MYCOHUNT

ANNEX I TO D8.1. REPORT GUIDELINES ON PREVENTION MEASURES

Guidelines for prevention and control of mould growth and mycotoxin production in cereals

Mia Eeckhout, Geert Haesaert, Sofie Landschoot, Nick Deschuyffeleer, Sarah De Laethauwer

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MYCOHUNT

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1 INTRODUCTION

In September 2003, a European study was completed on the occurrence of mould mycotoxins (produced by *Fusarium* species) in food. Also the dietary intake by the population of EU Member States was assessed. This study concluded that these *Fusarium* mycotoxins are widely distributed in the food chain, exposing the entire population to elevated levels of toxins. The major sources were products made from cereals (wheat and maize). Because these commodities are also used as animal feeding, measures need to be taken to protect animal health and to avoid adverse effects on livestock production. Therefore, the European Commission formulated Recommendation 2006/576/EC of August 17th 2006, containing guidance values for the presence of mycotoxins in products intended for animal feeding. Due to these thresholds, EU farmers are obliged to take measures to minimise *Fusarium* mycotoxin contamination. Early insight into the expected mycotoxin contamination at harvest is useful for the various stakeholders of the cereal supply chain, in order to reduce year-to-year losses by applying appropriate fungicides combined with other management strategies. Since moulds and mould spores are omnipresent in the environment (atmosphere, soil, etc.), it is impossible to completely eliminate mycotoxin contamination in cereal based commodities. However, it is of the utmost importance that all stakeholders involved realise that Good Agricultural Practices (GAP) represent the primary line of defence followed by Good Manufacturing Practices (GMP) during the handling, storage, processing and distribution of commodities intended for human food and animal feed (CAC/RCP 51-2003). The implementation of these Good Practices can lead to a further reduction of mycotoxin concentrations and reduce the exposure risk for humans and animals.

2 SCOPE

This practical guide is intended for people working in industry who are involved in the handling, storage, processing and distribution of cereal based commodities. The different measures that can be taken to reduce the presence/growth of moulds and the subsequent formation of mycotoxins in each of these steps will be discussed. Also the detection methods for mycotoxins and the maximum allowed concentrations will be listed. This information will allow companies to assess mycotoxin concentrations at the intake level. To fully limit the problem of mycotoxins in cereal based commodities, GMP must be combined with GAP. Therefore, this guide will also devote an entire chapter on how to avoid mould contamination during the cultivation of cereals on the field. People working in industry can use this information to consult the farmers and reduce the risk at an early stage in the production process.

3 MYCOTOXINS

3.1 DEFINITION, CLASSIFICATION AND TYPES OF MYCOTOXINS

The term 'mycotoxins' is usually reserved for the toxic secondary metabolites produced by moulds (fungi) that grow on crops and a variety of feed- and foodstuffs. The majority of these mould species are able to produce a variety of different mycotoxins. According to their chemical structure mycotoxins are classified into different groups; the **major mycotoxins** from public health and agricultural perspective belong to the following groups:

aflatoxins
ochratoxins
trichothecenes
zearalenone
fumonisins

The most important aflatoxin is **aflatoxin B1**, the most important ochratoxin is **ochratoxin A**. The most prevalent trichothecenes are deoxynivalenol (**DON**), nivalenol (**NIV**), the acetylated derivatives of DON and NIV (3-ADON and 15-ADON), **T-2 toxin** (T-2) and **HT-2 toxin** (HT-2).

3.2 SOURCES OF MYCOTOXINS

Contamination of feed and food commodities with mycotoxins can occur at two levels. The first source of contamination is **infection of the crop in the field** by mycotoxin producing moulds, for example *Fusarium* species. Several species of *Fusarium* colonise roots, stems, leaves, grains and heads of cereals and grasses. Field and weather conditions like temperature and humidity are key factors for mould growth and mycotoxin production. However, these conditions are very difficult to control and a thorough monitoring of the crop should be applied to prevent these moulds.

