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# Prevention strategies for trichothecenes

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**Abstract**

Contamination of cereal commodities with mycotoxins represents a significant hazard to consumer health and has thus received increasing attention from food safety authorities and legislators. For trichothecenes, and deoxynivalenol (DON) in particular, the imminent implementation of legislative limits has focused attention on ways to prevent entry of such mycotoxin contaminants into the food and feed chains. Knowledge of the pre- and post-harvest stages in the cereal production chain and in particular information on where prevention strategies can be implemented is being used to develop quality assurance systems for improving food safety. Information on the ecology of *Fusarium* species, breeding for resistance, more effective fungicides, potential for biological control and effective drying and storage and preservation systems, are all helping to develop effective preventative strategies for minimising consumer exposure to trichothecenes and other mycotoxins.
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## 1. Introduction

In the last 5 years, a concerted attempt has been made to examine the potential for effective control of trichothecenes, especially deoxynivalenol (DON), from entering the human and animal food chain. Resistant cultivars have not yet been developed and many fungicides are ineffective against *Fusarium* head blight (FHB) (known also as *Fusarium* ear blight) and more importantly, toxin accumulation. Recent studies have been carried out within the framework of hazard analysis critical control point (HACCP) approaches to identify critical control points (CCPs) in the cereal production process. Within HACCP system, for mycotoxin control, CCPs are key stages in production where control can and needs to be maintained to give a safe product with respect to trichothecene contamination. In Europe, *Fusarium culmorum* and *Fusarium graminearum* are the key fungal species responsible for cereal grain contamination with DON. Within an HACCP framework, information on the key components has included pre- and post-harvest factors. Weather conditions during flowering, type and amount of fungicides, ecology of the key species and moisture content at harvest are all key aspects that need to be considered. This paper will consider the key pre- and post-harvest components, which need to be considered for effective reduction of DON accumulation in cereal grain in Europe. Fig. 1 summarises some of the key components pre- and post-harvest, which need to be considered in reducing the risk from contamination with trichothecenes.

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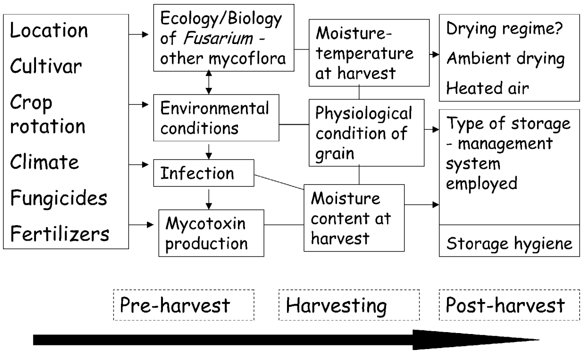


Fig. 1. Interactions that impact on mycotoxin contamination of cereals pre- and post-harvest.

## 2. Mycotoxin hazards pre-harvest

Consideration needs to be given to the growing crop together with the relevant environmental conditions and inputs into the system. The main mycotoxin hazards associated with wheat pre-harvest in Europe are the toxins that are produced by fungi belonging to the genus *Fusarium* in the growing crop. Mycotoxins produced by these fungi are collectively known as trichothecenes and include nivalenol (NIV), deoxynivalenol and T-2 toxin. *Fusarium* species are also responsible for a serious disease called *Fusarium* head blight, which can result in significant losses in crop yield and quality. Measures that are taken against *Fusarium* development in the field are currently mainly because of FHB rather than the mycotoxin risk. However, with EU legislation imminent, the consideration of mycotoxins is becoming increasingly important. It is important to note that although *Fusarium* infection is generally considered to be a pre-harvest problem, it is certainly possible for poor drying practices to lead to susceptibility in storage and mycotoxin contamination. In the case of wheat production, a number of these factors are of special relevance. These will be discussed below.

### 2.1. The nature of FHB

It is necessary first to consider the nature of *Fusarium* infection of wheat in the field. FHB is, in fact, a disease complex that means that it may be caused by individual species or a combination of related species. In northern Europe, disease is normally dominated by *F. culmorum* and in southern Europe, by *F. graminearum*, although this species is currently steadily spreading northwards. Other species implicated are *Fusarium avenaceum* and *Fusarium poae* (both toxin producers) and related *Microdochium nivale* varieties (not known to produce toxins). The problem of FHB seems to have been exacerbated by the increasing cultivation of bread wheats in Europe (Magan et al., 2002). At present, no durable, fully FHB-resistant wheat cultivars exist, so control relies on the use of cultivars with partial resistance together with field management and fungicide use (Diamond and Cooke, 2002). However, the development of resistant cultivars is an extremely important area, since it has been shown that wheat cultivars with resistance to the most aggressive, high DON-producing strains of *F. graminearum* and *F. culmorum* inhibited both disease progression and toxin production (Mesterhazy, 2002).

