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Chapter 11

Mycotoxins in Wheat and Mitigation Measures

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Abstract

Latest estimates for world cereal production in 2015 and EU-28 production in 2014 are approximately 2540 and 323 mil tons, respectively. The FAO estimated that the global wheat consumption is about 66 kg/per capita. Among the most important risks associated with wheat consumption are mycotoxins. It has been estimated that up to 25% of the world's crops grown for food and feed may be contaminated with mycotoxins. Despite efforts in controlling fungal growth, mycotoxin co-contamination represents an unavoidable risk, occurring pre- and postharvest and resulting in reduced nutritional value and possible risks for human and animal health. In addition to health risks, mycotoxins have a detrimental effect on the quality and the processing performance of wheat. Mitigation measures to manage the challenge of mycotoxins in wheat include strategies at pre- and postharvest. Preharvest events are predominantly dictated by environmental factors and good agronomic/cultural practices, whereas storage and processing are the major areas where contamination can be prevented at postharvest. Integrating as many management options as possible may minimize the risk of mycotoxin contamination in wheat and wheat products.

Keywords: wheat, mycotoxins, mitigation strategies, preharvest, postharvest

1. Introduction

Cereals and cereal by-products constitute a major part of the daily human and animal diet. Latest estimates for world cereal production in 2015 and EU-28 in 2014 are approximately 2540 and 323 mil tons, respectively [1]. According to the Food and Agriculture Organization of the United Nations (FAO), rice, maize, and wheat are staple foods for 4 bn people and make up about 60% of the world's food energy intake [2]. The FAO estimated that the global

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consumption for wheat is about 66 kg/per capita [3]. Among the most important risks associated with cereal consumption are mycotoxins, heavy metals, pesticide residues, and alkaloids. Richard et al. [4] estimated annual losses of $932 million in stored grain in the United States due to mycotoxin contamination. Cereal and cereal products can be contaminated with mycotoxins produced by a variety of fungi that colonize crops in the field or postharvest [5–8]. Mycotoxins are toxic secondary fungal metabolites that can cause a variety of adverse health effects in humans and animals, depending on the type of mycotoxin and the contamination levels. There are 300–400 mycotoxins known today. However, for practical consideration in food manufacturing, because of their worldwide occurrence and concern regarding human and animal diseases, the number is considerably less. The most important mycotoxins in wheat are mainly *Fusarium* toxins, such as deoxynivalenol (DON), zearalenone (ZEA), nivalenol (NIV), fumonisins (FUM), T-2, and HT-2 toxins [8–14]. Moreover, recent studies provided increased evidence for the presence of modified *Fusarium* mycotoxins and so-called emerging mycotoxins, particularly enniatins [15, 16]. Multi-mycotoxin contamination is the most common type of contamination [10, 14, 17–22]. This is a topic of great concern, as co-contaminated samples might still exert adverse health effects due to additive/synergistic interactions of the mycotoxins.

Mycotoxin regulations have been established in more than 100 countries, and the maximum acceptable limits vary greatly from country to country. The globalization of the trade in agricultural commodities and the lack of legislative harmonization have contributed significantly to the discussion about the awareness of mycotoxins entering the food supply chain. The European Union harmonized regulations for the maximum levels of mycotoxins in food and feed [23, 24]. Moreover, two EFSA scientific opinions recommended that the presence of modified and emerging mycotoxins must be considered by the European legislation in the near future [25, 26].

Fungal growth and mycotoxin contamination can occur during several steps of the food supply chain. Despite efforts in controlling fungal growth, mycotoxin co-contamination represents an unavoidable risk, occurring pre- and postharvest and resulting in reduced nutritional value and possible risks for human and animal health. In addition to health risks, fungal growth and mycotoxins have a detrimental effect on the quality and the processing performance of wheat. *Fusarium* damage may reduce wheat milling performance and affect flour yield and flour ash, with a strong negative effect on flour brightness, and baking performance [27–29].

Many factors with pre- and postharvest origins must be taken into account to manage the challenge of mycotoxins in wheat. Preharvest events are predominantly dictated by environmental factors and good agronomic/cultural practices, whereas storage and processing are the major areas where contamination can be prevented at postharvest level. The aim of this chapter is to present an overview of the most recent findings on wheat mycotoxin contamination and of the main pre- and postharvest strategies as mitigation measures, focusing on those more consolidated and used by the wheat industry chain. Other promising measures, but still studied at research level, will be presented with papers and reviews to which the reader is directed for specific insights.

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2. Mycotoxin occurrence in wheat

The major mycotoxins occurring in wheat, at levels of potential concern for human and animal health, are *Fusarium* mycotoxins [8–14] (**Figure 1**).