After harvest, a second contamination with mycotoxins can take place by **infection of the harvested and processed products with storage moulds**, for example by *Aspergillus* or *Penicillium* species. Infection with storage moulds occurs when storage and/or drying conditions are not optimal. Of these storage conditions, temperature and humidity are the most determining factors for mould growth and mycotoxin production. Also the moisture content of the commodities, especially in cereal grains, plays a crucial role. Storage conditions should be very well fine-tuned to avoid mould growth.

In Table 1, an overview is given of the main mycotoxin producing moulds, their growing medium (crop or host plants in case of field moulds; commodities in case of storage moulds) and the most important mycotoxins they can produce in these feed and food products.

Table 1: An overview of the main mycotoxin producing moulds in the field and during storage, their growing medium and the mycotoxins they can produce.

Field			Storage		
Species	**Crop/Host**	**Mycotoxins**	**Species**	**Commodity**	**Mycotoxins**
Fusarium spp.	Wheat Maize Barley Oat Grasses	Trichothecenes Zearalenone Fumonisins Moniliformin Enniatins	*Aspergillus spp.*	Cereal products Cereal by-products Maize products Maize by-products Nuts Dried fruit Fruit and fruit juice (mainly apple)	Aflatoxins Ochratoxins Patulin
Alternaria spp.	Wheat Barley Potato Tomato Apple Citrus fruits Sunflower Rapeseed	Alternariol Altenuene Altertoxins Tenuazonic acid Alternariol monomethyl ether	*Penicilium spp.*	Cereal products Cereal by-products Maize products Maize by-products Nuts Dried fruit Fruit and fruit juice (mainly apple)	Ochratoxins Citrinin Patulin
Claviceps spp.	Cereals Grasses	Ergot alkaloids	*Alternaria spp.*	Fresh fruits Fresh vegetables Oil products	Alternariol Altenuene Altertoxins Tenuazonic acid Alternariol monomethyl ether

3.3 PROBLEMS CAUSED BY MYCOTOXINS

Because of their economic importance for maize, wheat and other small grain cereals in Europe, most topics in this practical guide mainly focus on the *Fusarium* species and their mycotoxins. *Fusarium* mycotoxins can cause two types of problems: health problems for humans and animals and technological problems.

3.3.1 HEALTH PROBLEMS FOR HUMANS AND ANIMALS

Poisoning or intoxication with mycotoxins usually occurs by eating contaminated food or feed. Since mycotoxins are usually not degraded during digestion or temperature treatments, these compounds can accumulate to high doses in food and feed products, in this way forming a serious risk for both humans and animals.

Many symptoms are associated with mycotoxin intoxication (mycotoxicosis), most of which are well described in animals. However, a direct connection between mycotoxicosis and human illness was rarely demonstrated. Mycotoxicosis can occur at two levels: **acute illness** due to intake of high levels of mycotoxins and **chronic illness** due to regular low level intake of mycotoxins.

Symptoms of acute mycotoxicosis are nausea, vomiting, abdominal pain and diarrhoea.

Symptoms of chronic mycotoxicosis are reduced food/feed intake, reduced growth and development, suppression of the immune system, some types of cancer, foetal malformation, birth defects, disturbed embryonic development during pregnancy and toxic effects on fertility.

3.3.2 TECHNOLOGICAL PROBLEMS

Mycotoxins not only affect higher organisms, but also micro-organisms suffer from their toxic activity. Hence, mycotoxins may generate technological problems in the production of beer, where micro-organisms are used in the **fermentation** process. Mycotoxins also inhibit enzymatic activity, this is of importance in **malting** and **baking** processes of cereals. In fact, the presence of moulds and their mycotoxins generally reduce grain quality, in this way also influencing the processing qualities of the grain.

3.4 TOXIC LEVELS OF MYCOTOXINS

The toxicity of mycotoxins manifests at two levels: an **acute and a chronic dose** of mycotoxin intake. These two levels are determined by respectively the LD_{50} (acute dose at which 50% of the tested animals die within 24 hours) and the TDI (tolerable daily intake).