### 2.2. Resistant cultivars

One area of particular interest is the development and use of resistant wheat cultivars. In recent studies by Mesterhazy (2002), wheat varieties most resistant to FHB were shown to reduce DON production to near zero. In fact, resistance seemed to depend to a great extent on inhibition of toxin production directly, since the most aggressive disease-causing fungal strains were also those producing the highest levels of DON. This author suggested that an increased availability of such resistant varieties, coupled with the use of appropriate fungicides, was the key in an integrated approach to mycotoxin control associated with *Fusarium*. Such a strategy, with emphasis on mycotoxin control, could represent a CCP in the pre-harvest stage.

### 2.3. Field management

Appropriate field management and preparation is particularly relevant to FHB and mycotoxin control. Appropriate strategies include deep ploughing as a method of removing residual fungal material from the surface (present in debris from the previous crop). Crop rotation is also important and is intended to break the chain of production of infectious material, for example by using wheat/legume rotations. The use of maize in a rotation is to be avoided as maize is also susceptible to *Fusarium* infection and can lead to carry-over onto wheat via stubble/crop residues. In Mediterranean climates, it is good practice to leave ploughed land exposed to autumn sunshine as a means of destroying fungal material that could otherwise infect the following crop.

There is evidence that infection of wheat flowers (the usual initiation point of FHB infection) can be brought about by water splash of soil-borne material onto the plants during anthesis, so irrigation methods that prevent excessive splashing may be an important control point in some instances. This may be managed by the type of irrigation used or more practically by the timing of irrigation to avoid the anthesis stage. Lodging also seems to be an important risk factor in mycotoxin production. A recent study carried out for the Home Grown Cereal Authority (HGCA) showed that when lodging occurred, DON production was very high irrespective of any fungicide treatment used in the study (Nicholson et al., 2003).

### 2.4. Environmental conditions

Environmental conditions such as relative humidity and temperature are known to have an important effect on the onset of FHB. For example, it has been shown that moisture conditions at anthesis are critical in *Fusarium* infection of the ears, and Lacey et al. (1999) have shown that *Fusarium* infection in the UK is exacerbated by wet periods at a critical time in early flowering in the summer, which is the optimum window for susceptibility. Equally, drought-damaged plants are more susceptible to infection. However, until recently, very little was known about the threshold limits for mycotoxin production in the FEB system. Recent work by Hooker and Schaafsma (2003) in Canada and by Detrixhe et al. (2003) in Belgium and Rossi et al. (2003) in Italy has shown that agrometeriological information preceding and during ripening can be used for predicting risk of DON contamination of wheat by *F. graminearum* and *F. culmorum*, respectively.

Magan et al. (2003) have produced two-dimensional response profiles for growth and DON and nivalenol production by *F. culmorum* in relation to water activity ($a_W$) and temperature for the first time. They found that the conditions under which both toxins were produced were far more restrictive than the conditions allowing growth of the fungus. For example, production occurred in the relatively narrow $a_W$ range 0.995–0.95 while growth persisted to 0.90 $a_W$. However, the optimal conditions for DON and NIV production, 25 °C at 0.995 and 0.981 $a_W$, respectively, were within the range optimal for growth. These $a_W$ levels correspond to water contents of approximately 30% and 26%, respectively, and are within normal ranges for harvested grain in wet years. Toxin production was significantly higher at 25 °C compared to 15 °C. Both toxins continued to accumulate for the duration of the studies (40 days).

### 2.5. Fungicide use

Part of the integrated control of FHB in wheat production involves the use of fungicides, but this introduces a complication as far as trichothecenes are concerned as there is evidence that under certain conditions, fungicide use may actually stimulate toxin production. This is a particularly dangerous situation, since circumstances may arise where the obvious manifestations of FEB are reduced or even eliminated and yet high levels of mycotoxins may be present. Clearly, grain affected in this way cannot be identified by visual inspection for signs of FHB (e.g., pink grains) and, in fact, cannot be identified until a specific mycotoxin analysis is carried out.