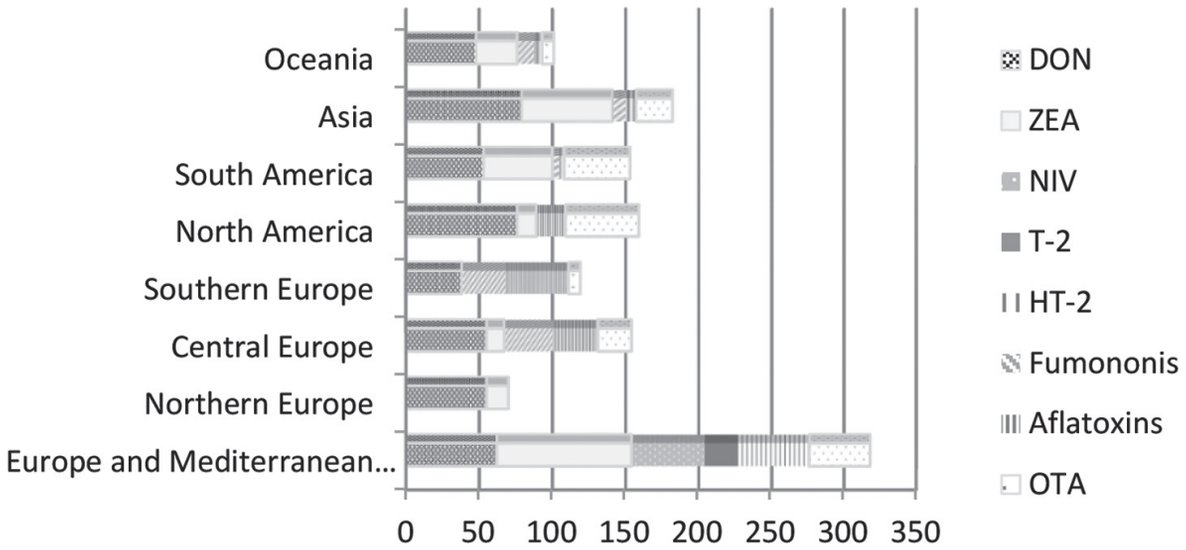


Figure 1. World mycotoxin occurrence (% of positive samples) in wheat and wheat bran (modified from Ref. [8]).

Results from worldwide mycotoxin occurrence studies indicate that DON is the most common mycotoxin contaminant of wheat and wheat-based products. Moreover, results highlighted the presence of considerable differences regarding the type and prevalence of mycotoxin contamination in different regions of the world, confirming that contamination is strongly dependent on regional climatic conditions [10, 14, 17–22]. Differences in mycotoxin occurrence and concentration between distant geographical areas are uncontroversial. Within each geographical area, seasonal and local weather conditions during critical crop growing stages are of great importance to explain the variation in mycotoxin occurrence. In general, environmental conditions, such as excessive moisture, temperature extremes, humidity, drought conditions, insect damage, crop systems, and some agronomic practices, can cause stress and predispose wheat in the field to mold and determine the severity of mycotoxin contamination [20, 30–32]. Moreover, the high variability in the occurrence and level of mycotoxins may be the results of several factors, such as the years of the surveys, the annual weather fluctuations, and the storage conditions (**Figure 2**).

Data on the occurrence of *Fusarium* mycotoxins in durum wheat are quite limited. Available data indicated that durum wheat was generally more contaminated than common wheat, but, with the exception of a few samples, no durum wheat sample was noncompliant to the maximum permitted level for DON and ZEA [33].

Another important point highlighted from studies on the worldwide mycotoxin occurrence in wheat and cereals is that the levels of detected mycotoxins are extremely variable. Average

levels of mycotoxin contamination may be low and rarely exceed risk threshold levels, but as the content range is very wide, several samples may exceed the maximum or recommended levels for mycotoxin contamination (**Table 1**) [11, 14, 17, 18, 20, 22, 34].

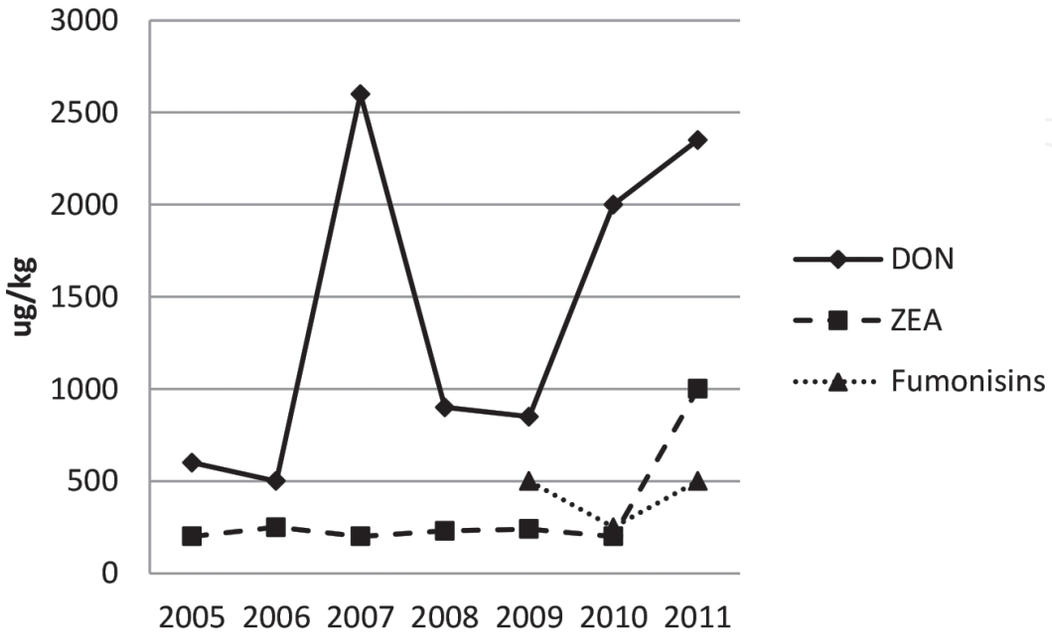


Figure 2. Year-by-year average mycotoxin concentration in wheat and wheat bran samples (modified from Ref. [20]).