Table 2 gives the acute oral toxic levels (LD_{50}) of mycotoxins, observed in different organisms. Table 3 gives the tolerable daily intake for humans, as set by European Commission Regulation 1881/2006.

Table 2: Acute oral toxic levels (LD_{50}) of mycotoxins (/kg body weight) in different organisms.

Mycotoxin	Organism	LD_{50} (/kg body weight)
Aflatoxin B1	Pigs	0.6 mg
	Chickens	6.3 mg
	Mice	9 mg
Ochratoxin A	Pigs	1 mg
	Chickens	3.3 mg
	Turkeys	5.9 mg
Deoxynivalenol (DON)	Poultry	140 mg
	Mice	46-78 mg
Zearalenone	Mice and rats	4000-20000 mg
T-2 toxin (T-2)	Pigs	5 mg
	Chickens	2-6 mg
	Rodents	5-10 mg
HT-2 toxin (HT-2)	Chickens (1-day old)	7.2 mg
	Mice	6.5 mg
Fumonisin B1		Unknown

Table 3: Tolerable daily intake (/kg body weight) for humans, as determined by the European Commission Regulation 1881/2006.

Mycotoxin	TDI (/kg body weight/day)
Aflatoxin	1 ng
Ochratoxin A	17.1 ng (120 ng/kg body weight/week)
Patulin	0.4 µg
Deoxynivalenol (DON)	1 µg
Nivalenol	0.7 µg
Zearalenone	0.2 µg
T-2 + HT-2 toxin	0.06 µg
Fumonisin B1/B2/B3	2 µg

3.5 EC REGULATION AND RECOMMENDATION ON MAXIMUM MYCOTOXIN LEVELS IN FOOD AND ANIMAL FEED

In 2006, the European Commission has set maximum levels and guidance levels for mycotoxins in products, respectively intended for human consumption (Commission Regulation 1881/2006) and for animal feed (Commission Recommendation 576/2006). Table 4 shows the **maximum level** of aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone and fuminisins in a number of **cereal-based foodstuffs**. Table 5 gives **guidance values** of deoxynivalenol, zearalenone, HT/T-2 toxins, ochratoxin A, fumonisins and aflatoxin B1 in some **cereal-based feedstuffs**. However, it should be mentioned that mycotoxin legislation is a dynamic issue and that in the near future threshold legislation might be fine-tuned and complemented with additional guidelines for other toxins.

Table 4: Maximum levels for mycotoxins in unprocessed cereals and finished products intended for human consumption (more details in Commission Regulation 1881/2006).

Foodstuffs	Maximum levels (µg/kg)		
	AFB1	**AFB1+B2+ G1+G2**	**OTA**
* Unprocessed cereals	2.0	4.0	5.0
* All cereals and all products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption with the exception of:	2.0	4.0	3.0
- maize and rice to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs	5.0	10.0	3.0
- processed cereal-based foods and baby foods for infants and young children	0.1		0.5
- dietary foods for special medical purposes intended specifically for infants	0.1		0.5
	DON	**ZEN**	**FUM***
* Unprocessed cereals other than durum wheat, oats and maize	1250	100	
* Unprocessed durum wheat and oats	1750	100	
* Unprocessed maize	1750	200	4000
* Cereal flour, bran and germ	750	75	
* Maize flour	750	200	1400
* Bread, pastries, biscuits, cereal snacks and breakfast cereals	500	50	800
* Processed cereal-based foods and baby foods for infants and young children	200	20	200

AFB1: aflatoxin B1; AFB1+B2+G1+G2: aflatoxin, sum of B1, B2, G1 and G2; OTA: ochratoxin A; DON: deoxynivalenol; ZEN: zearalenone; FUM: fumonisins, sum of B1 and B2

** only maize products*

Table 5: Guidance values for mycotoxin levels in products intended for animal feed, relative to a feedingstuff with moisture content of 12% (Commission Recommendation 576/2006 and Commission Directive 100/2003).

