., Melchinger A.E., Klein, D. and Bohn, M., 2002. Relationship between European corn borer resistance and concentration of mycotoxins produced by *Fusarium* spp. in grains of transgenic Bt maize hybrids, their isogenic counterparts, and commercial varieties. Plant Breeding 121: 146-154.

Marasas, W.F.O., 2001. Discovery and occurrence of the fumonisins: a historical perspective. Environmental Health Perspectives 109: 239-243.

Marín, S., Magan, N., Bellí, N., Ramos, A.J., Canela R. and Sanchis, V., 1999. Two-dimensional profiles of fumonisin B_1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain. International Journal of Food Microbiology 51: 159-167.

Marín, S., Magan, N., Ramos, A.J. and Sanchis, V., 2004. Fumonisin-producing strains of *Fusarium*: a review of their ecophysiology. Journal of Food Protection 67: 1792-1805.

Marín, S., Ramos, A.J., Cano-Sancho, G. and Sanchis, V., 2013. Mycotoxins: occurrence, toxicology, and exposure assessment. Food and Chemical Toxicology 60: 218-237.

Masoero, F., Moschini, M., Rossi, F., Prandini, A. and Pietri, A., 1999. Nutritive value, mycotoxin contamination and *in vitro* rumen fermentation of normal and genetically modified corn (Cry1Ab) grown in Northern Italy. Maydica 44: 205-209.

Maupin, L.M., Clements, M.J., Walker, S.L. and White, D.G., 2001. Effects of CryIA(b) on *Aspergillus* ear rot and aflatoxin in commercial corn hybrids. Phytopathology 91: S59.

Miller, J.D., 2001. Factors that affect the occurrence of fumonisin. Environmental Health Perspectives 109: 321-324.

Munkvold, G.P., 2003. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. European Journal of Plant Pathology 109: 705-713.

Munkvold, G.P. and Desjardins, A.E., 1997. Fumonisins in maize: Can we reduce their occurrence? Plant Disease 81: 556-565.

Munkvold, G.P. and Hellmich, R.L., 1999. Genetically modified insect resistant corn: implications for disease management. APSnet Features. Available at: http://tinyurl.com/kuqq2ae.

Munkvold, G.P., Hellmich, R.L. and Rice, L.G., 1999. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and non-transgenic hybrids. Plant Disease 83: 130-138.

Munkvold, G.P., Hellmich, R.L., and Showers, W.B., 1997. Reduced *Fusarium* ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology 87: 1071-1077.

Nemali, K.S., Bonin, C., Dohleman, F.G., Stephens, M., Reeves, W.R., Nelson, D.E., Castiglioni, P., Whitsel, J.E., Sammons, B., Silady, R.A., Anstrom, D., Sharp, R.E., Patharkar, O.R., Clay, D., Coffin, M., Nemeth, M.A., Leibman, M.E., Luethy, M. and Lawson, M., 2015. Physiological responses related to increased grain yield under drought in the first biotechnology-derived drought-tolerance maize. Plant, Cell and Environment 38: 1866-1880.

Odvody, G.N. and Chilcutt, C.F., 2002. Aflatoxin and insect response in South Texas of near-isogenic corn-hybrids with Cry1Ab and Cry2Ab events. In: Proceedings aflatoxin/fumonisin elimination and fungal genomics workshops. 23-26 October 2001, Phoenix, Arizona. Mycopathologia 155: 107.

Odvody, G.N. and Chilcutt, C.F., 2003. Aflatoxin and insect response of near-isogenic non-Bt and Cry1A(b) (Mon810 and Bt11) commercial corn hybrids in South Texas in 2001-2002. In: Robens, J.F. and Dorner, J.W. (eds.) Proceedings 3rd Fungal genomics, 4th Fumonisin elimination, and 16th Aflatoxin elimination workshop. 13-15 October 2003, Savannah, Georgia. USDA-ARS, Beltsville, MD, USA, p. 120.