Mycotoxins	Contaminated samples, % (n of tested samples)	Content, average of positive (ppb)	Maximum level (ppb)	EU maximum levels* (ppb)
DON	68 (770)	960	15976	UW: 1250 W: 750
ZEA	37 (645)	98	3274	UW: 100 W: 75
T-2	22 (342)	21	163	T-2+HT-2** UW: 100 W: 50
FUM	14 (331)	356	5334	–
AFLA	16 (396)	5	161	4
OTA	14 (278)	3	9	UW: 5 W: 3

AFLA, aflatoxins; DON, deoxynivalenol; FUM, fumonisins; OTA, ochratoxin A; T-2, T-2 toxin; ZEA, zearalenone; A_w, water activity; n.a., not available; W, wheat for direct human consumption; UW, unprocessed wheat.

*Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

**Indicates recommendations (2013/165/EU: Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products).

Table 1. Results of mycotoxin occurrence in wheat in 2015 (modified from Ref. [22]).

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Another important point highlighted from mycotoxin researches is that mycotoxin co-contamination is more the rule than the exception. Several studies reported a high incidence of multi-mycotoxin contamination in cereals and agricultural commodities [10, 14, 17–22]. A recent survey showed that in 2015, 46% of wheat samples were co-contaminated by two to six mycotoxins [35]. A study carried out in Italy showed that at least 80% of wheat samples were contaminated with one mycotoxin, while two mycotoxins were found in 27% of contaminated samples; 38% of the analyzed samples were contaminated with three or more mycotoxins [36]. Multi-mycotoxin contamination is a topic of great concern, as co-contaminated samples, although at lower levels than those indicated by EU regulations, might still exert adverse effects on animals due to additive/synergistic interactions of the mycotoxins.

A further scenario is represented by the climate changes. Estimates suggest that climate change will reduce wheat production globally by 29–34% by 2050 in developing countries [37]. This will have a great impact on food security. In terms of food safety and mycotoxin contamination, although aflatoxin is the mycotoxin that is most likely to increase under near-future climate scenario, problems concerning also *Fusarium* toxins may represent a challenge if the temperature increases in cool or temperate climate countries [38, 39].

In terms of mycotoxin contamination, new issues for cereal safety include both emerging mycotoxins and modified forms [15, 16, 25, 26, 40]. Mycotoxin contamination by emerging *Fusarium* mycotoxins, such as beauvericin and enniatins, represents a problem of global concern, especially in Northern Europe [15, 25, 36, 40]. Modified mycotoxins represent another emerging topic. Plant metabolites have been identified so far for DON, NIV, fusarenon-X, T-2 toxin, HT-2 toxin, ZEA, ochratoxin A (OTA), destruxins, fusaric acid, and modified fumonisins have been found, especially in wheat and other cereal commodities [41–46]. The acetylated derivatives of DON, 3-ADON, and 15-ADON are frequently detected in DON-contaminated grains [47].

3. Strategies to mitigate mycotoxin contamination

Fungi can invade, colonize, and produce mycotoxins during either preharvest or postharvest stages [5–8]. Therefore, to properly manage mycotoxin contamination in wheat, the primary strategy is the prevention, by reducing fungi proliferation in field and during storage [48–51]. Commonly and usually, mycotoxinogenic fungi are divided into two groups: preharvest (mainly *Fusarium* species) and postharvest (mainly *Aspergillius* and *Penicillium* species) fungi. During storage, fungi and insects may cause further deterioration. Fungi, such as *A. clavatus, A. fumigatus, Chaetomium, Scopulariopsis, Rhizopus, Mucor,* and *Absidia,* do not infect intact crops, but can easily attack damaged grains and, in the presence of high moisture content, may be responsible of advanced deterioration [52].

There are several possibilities for mitigating mycotoxin contamination. Preharvest events are predominantly dictated by environmental factors and good agronomic/cultural practices. Conditions, such as excessive moisture, temperature extremes, humidity, drought conditions, insect damage, crop systems, and some agronomic practices, can cause stress and predispose

plants in the field to mold and determine the severity of mycotoxin contamination [5, 31, 53]. *Fusarium* sp. are generally associated with a cool and excessively wet growing season [31, 54]. Wheat storage and processing are the major areas where contamination can be managed and mitigated at postharvest level, keeping in mind that postharvest contamination is also the result of preharvest presence of fungal contamination. The main strategies that need to be considered and implemented to mitigate mycotoxin accumulation pre- and postharvest are summarized in **Figure 3**.