Mycotoxin	Products intended for animal feed	Guidance value (mg/kg) *
DON	Feed materials:	
	- cereals and cereal products with the exception of maize by-products	8
	- maize by-products	12
	Complementary and complete feedingstuffs with the exception of:	5
	- complementary and complete feedingstuffs for pigs	0.9
	- complementary and complete feedingstuffs for calves (< 4 months), lambs and kids	2
Zearalenone	Feed materials:	
	- cereals and cereal products with the exception of maize by-products	2
	- maize by-products	3
	Complementary and complete feedingstuffs:	
	- for piglets and gilts (young sows)	0.1
	- for sows and fattening pigs	0.25
	- for calves, dairy cattle, sheep (including lamb), and goats (including kids)	0.5
HT/T-2 toxin	Complementary and complete feedingstuffs:	
	- for piglets and calves	0.2
	- for poultry	0.4
	- for fattening pigs	0.5
Ochratoxin A	Feed materials: cereal and cereal products	0.25
	Complementary and complete feedingstuffs:	
	- for pigs	0.05
	- for poultry	0.1
Fumonisin B1 + B2	Feed materials: maize and maize products	60
	Complementary and complete feedingstuffs:	
	- for pigs, horses, rabbits and pet animals	5
	- for fish	10
	- for poultry, calves (< 4 months), lambs and kids	20
	- for adult ruminants (> 4 months) and mink	50
Aflatoxin B1	All feed materials	0.02
	Complete (and complementary) feedingstuffs for cattle, sheep and goat with the exception of:	0.02
	- complete feedingstuffs for dairy animals	0.005
	- complete feedingstuffs for calves and lambs	0.01
	Complementary and complete feedingstuffs for pigs and poultry (except young animals)	0.02
	Other complete feedingstuffs	0.01
	Other complementary feedingstuffs	0.005

** relative to a feedingstuff with a moisture content of 12%*

3.6 DETECTION OF MYCOTOXINS

Because of the serious health risks and technological problems, it is very important to monitor mycotoxin levels during the total cereal production chain. Monitoring involves a correct sampling method as well as an accurate detection technique.

3.6.1 SAMPLING

A correct and representative sampling method is very important to allow precise detection of mycotoxins and good decision making. The size of the sample is of special importance to be representative for the whole cereal lot. The European Commission has set rules and methods of sampling and analysis for the control of mycotoxin levels in foodstuffs (all details in Commission Regulation 401/2006), including sample weight and sampling method in different sizes of cereal lots (Table 6).

The most common sampling method consists of incremental samples (normally 100 g), taken throughout the (sub)lot. The incremental samples are then brought together in the aggregate sample and carefully homogenised (distribution of mycotoxins is usually not homogeneous). Subsamples are taken for laboratory analysis (e.g. mycotoxins), and for enforcement, trade and reference purposes. Each sample must be placed in a clean, inert and dry container. All necessary precautions should be taken to avoid changes in the composition of the samples. The samples are sealed and labelled/identified following the rules of the Member State.

Incremental samples (100g)
throughout the lot

↓

Aggregate sample
(homogenised)

↓

Subsamples
(lab, replicate, reference)

↓

Sealed and labelled

The Commission Regulation 401/2006 does not mention the specific techniques and instruments that should be used for the sampling of cereals. However, a guidance document describing the sampling of cereals for mycotoxins is available (http://ec.europa.eu/food/food/chemicalsafety/contaminants/guidance-sampling-final.pdf). This guidance document focuses on the sampling of large batches in ships, warehouses and silos, but also an overview of instruments for sampling smaller product batches is given.

Table 6: Method of sampling for cereals and cereal products, depending on the lot weight: division into sublots, number of incremental samples and determined weight of the aggregate sample (all details in Commission Regulation 401/2006).