In fact, research carried out on fungicide use in terms of FHB and mycotoxin development has produced very interesting results. In particular, fungicides in common use have been shown to have differential effects against toxin-forming *Fusarium* species and related non-toxin-forming pathogens such as *M. nivale* on ears (Simpson et al., 2001). The outcome of the use of fungicides seems to depend on the fungal species present and the effect that the particular fungicide has on these species. For example, in recent work commissioned by the Home Grown Cereal Authority, in an experimental situation where *F. culmorum* and *M. nivale* were both present, the use of azoxystrobin showed a significant reduction in disease levels while increasing the levels of DON present in grain (Nicholson et al., 2003). This was believed to be the result of selective inhibition of *M. nivale* by azoxystrobin. *M. nivale* is a natural competitor of toxin-forming *Fusarium* species, particularly *F. culmorum*. Removal of *M. nivale* by the fungicide probably allowed development of the toxigenic species in its place with concomitant increase in toxin formation. This is an important finding as it indicates that the impact of the fungicide is not directly related to mycotoxin production. Other fungicides such as tebucanazole and metconazole appeared to work in the opposite way. They selectively inhibited *F. culmorum*, while having far less effect on *M. nivale*. The efficacy of the fungicides in these situations was found to be directly correlated with dose level. It follows from these findings that where FEB is caused by *Fusarium* species in the absence of *Microdochium*, disease development is associated with higher levels of toxin.

Simpson et al. (2001) also carried out work with azoxystrobin and reported similar findings. Importantly, mixtures of azoxystrobin with both prochloraz and fluquinconazole were found to be less effective against *F. culmorum* than *M. nivale*. Other fungicides that have been shown to stimulate toxin production under certain conditions include tridemorph, which stimulated T-2 toxin production by *Fusarium sporotrichoides*, tubiconazole, which stimulated production of monoacetyl deoxynivalenol (3-AcDON), and difenoconazole, which stimulated production of the same toxin by *F. culmorum*. Recent in vitro studies looking at a range of *F. culmorum* strains from across Europe showed a stimulation in DON production in the presence of epoxiconazole and propiconazole (Magan et al., 2002).

In general studies, it was found that full-dose application of fungicides with main activity against *Fusarium* species and timed at mid-anthesis (mid-flowering) were most effective at inhibiting both FHB and DON and NIV production. Fungicides used in the studies appeared to be equally effective against DON and NIV-producing strains of *F. culmorum*.

### *2.6. Biological control agents*

Recent studies have been carried out to screen a range of potential biocompetitive microorganisms to *Fusarium* pathogens of cereal ears and maize (Dawson et al., 2002a, 2002b). This has shown that there are potential microorganisms that can decrease sporulation of *Fusarium* species on cereal stubble and thus decrease the pool of inoculum for infection. Studies to control head blight and DON production have shown that a number of competitive microorganisms existing are effective at decreasing both significantly. Potential does exist in this area as there is a very narrow window of about 5–10 days during which protection is needed. Targeted spraying of biocontrol agents to flowering ears may give the necessary protection required. Potential for commercialisation of these candidates is now being examined.

## 3. Problems related to use of HACCP pre-harvest

The application of HACCP-type principles is at its most problematic in the pre-harvest stages. There are a number of reasons for this:

- The field situation with regard to FHB and mycotoxin development is complex and scientific knowledge is still lacking.
- At present, no control measures exist that can guarantee elimination of FHB and mycotoxin production.
- Economic factors impinge on farming practices. In particular, field management, cropping systems and chemical input may directly reflect economic pressures rather than best practice for disease elimination.
- In food-processing plants, where HACCP was first developed, definitive control measures can be devised and implemented to close limits. The same levels of control cannot be exercised in the field situation; one obvious example is temperature and moisture levels (i.e., the weather conditions). In fact, some experts believe that the attempt to apply HACCP to the pre-harvest situation is fundamentally flawed.

## 4. Post-harvest management

Grain at harvest and entering initial storage contains a wide range of potential spoilage and toxigenic organisms. The population present depends largely on the field conditions and harvesting process and will be further modified during storage. Sometimes, grain is kept for short periods of time on farm in buffer storage before drying. This can result in conditions conducive to *Fusarium* growth and further mycotoxin contamination. Thus, poor post-harvest management can result in rapid quality loss as well as risk from mycotoxins. This is a particular problem in wet harvest years.

### 4.1. Harvest

Harvest is the first stage in the production chain where moisture management becomes the dominant control measure in the prevention of mycotoxin development. Moisture management will involve accurate and prompt measurement methods and procedures in place to efficiently undertake bulk drying where necessary. Another equally important control measure at this stage will be an effective assessment of the crop for the presence of disease such as FEB. This will need to be accompanied by an efficient strategy for separation of diseased material from healthy grain.