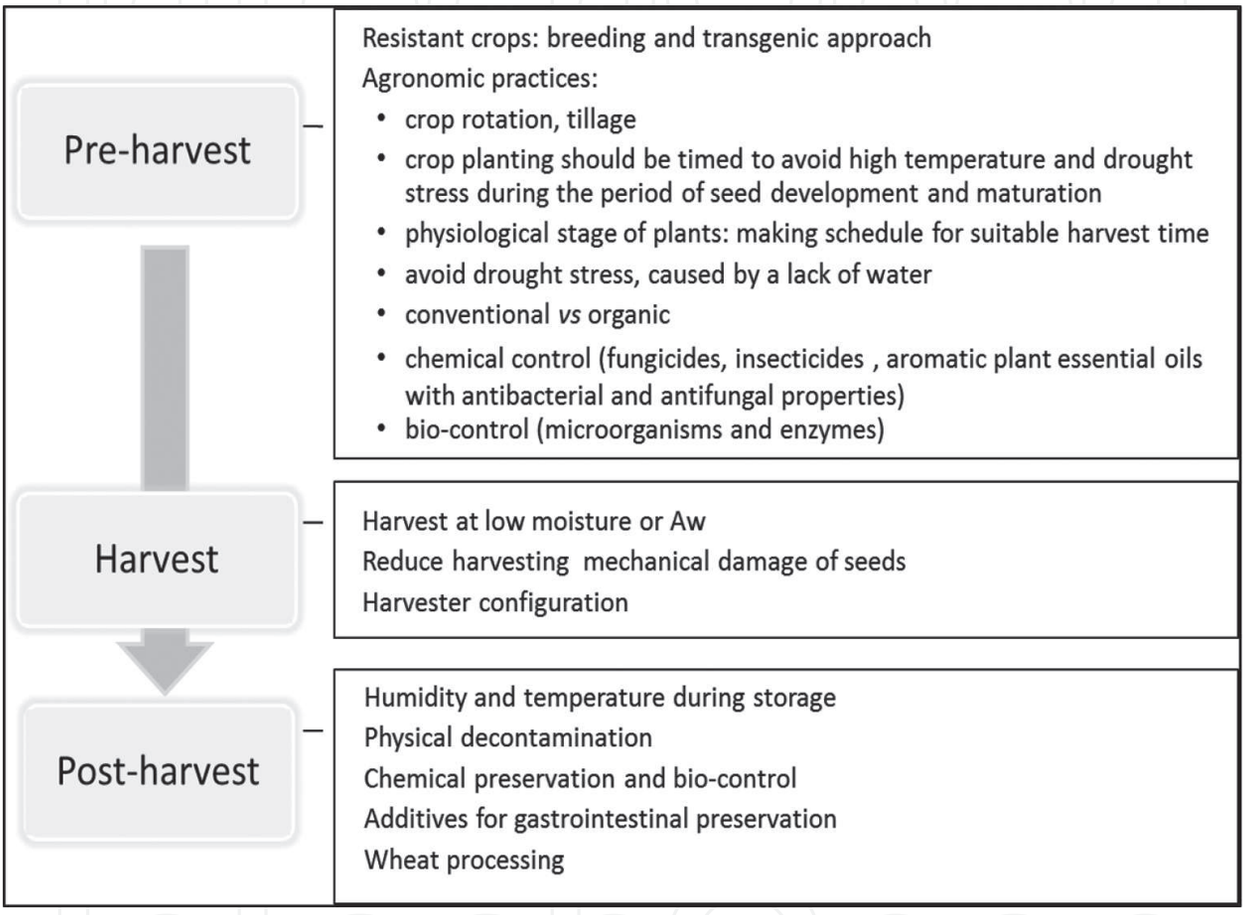


Figure 3. More consolidated and emerging strategies to reduce mycotoxigenic fungi and mycotoxin contamination in wheat.

4. Preharvest mitigation measures and management

One of the main wheat diseases associated with mycotoxin contamination is *Fusarium* head blight (FHB) caused by several species of *Fusarium* fungi, mainly *Fusarium graminearum, Fusarium culmorum,* and *Fusarium avenaceum.* The control of infection by *Fusarium* fungi in field is the first critical step in mitigating mycotoxin accumulation in the harvested products. To reduce the risk of *Fusarium* fungi and mycotoxin contamination, the most important pre-harvesting strategy is the application of appropriate good agriculture practices, such as crop selection, crop rotation, tillage, irrigation, and the proper use of chemicals [53].

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Crop selection: The use of genetic varieties more resistant to *Fusarium* sp. represents an effective management strategy to mitigate the mycotoxin challenge in wheat. There are differences in the susceptibility of wheat variety to *Fusarium* and differences in the degree of mycotoxin contamination. Moreover, differences between crops appear to differ between countries which can be related to differences in the genetic pool within each country and the different environmental and agronomic conditions in which crops are cultivated [48]. Wheat lines have been produced and provide good resistance to *Fusarium* sp. [55, 56]. For an important impact in terms of wheat security and safety, breeding for resistance must provide good resistance to *Fusarium* sp. without adversely affecting quality and agronomic properties. In addition to breeding programs, the increase in *Fusarium* resistance through developing genetically modified plants is another approach. It is well documented that transgenic resistance against toxigenic fungi or their toxins may be improved by using three basic strategies: enhance resistance to insect attack, induce mycotoxins detoxification pathways, and reduce mycotoxin accumulation by interfering with the biosynthetic pathway [57]. The topic of breeding for resistance and transgenic resistance would require a full manuscript. These topics have been specifically and extensively reviewed by several Authors to which the reader is directed [58, 59]. Despite progress made in prevention through breeding of resistant varieties and improvement in agronomic practices [31, 57], hazardous concentrations of mycotoxins may further occur as a result of annual weather fluctuations.