Lot weight (tonnes)	Weight or number of sublots	Number of incremental samples (100g)	Aggregate sample weight (kg)
≤ 0.05	-	3	1
> 0.05 - ≤ 0.5	-	5	1
> 0.5 - ≤ 1	-	10	1
> 1 - ≤ 3	-	20	2
> 3 - ≤ 10	-	40	4
> 10 - ≤ 20	-	60	6
> 20 - ≤ 50	-	100	10
> 50 - ≤ 300	100 tonnes	100	10
> 300 - ≤ 1500	3 sublots	100	10
> 1500	500 tonnes	100	10

3.6.2 DETECTION METHODS

For the detection of mycotoxins in cereal-based products, further preparation of the sample is necessary. **Sample preparation** includes **grinding**, **extraction** of the mycotoxins and a **purification** of the extract (clean-up). Usually, mycotoxins are extracted from the grinded sample with a (various) combination of solvents. Most common used solvents are acetonitrile and methanol. Subsequently, the extracts are purified to remove impurities that can impair the effectiveness of the subsequent mycotoxin detection method.

Also for the actual **detection** of mycotoxins in a sample different techniques exist, the most common are discussed here. **ELISA** (enzyme-linked immunosorbent assay) is an immune-based technique that is rapid and easy to perform. After a number of reaction and washing steps, a blue coloured solution is obtained, of which the colour intensity is inversely proportional to the mycotoxin concentration in the sample. This colour intensity is measured with an ELISA reader and compared to a standard series to obtain the level of mycotoxins in the sample. Other immune-based methods consist of dip-sticks and are semi-quantitative methods that can be used on site to determine the presence of mycotoxins.

Both **HPLC** (High-Performance Liquid Chromatography) and **LC-MS** (Liquid Chromatography coupled with Mass Spectrometry) separate and detect mycotoxins

that are present in a sample, making use of the chemical and molecular properties of the mycotoxins. These techniques are very sensitive, precise and reliable but require a lot of knowledge and skills. The needed equipment is also very expensive.

The application, advantages and disadvantages of these three common methods are compared in Table 7.

Table 7: Comparison between the three most common methods to detect mycotoxins in cereal-based products.

Method	Mycotoxins	Advantage	Disadvantage
ELISA	Aflatoxins Ochratoxin A Fumonisins Zearalenone Deoxynivalenol Citrinin T-2 toxin	Rapid High throughput Low sample volume Simple Portable Often no clean-up needed	Matrix dependent Cross reactivity Extensive validation needed Single mycotoxin detection
HPLC	Fumonisins Zearalenone Trichothecenes Ochratoxin A Aflatoxins	Sensitive Reliable Minimum variability	Time-consuming Substantial clean-up necessary
LC-MS	Trichothecenes Zearalenone Fumonisins Enniatins Ergot alkaloids Ochratoxins Aflatoxins Moniliformin	High sample throughput High selectivity High sensitivity Multiple analysis (tandem MS)	Matrix interference (sample clean-up necessary) Expensive Time-consuming

Most analyses of mycotoxins are still performed using the above mentioned conventional methods, however, newly emerging techniques as biosensor methods are being developed to assess mycotoxin levels. A biosensor combines a biochemical reaction (ELISA - antibody) and a physicochemical (electrochemical, optical (light), mass sensitive (weight), calorimetric (heat)) detector for the quantification of mycotoxin levels. Biosensors are able to provide rapid, sensitive, robust and cost-effective quantitative methods for on-site testing.

4 FUSARIUM HEAD BLIGHT: SOURCE OF MYCOTOXINS

One of the major sources of mycotoxins in the food and feed chain are *Fusarium* species. Usually, the contamination with *Fusarium* mycotoxins occurs in the field when the cereal crop gets infected with Fusarium head blight (FHB). FHB is a fungal disease **in wheat and other small grain cereals** (barley, oat, triticale, etc.) that is often caused by a mixture of *Fusarium* species. FHB has a high economic impact, causing two types of problems: **yield and quality losses**. Significant losses in grain yield are due to flower abortion, decrease in grain test weight and highly damaged grains eliminated during threshing. Additionally, FHB can result in the loss of grain quality, either by affecting grain processing qualities or by producing plenty of mycotoxins. Growing wheat and other cereals is therefore faced with the challenge of keeping contamination with *Fusarium* species and related mycotoxins as low as possible.