Odvody, G.N., Chilcutt, C.F., Parker, R.D. and Benedict, J.H., 2000. Aflatoxin and insect response of near-isogenic Bt and non-Bt commercial corn hybrids in South Texas. In: Robens, J.F. (ed.) Proceedings 2000 aflatoxin/fumonisin workshop. 25-27 October 2000, Yosemite, CA, USA. USDA-ARS, Beltsville, MD, USA, p. 121.

Ostry, V., Malír F. and Pfohl-Leszkowicz, A., 2015. Comparative data concerning aflatoxin contents in Bt maize and non-Bt isogenic maize in relation to human and animal health-a review. Acta Veterinaria Brno 84: 47-53.

Ostry, V., Ovesna, J., Skarkova, J., Pouchova, V. and Ruprich, J., 2010. A review on comparative data concerning *Fusarium* mycotoxins in Bt maize and non-Bt isogenic maize. Mycotoxin Research 26: 141-145.

Papst, C., Utz, H.F., Melchinger, A.E., Eder, J., Magg, T., Klein, D. and Bohn, M., 2005. Mycotoxins produced by *Fusarium* spp. in isogenic Bt vs. non-Bt maize hybrids under European corn borer pressure. Agronomy Journal 97: 219-224.

Pazzi, F., Lener, M., Colombo, L. and Monastra, G., 2006. Bt maize and mycotoxins: the current state of research. Annals of Microbiology 56: 223-230.

Picot, A., Barreau, C., Pinson-Gadais, L., Caron, D., Lannou, C. and Richard-Forget, F., 2010. Factors of the *Fusarium verticillioides*-maize environment modulating fumonisin production. Critical Reviews in Microbiology 36: 221-231.

Pietri, A. and Piva, G., 2000. Occurrence and control of mycotoxins in maize grown in Italy. In: Proceedings of the 6th International Feed Production Conference, Food Safety: Current Situation and Perspectives in the European Community, 27-28 November 2000, Piacenza, Italy, pp. 226-236.

Pinson, L., Plancke, M.P., Richard-Forget, F. and Fleurat-Lessard, F., 2002. *Fusarium* mycotoxins in isogenic and Bt maize varieties grown in different geographic areas in France. In: Advanced in Stored Product Protection. Proceedings of the 8th International working conference on stored product protection, 22-26 July 2002, York, UK, pp. 511-516.

Qaim, M., 2009. The economics of genetically modified crops. Annual Review of Resource Economics 1: 665-693.

Rule, D.M., Nolting, S.P., Prasifka, P.L., Storer, N.P, Hopkins, B.W., Scherder, E.F., Siebert, M.W. and Hendrix III, W.H., 2014. Efficacy of pyramided Bt proteins Cry1F, Cry1A.105, and Cry2Ab2 expressed in SmartStax corn hybrids against lepidopteran insect pests in the Northern United States. Journal of Economic Entomology 107: 403-409.

Sanchis, V., Marín, S., Magan, N. and Ramos, A.J., 2006. Ecophysiology of fumonisin producers in *Fusarium* Section *Liseola*. Advances in Experimental Medicine and Biology 571: 115-122.

Schaafsma, A.W., Hooker, D.C., Baute, T.S. and Illincic-Tamburic, L., 2002. Effect of Bt-corn hybrids on deoxynivalenol content in grain at harvest. Plant Disease 86: 1123-1126.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. and Dean, D.H., 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62: 775-806.

Scientific Cooperation on Questions Relating to Food (SCOOP), 2003. Reports on tasks for scientific cooperation. Report of experts participating in Task 3.2.10. Collection of occurrence data of *Fusarium t*oxins in food and assessment of dietary intake by the population of EU Member States. European Commission, DG Health and Consumer Protection. Available at: http://ec.europa.eu/food/fs/scoop/index_en.html.

Selwet, M., 2011. Maize plants infestation by *Fusarium* spp. and deoxynivalenol in genetically modified corn hybrid and traditional maize cultivars. Polish Journal of Microbiology 60: 317-321.