In field management: Appropriate field management practices may be effective to mitigate mycotoxin contamination in wheat [60]. When crop rotation is considered, maize should be avoided in the rotation, as maize is very susceptible to *Fusarium* sp. and the presence of maize residues appears to be an important factor contributing to DON contamination of wheat [57, 61]. The incidence and severity of *Fusarium graminearum* and DON contamination levels are higher in wheat grown after maize or wheat compared with wheat grown after soybeans [61, 62]. Moreover, the great differences in the frequency of isolation of *Fusarium* sp. and *F. graminearum* among years suggest the importance of annual climatic conditions in promoting the colonization and survival of these fungi. Other studies found no evidence that wheat following wheat is more at risk than wheat following a non-cereal crop, since some pathogenic *Fusarium* species isolated from cereals can also have pathogenicity toward non-cereal crops [63, 64]. The incidence of *F. avenaceum*, which is another of the most commonly isolated *Fusarium* species from FHB-infected ears of wheat in Canada, was lower in wheat grown continuously compared to wheat grown in crop rotation [65]. Crop rotation in conjunction with tillage techniques may further mitigate *Fusarium* and mycotoxin contamination. Higher levels of *Fusarium* and DON contamination in wheat have been reported with minimum tillage or no-till compared to conventional tillage [61, 63]. This effect can be attributed to inoculum survival and the concentration of *Fusarium* sp. in the soil [66, 67]. However, not significant effect of tilling has been reported when wheat was grown after soybeans [60].

Irrigation management is another critical point to mitigate preharvest mycotoxin contamination. All plants in the field need adequate water supply. Drought stress and also an excess irrigation are favorable conditions for *Fusarium* infection. Drought stress should be avoided during the period of wheat seed development and maturation; therefore, crop planting should be timed accordingly. Excessive moisture in irrigated wheat fields during flowering and early

grain fill period is a favorable condition for *Fusarium* infection [68, 69]. Nevertheless, the effect of moisture in increasing the levels of DON contamination is not consistent among published studies [69–73].

Use of chemical and biological compounds: Mold infection can be controlled by the appropriate use of fungicides. Fungicide treatment reduces wheat *Fusarium* infection and DON contamination [74–76]. Recently, Scarpino et al. [77] reported that azole fungicides, the most effective active substances in the reduction of DON, also consistently reduce the main emerging and modified mycotoxins of winter wheat in temperate areas. However, as far as the effectiveness of fungicide application to control mycotoxin contamination by *Fusarium* species, conflicting evidence has been reported. A meta-analysis carried out by Paul et al. [78] reported results ranging from no detectable effects to substantial reduction in both *Fusarium* head blight and DON with triazole-based fungicides. Overall results indicate that the variability of fungicide effects is related to several factors, such as cultivar resistance, the type of fungicide used, fungicide timing, pathogen aggressiveness, and different environmental and agronomic conditions. A greater fungicide efficacy in reducing FHB and DON has been reported in moderately resistant cultivars than in susceptible ones [79]. These results confirm that the efficacy of each mitigating approach must be considered within an integrated strategy for an effective management of *Fusarium* and mycotoxin control in wheat. As a tool of chemical control, several aromatic plant essential oils have been tested for their antibacterial and antifungal properties [80–83]. Results demonstrated a different antifungal activity and efficacy of these compounds, but more research is needed on this topic.

The chemical control of fungal infection and mycotoxin contamination may be only partly effective; therefore, biological control as an additional strategy has been considered and evaluated [53]. The efficacy of bacterial and fungal antagonist against *Fusarium* sp. has been reported in vitro, in the greenhouse, and in the field [84–92]. Biological antagonists can be sprayed directly at the flowering stage to limit the growth of fungal toxin producers. Wegulo et al. [53] concluded that the application and efficacy of the biological control for *Fusarium* infection and mycotoxin control pose challenges similar to those posed by fungicide application.

The use of biological control strategies to reduce mycotoxin challenge in wheat can be especially useful in organic production where synthetic fungicides cannot be used. The increased demand for organically produced food asks for scientific assessments of the safety of products from different farming systems, such as organic *vs.* conventional. Brodal et al. [93] published very recently an extensive review of studies comparing the content of DON, HT-2+T-2 toxins, ZEA, NIV, OTA, and fumonisins in cereal grains from organic and conventional farming systems. Inconsistent results have been reported regarding the DON, ZEA, NIV, and T-2+HT-2 content in wheat from the two farming systems (**Figure 4**).

Although no significant differences have been found in the majority of mycotoxin comparisons, several studies showed a tendency of a lower mycotoxin content in organically than in conventionally produced wheat. Moreover, results indicate that organic systems appear generally able to maintain mycotoxin contamination at low levels, despite no use of fungicides. The inconsistency of the results confirm that several preharvest factors, such as those previously described, may have more influence on the mycotoxin levels than the type of farming.