4.1 SYMPTOMS IN WHEAT

The first symptoms of FHB in wheat include a brown, dark purple to black discoloration at the base of a floret within the spikelets of the head. As the infection progresses, the diseased spikelets become light tan or bleached in appearance (Figure 1). The infection may be limited to one spikelet, but can also colonise the entire head. The base of the infected spikelets often develop a dark brown colour. When weather conditions have been favourable for pathogen reproduction, the fungus may produce small orange clusters of spores or black reproductive structures, called perithecia, on the surface of the glumes. Infected kernels are often shrivelled, white, and chalky in appearance, known as “tombstone” kernels. Kernels that are colonised by the pathogen during late kernel development may not appear to be affected, but may still be contaminated with mycotoxins. During prolonged wet periods, pink to salmon orange spore masses of the fungus can be seen on infected spikelets, glumes and wheat kernels (Figure 1).

Figure 1: Wheat heads with symptoms of FHB. Diseased spikelets become bleached or tan in appearance (a, b), grain damaged by FHB (c, left) and healthy kernels (c, right).

4.2 INVOLVED PATHOGENS

Fusarium head blight (FHB) is caused by **a mixture of several *Fusarium* species**. Until now, 17 species of *Fusarium* have been described to be potentially associated with FHB symptoms. The main causal agents of FHB in Europe are *Fusarium avenaceum* (in sexual stage known as *Gibberella avenacea*), *F. culmorum*, *F. graminearum* (sexual stage: *Gibberella zeae*), *F. poae* and *Microdochium nivale* (sexual stage: *Monographella nivalis*). The fungal species making up the pathogenic complex may differ from one region to another. Their geographical distribution is particularly related to temperature and moisture requirements. Crop husbandry practices also have an important influence on the predominance of FHB pathogens (see also 6. GAP).

4.3 LIFE CYCLE

Fusarium species reside and multiply on infected crop residues of small grain cereals, maize and other host plants (e.g. grass species). Some *Fusarium* species can **survive in soil** as mycelium or resting spores. Reproduction and spore formation occurs during mild periods. In the asexual stage conidia are produced, whereas in the sexual stage ascospores are produced (Figure 2). **Conidia** are typically transported **by rain drops**. Because these conidia are entrapped in rain drops, they cannot be easily transported by wind. On the contrary, **ascospores** can be easily picked up **by the wind** and transported over long distances. Wheat crops are susceptible to infection from the flowering period up to hard dough stage of kernel development. During moist weather, spores of the fungi are windblown or splashed onto the heads of cereal crops, in this way infecting susceptible wheat heads. Under wet conditions mycelia can spread over the exterior surfaces of the floret. FHB has commonly one cycle per year. However, early infections may produce air-borne spores, which are responsible for a secondary spread of the disease, especially if the crop has uneven flowering due to late tillers (Figure 2).