Sobek, E.A. and Munkvold, G.P., 1999. European corn borer (*Lepidoptera*: *Pyralidae*) as vectors of *Fusarium moniliforme*, causing kernel rot and symptomless infection of maize kernels. Journal of Economic Entomology 92: 503-509.

Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L. and Kizek, R., 2010. Deoxynivalenol and its toxicity. Interdisciplinary Toxicology 3: 94-99.

Takahashi-Ando, N., Kimura, M., Kakeya, H., Osada, H. and Yamaguchi, I., 2002. A novel lactonohydrolase responsible for the detoxification of zearalenone: enzyme purification and gene cloning. Biochemical Journal 365: 1-6.

Traore, S.B., Carlson, R.E., Pilcher, C.D. and Rice, M.E., 2000. Bt and non-Bt maize growth and development as affected by temperature and drought stress. Agronomy Journal 92: 1027-1035.

United States Department of Agriculture Economic Research Service (USDA-ERS), 2015. Adoption of Genetically Engineered Crops in the U.S. Overview: Recent trends in GE adoption. Available at: http://tinyurl.com/9qm9ssv.

Valenta, H., Dänicke, S., Flachowsky, G. and Böhme, T., 2001. Comparative study on concentrations of deoxynivalenol and zearalenone in kernels of transgenic Bt maize hybrids and non-transgenic maize hybrids. Mycotoxin Research 17: 15-18.

Wiatrak, P.J., Wright, D.L., Marois, J.J. and Wilson, D., 2005. Influence of planting date on aflatoxin accumulation in Bt, non-Bt, and tropical non-Bt hybrids. Agronomy Journal 97: 440-445.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D., 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. American Journal of Clinical Nutrition 80: 1106-1122.

Williams, W.P., Krakowsky, M., Windham, G., Balint-Kurti, P., Hawkins, L. and Henry, W.B., 2008. Identifying maize germplasm with resistance to aflatoxin accumulation. Journal of Toxicology, Toxin Reviews 27: 319-345.

Williams, W.P., Windham, G.L., Buckley, P.M. and Daves, C.A., 2002. Aflatoxin accumulation in conventional and transgenic corn hybrids infested with Southwestern corn borer (*Lepidoptera*: *Crambidae*). Journal of Agricultural and Urban Entomology 19: 227-236.

Williams, W.P., Windham, G.L., Buckley, P.M. and Daves, C.A., 2006. Aflatoxin accumulation in corn hybrids infested at different growth stages with Southwestern corn borer (*Lepidoptera*: *Crambidae*). Journal of Agricultural and Urban Entomology 23: 97-103.

Williams, W.P., Windham, G.L., Buckley, P.M. and Perkins, J.M., 2005. Southwestern corn borer damage and aflatoxin accumulation in conventional and transgenic corn hybrids. Field Crops Research 91: 329-336.

Williams, W.P., Windham, G.L., Krakowsky, M.D., Scully, B.T. and Ni, X., 2010. Aflatoxin accumulation in Bt and non-Bt maize testcrosses. Journal of Crop Improvement 24: 392-399.

Windham, G.L., Williams, W.P. and Davis, F.M., 1999. Effects of the Southwestern corn borer on *Aspergillus flavus* kernel infection and aflatoxin accumulation in maize hybrids. Plant Disease 83: 535-540.

Wu, F., 2006. Mycotoxin reduction in Bt Corn: potential economic, health, and regulatory impacts. Transgenic Research 15: 277-289.

Wu, F., 2007. Bt corn and impact on mycotoxins. CAB Reviews 2: 1-8.

Wu, F., 2014. Global impacts of aflatoxin in maize: trade and human health. World Mycotoxin Journal 8: 137-142.

Wu, F., Miller, J.D. and Casman E.A., 2004. The economic impact of Bt corn resulting from mycotoxin reduction. Journal of Toxicology, Toxin Reviews 23: 397-424.