### 4.2. Interactions of fungi during drying and storage

Until recently, surprisingly little work has been carried out on the ways that spoilage fungi interact with each other in the stored grain ecosystem and the effect that this has on mycotoxin production. Recent studies have, in fact, indicated that important inter- and intra-specific interactions occur, depending on the species present and the prevailing environmental conditions. Most importantly, the dominance of certain species has been shown to shift under changing conditions, particularly with changes in water content. For example, Magan et al. (2003) studied competition for resources and niche overlap between *F. culmorum*, other Fusaria and contaminant species. They concluded that the system was in a state of dynamic flux with niche overlap altering in direct response to temperature and $a_W$ level. In general, the results indicated that the fungi present tended to occupy separate niches, based on resource utilization, and this tendency increased with drier conditions. Production of DON and NIV by *F. culmorum* was significantly inhibited by the presence of some other fungi (Hope and Magan, 2003).

It should be borne in mind that insects may also be present in the stored grain ecosystem, and these may also interact with fungal species. Insects' damage may make the grain more susceptible to fungal colonization and mycotoxin production. Insects may also act as disseminators of fungal spores and generally show high tolerance to the presence of some mycotoxins.

### 4.3. Use of preservatives

At present, the use of chemical preservatives in wheat-based food production only becomes important in the latter processing stages, e.g., the use of propionates in breads. This is likely to remain the case, given the current pressure to reduce the use of chemical additives generally in the food industry. However, recent work has taken an alternative view by looking at the potential of using antioxidants, essential oils from plants and other natural products from bacteria and fungi. There are many economic and technological hurdles associated with this type of approach. However, tests on wheat grain, butylhydroxyanisole (BHA), propyl paraben (PP), cinnamon oil and resveratol gave greater than 90% reduction in DON and NIV accumulation. Resveratol (an antioxidant), in particular, showed a wide spectrum of mycotoxin control, although this is a relatively expensive product (Fanelli et al., 2003).

### 4.4. The storage situation

Generally, grain stored at a moisture content equivalent to less than 0.70 $a_W$ ($<$14.5% moisture by weight) will not be subject to fungal spoilage and mycotoxin production. However, grain is often harvested at moisture levels far in excess of this and is often traded on a wet weight basis. In addition, there are still some technological challenges associated with bulk drying and storage of grain and instances of poor practice and negligence. The mycotoxins hazard is therefore associated with a significant risk in grain production in the post-harvest situation.

Harvested wheat grain may pass through the hands of a number of "owners" on its way to the primary processor. In perhaps the simplest case, it will remain on-farm in store or buffer storage for short time periods before being passed directly to the processing facility. In other cases, it may pass through the hands of grain merchants or to third party drying facilities if it has been harvested wet and no on-farm drying facilities are available. In these cases, it will be stored at a number of different geographical locations with transportation steps in between. During all of these stages, the grain could become susceptible to fungal spoilage if the storage conditions are not strictly controlled. In most instances, the key to this is drying of freshly harvested material down to 0.70 $a_W$ and maintaining the grain in this condition. The most important control measures relevant to storage stages may be listed as follows:

- Regular and accurate moisture determination.
- Efficient and prompt drying of wet grain. This will relate to holding time/temperature prior to drying as well as the actual drying conditions.
- Infrastructure for quick response, including provision for segregation and appropriate transportation conditions.
- Appropriate storage conditions at all stages in terms of moisture and temperature control, and general maintenance of facilities for prevention of pest and water ingress.
- Ability to efficiently identify and reject material below specified standards in terms of both fungal disease and, at some stages, mycotoxin levels (e.g., when passing onto a third party).
- Operation of approved supplier systems. This involves setting specifications for acceptance/rejection.

Although complex, the post-harvest stages in the wheat commodity chain, including drying, storage, transport, milling and baking, are far more conducive to a "classic"-type HACCP analysis than the pre-harvest stages. Unlike pre-harvest, these stages are characterized by the ability to apply definitive control measures, to set critical limits and to initiate monitoring procedures. In particular, flour milling and baking can be viewed as straightforward food-processing procedures immediately accessible to the HACCP approach.

### *4.5. Processing—milling and bread making*

The overall post-harvest situation becomes even more complex if the stages relating to processing are included. In the case of wheat, primary processing will normally be milling to produce flour for bread making. The flour produced at the mill is susceptible to the same mycotoxin hazards as grain under similar environmental conditions. Therefore, the production, storage and transportation of flour require essentially the same type of management as is required for grain. However, due to the way the bread-making industry operates, the majority of flour produced will be in storage for a short period of time. Preparatory stages at flour mills include removal of defective and foreign material, and this could in principle act as a control measure for mycotoxin contamination. Current research work is looking at the influence of the milling process on mycotoxins. It is possible that the removal of certain grain components during milling could result in the reduction in toxin levels in contaminated grain. The bread making (baking) process itself, based on modern methods such as the Chorleywood bread making process (CBMP), does not appear to present any significant risk factors in relation to mycotoxin development.

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