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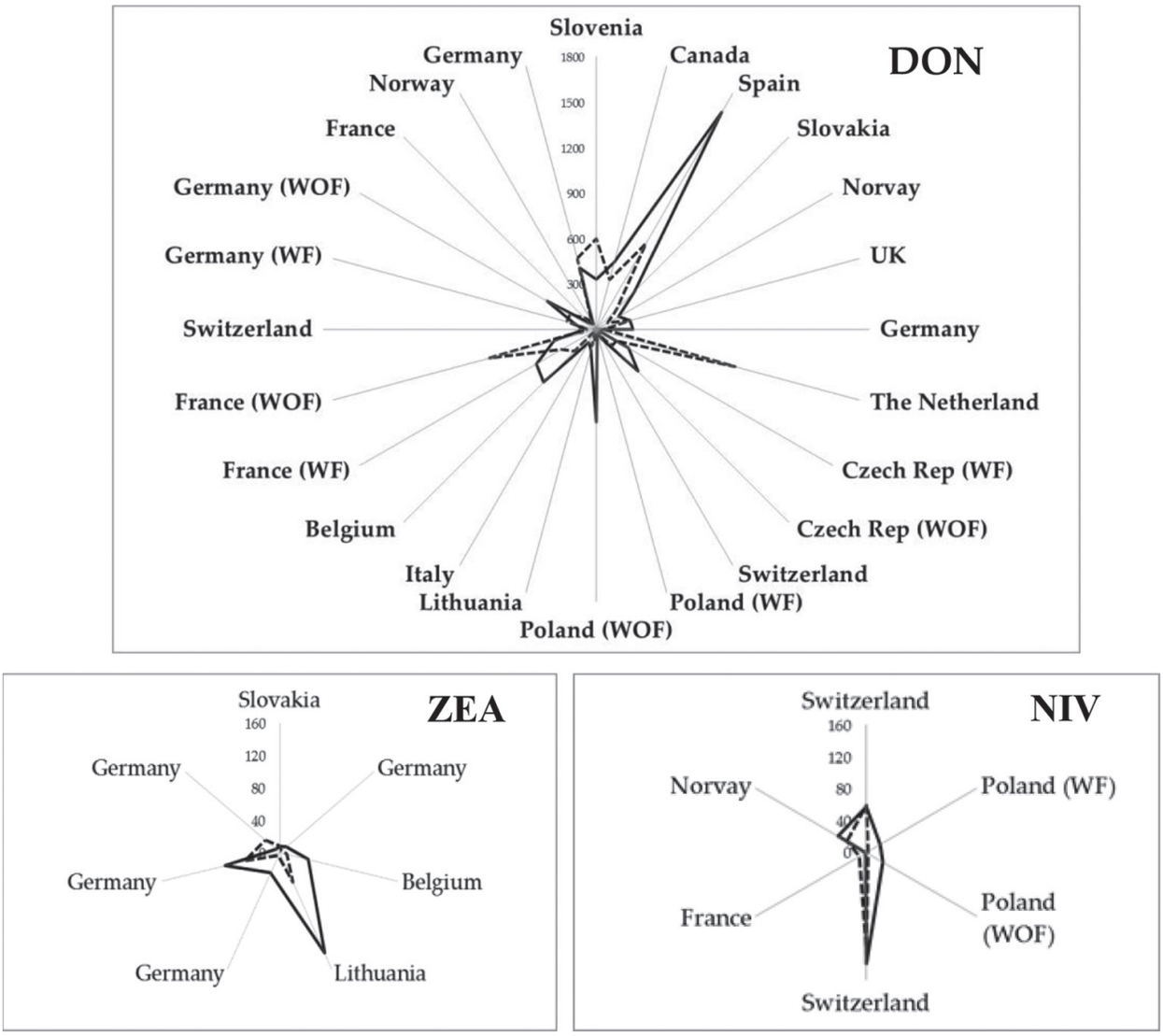


Figure 4. Mycotoxin contamination (μg/kg) in wheat from organic (- - -) and conventional (—) production (W: with fungicide; WOF: without fungicide) (modified by Ref. [93]).

To conclude, there are several preharvest practices and management approaches to reduce the risk of mycotoxin contamination in wheat, whose combination in an integrated strategy represents the best mitigation measure. All preharvest practices can be controlled, while climatic and environmental conditions cannot. Computer models, integrating field parameters and weather variables (temperature, rainfall, and moisture level) have been developed to predict the occurrence and risk of *Fusarium* and mycotoxin contamination in wheat [94–98]. Moreover, forecasting systems have been developed to optimize the use and application of chemical treatments [53].

5. Harvest and postharvest mitigation measures and management

Controlling harvest and storage conditions is critical to effectively prevent mold growth and mycotoxin production in wheat postharvest. Harvesting strategies, moisture, water activity

(A_w), temperature, storage period, contamination rate, broken grains, insect presence, and oxygen rate are the main critical points to manage in order to mitigate the mycotoxin risks postharvest [48, 50–52, 99].

Harvest management: Wheat should be harvested as soon as possible to reduce fungal growth and spread during favorable weather conditions. Management strategies during harvest include wheat harvest at low moisture or A_w, reduced mechanical seed damage, and the use of different grain harvest strategies to remove diseased kernels which are often lighter than the healthy ones. The use different harvesting configurations, with varying fan speeds and shutter openings, resulted in lower *Fusarium*-damaged wheat kernels and DON content in harvested wheat [99, 100]. The removal of damaged grain implies a loss in the yield of harvested grain, but results in better storage conditions and improvement in grain safety offsetting the economic losses.

Postharvest management: Efficient drying and storage of wheat in silos free of insect pests and moldy material are critical points to reduce mycotoxin contamination. Harvested grain must be dried to <14.5% moisture content and at a relative humidity of 70% to avoid mold spoilage or increase of preharvest contamination with mycotoxins [48, 51, 101, 102]. Besides humidity, the temperature during storage is another critical point for fungal growth and activity. During storage, humidity and temperature are strictly related and cause changes in the microclimate conditions favoring or inhibiting fungal growth and colonization and influencing the pattern of mycotoxin contamination [49, 51, 103]. A comparison of environmental conditions for fungal growth and toxin production by some common fungal species is reported in **Table 2**.

	T, °C		**pH**		**Optimal A_w**	
Species (mycotoxins)	**G**	**TP**	**G**	**TP**	**G**	**TP**
A. parasiticus (AFLA)	Range: 10–43 Optimum: 32–35	12–40	Range: 2.1–11.2 Optimum: 3.5–8.0	Range: 3.5–8.0 Optimum: 6.0	0.84	0.87
A. flavus (AFLA)	Range: 10–43 Optimum: 32–35	12–40	Range: 2.1–11.2 Optimum: 3.5–8.0	Range: 3.5–8.0 Optimum: 6.0	0.80	0.82
Fusarium species (T-2, DON, NIV, ZEA)	24–26	24–26	2.4 at 30°C and 3.0 at 25°C and 37°C	2.4–3.0	0.90	0.90
P. verrucosum (OTA)	Range: 0–31 Optimum: 20	4–20	Range: 2.0–10.0 Optimum: 6.0–7.0	n.a.	0.80	0.86

AFLA, aflatoxins; DON, deoxynivalenol; NIV, nivalenol; OTA, ochratoxin A; T-2, T-2 toxin; ZEA, zearalenone; A_w, water activity; n.a., not available.

Table 2. Comparison of environmental conditions for fungal growth (G) and toxin production (TP) by some common fungal species (modified from Ref. [104]).

In wheat, positive relationships between dry matter losses caused by *F. graminearum* under different environmental conditions (temperature, humidity, A_w) and the level contamination with DON have been reported [49, 51]. Moreover, it has been shown that the pattern and the

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levels of mycotoxin production in wheat grains by various *Aspergillus* sp. are different in relation to different relative humidity values and storage periods [101].

Use of physical, chemical, and biological decontaminating methods: Despite efforts to control, mitigate, and reduce fungal and mycotoxin contamination, wheat mycotoxin contamination is unavoidable and unpredictable, and postharvest decontaminating approaches can offer a last resort. Different decontaminating methods can be used to eliminate or reduce mycotoxin content in cereals before their entry in the food supply chain (**Table 3**).

Strategy	Effects	References
Physical decontamination		
- Sorting, dehulling, debranning, milling, irradiation, heating, or combined approaches	Removing of highly contaminated fractions or mycotoxin repartitioning from bulk wheat	[8, 105–112]
- Inorganic or organic mycotoxin binders	Reduced food mycotoxin bioavailability	[113–116]
Chemical decontamination	Conversion of mycotoxins via chemical reactions	[48, 51, 80, 106–118]
Microbial based methods	Microbial transformation, biodegradation	[51, 84, 106, 119, 120]

Table 3. Mycotoxin contamination: main post-harvest physical, chemical, and biological based decontamination strategies.

Jard et al. [120] underlined that the decontaminating approaches must consider several topics concerning safety issues: they must not generate toxic products, ensure the nutritional value of the food, and should not induce negative modification for food processing.

A wide variety of chemical decontamination processes including oxidation, reduction, ammonization, alkalization, acidification, and deamination has been reported [48, 121]. These methods have some limitations concerning safety issues, efficacy coupled with cost and regulatory implication. The use of chemical methods for the decontamination of cereals that exceed the mycotoxin threshold limits are not allowed in the European Union [122]. In the United States of America, only ammonization is licensed for detoxifying aflatoxins [123, 124]. In addition to chemical methods, natural plant extracts and spices are known to prevent mold growth and mycotoxin production. In recent years, the use of essential oils as natural food preservatives to control mold and mycotoxin contamination is gaining interest [117]. Several essential oils have been found to be effective in controlling growth of several *Fusarium* sp. and production of mycotoxin in stored wheat [125, 126]. However, more studies should be performed to identify the components of essential oils with modulatory activity on the growth and toxin production of *Fusarium* sp.

Currently, many researches have been carried out to evaluate the possible use of biological agents or biological transformations for mycotoxin detoxification, as an alternative to the chemical one. This approach includes fungal, microbial, and enzymatic degradation of mycotoxins. Several very recent reviews on this topic can be found in the literature to which the

reader is directed for specific insights [84, 118, 119, 127, 128]. Despite the many publications on this topic, this promising approach is still at a research level and far from an immediate outcome and application in practice for mycotoxin detoxification of food at industrial level. More research is needed to fully understand mycotoxin biotransformation mechanisms, to evaluate the toxicity of metabolites and the feasibility of application in wheat industry. All these topics must be considered and evaluated keeping in mind the existing regulatory issues for food safety.

Physical decontamination reducing mycotoxins in wheat can be carried out during industrial processing. For the wheat milling industry, the precise knowledge of the fate of mycotoxins during milling is vital and may provide a sound technical basis to conform to legislation requirements, support risk management and regulatory bodies in order to reduce human and animal exposure to mycotoxins, and reduce the risk of severe adverse market and trade repercussions. Wheat sorting, cleaning, debranning, and milling influence mycotoxin repartitioning in wheat milling fractions entering the food chain. The effects of wheat milling and thermal processes on the fate of mycotoxins have been extensively studied [8, 33, 105–112, 121, 129–133]. Published data confirm that milling reduces mycotoxin concentration in fractions used for human consumption, but concentrates mycotoxins into fractions commonly used as animal feed. Physical and mechanical processes, such as sorting and cleaning prior to milling, reduce mycotoxin contamination in wheat by removing kernels with extensive mold growth, broken kernels, fine materials, and dust. The results indicate that the effect of premilling processes and the efficiency of mycotoxin removal are extremely variable. The concentration of mycotoxins in cleaned wheat ranges from 7 to 63% for DON, from 7 to almost 100% for NIV, and from 7 to 40% for ZEA, of the contamination level in unclean grains [28, 134, 135]. A reduction of 62 and 53% of T-2 and HT-2, respectively, has been reported in wheat grains after cleaning [136]. Several factors may be involved in this response, such as the initial condition of the grains, the type and extent of the contamination, and the type and efficiency of the cleaning process. Debranning before cleaning is used in industrial processing to enhance the milling performance of wheat and the degree of refinement of flour and semolina [137]. Debranning before milling further reduces the level of mycotoxin content in wheat grain. As for the cleaning and sorting procedures, the effect of debranning and the efficiency of mycotoxin removal are extremely variable. A reduction of DON in debranned wheat ranging from 15 to 78% has been reported [134, 138–140]. Despite the high variability in removal efficiency of mycotoxin, overall results indicate that the physical processes that are carried out before milling (such as sorting, cleaning, and debranning) are very efficient methods to reduce wheat mycotoxin content before milling. As in cleaning and debranning, in the milling process there is no step that destroys mycotoxins; however, mycotoxin contamination may be redistributed in milling fractions [141–143].

Overall results regarding the efficacy of mycotoxin reduction/repartition wheat industrial processing showed a high variability and sometimes appear conflicting. This is related to the type of mycotoxins, the level and extent of fungal contamination, and a failure to understand the complexity of the milling technology. The knowledge of mycotoxin repartitioning in wheat milling fractions is largely limited to DON, using different approaches (artificially vs. naturally contaminated wheat; wide range of mycotoxin contamination levels; laboratory;

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semi-industrial; and industrial milling), but there is still a lack of data for other mycotoxins. Fewer data are available regarding the distribution of other mycotoxins and modified mycotoxins in milling fractions [45, 142–146], but a similar scenario has been found, such as mycotoxins concentration in milling fractions intended for animal feed.

6. Conclusions and future perspectives

Mycotoxins in wheat represent a significant health risk to animal health and significant issues for a safe food supply chain. Regarding this topic, mycotoxin regulations have been established in more than 100 countries, and maximum acceptable limits have been fixed for food and feed. Mycotoxin co-contamination in wheat is a reality, and future attention should be paid not only to the mycotoxins believed to be the most likely to occur, but also to emerging and modified mycotoxins. The co-occurrence of several mycotoxins, with specific chemical traits and modes of action, is a serious health problem because of potential additive and/or synergistic effects. The impact of mycotoxins entering the food chain could increase in the next future. Most predictions indicate that the climate change scenarios, with global warming, could affect agriculture and increase the threat from fungal invasion of crops. Regarding this topic, there is a need to improve predictive models for mycotoxin contamination in wheat, integrating field parameters and weather variables.

Strategies to mitigate and reduce mycotoxin contamination in wheat include approaches at pre- and postharvest levels. The efficacy of each mitigating approach is highly variable depending on several factors, such as the type of approach, the type and level of mycotoxin contamination, the crop variety and agronomic practices, storage condition, etc. Integrating as many management options as possible is the key to minimize the risk of mycotoxin contamination in wheat and wheat products. However, it must be underlined that even if pre- and postharvest practices can be controlled, there is an unpredictable factor that influence mycotoxin occurrence in wheat, namely the climatic and environmental conditions. Therefore, despite efforts to control and reduce fungal and mycotoxin contamination, wheat mycotoxin contamination is unavoidable and unpredictable and postharvest decontaminating approaches can offer the last resort. The use of these strategies must not be detrimental for the wheat quality and safety, and must comply with the existing regulatory requirements.

The high variability in the efficacy of mitigating strategies increases awareness and ongoing surveillance for mycotoxins. At industrial level, an effective approach to manage the mycotoxin challenge in wheat requires regular, effective, economical, and straight forward wheat sampling and analytical diagnostic tools which can be used to monitor mycotoxin contamination, rapidly identify material below specified standards, and make justified management decisions regarding what to do with wheat lots that may be contaminated with mycotoxins. Sampling is the greatest source of error in quantifying mycotoxin contamination because of the difficulty in obtaining samples from large grain consignments and of the uneven distribution of mycotoxins within a commodity [147]. The Commission Regulation 401/2006/EC provides precise details regarding the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs [148]. The development of rapid methods for use in

the field represents a future challenge, but such methods would allow for "decision-making" regarding the safe use of wheat and wheat by-products. Moreover, more research on the development and application of multi-mycotoxin analytical methods should be encouraged in order to obtain a more accurate picture of the extent of multi-mycotoxin contamination.

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