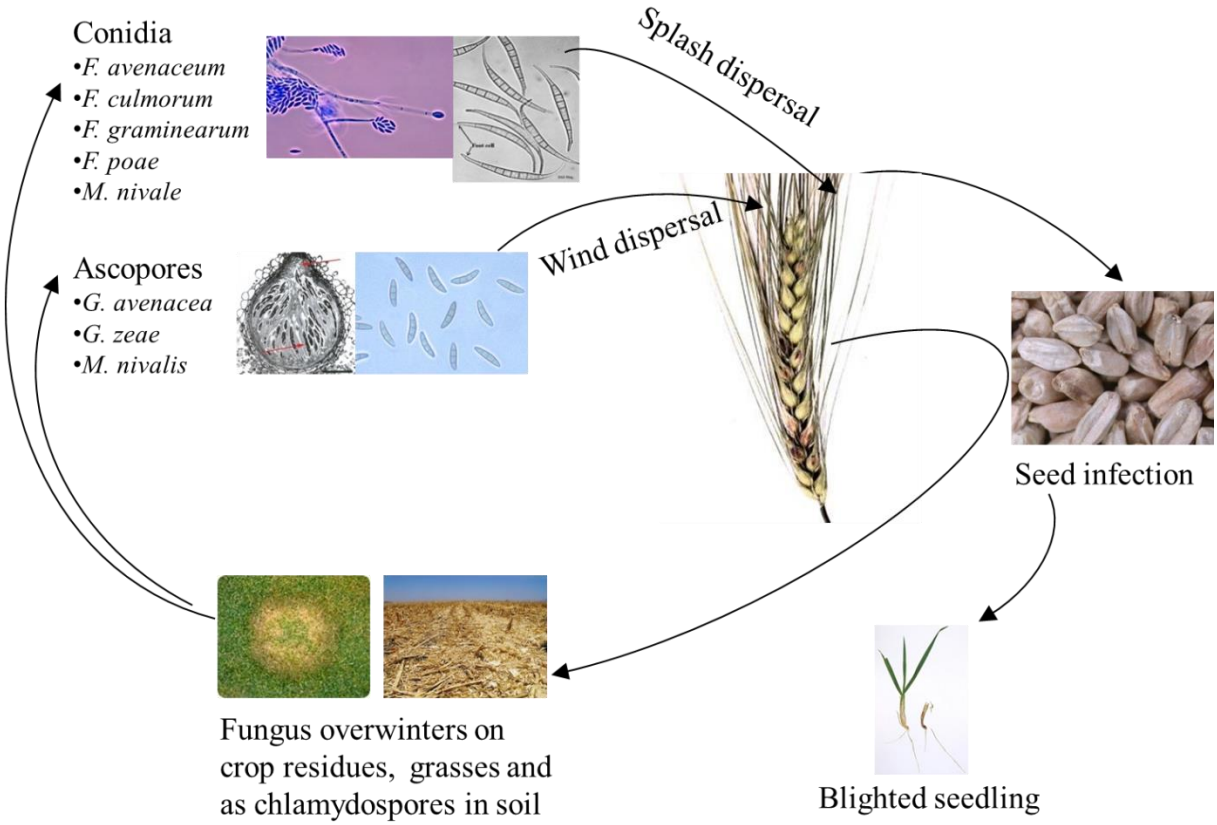


Figure 2: Life cycle of *Fusarium* species.

4.4 RELATION BETWEEN FHB AND MYCOTOXIN PRODUCTION

Although FHB may cause grain yield losses, the interest in FHB is primarily fuelled by the ability of the majority of the *Fusarium* species to produce a diverse spectrum of mycotoxins. These secondary fungal metabolites can accumulate to significant doses and as such cause a serious obstacle for human and animal health.

F. avenaceum, *F. culmorum*, *F. graminearum* and *F. poae* can produce mycotoxins, whereas *M. nivale* appears not to produce mycotoxins. One of the most common and known toxins produced by *Fusarium* species is deoxynivalenol (DON).

Mycotoxin production by moulds is a complex process that is currently not fully understood. **Each *Fusarium* species can produce several mycotoxins**. Trichothecenes, fumonisins, the oestrogenic zearalenone and moniliformin are the most important mycotoxins produced by *Fusarium* species. Trichothecenes, the main mycotoxins found in small-grain cereals, are a family of more than 200 toxins, of which T-2 toxin (T-2), HT-2 toxin (HT-2), diacetoxyscirpenol (DAS), deoxynivalenol (**DON**), nivalenol (NIV) and their acetylated derivatives (3-ADON and 15-ADON) are most prevalent.

The relationship between visual symptoms of FHB and DON content is highly variable, ranging from none to a very strong positive relationship. Differences in relationship may be due to differences among wheat varieties, weather conditions, pathogen population, crop production and disease management practices, as well as other unknown random effects. According to a research study at University College Ghent, there is overall a significant positive relationship between visual symptoms of FHB and DON content. Nevertheless, **caution should be taken when using disease symptoms as a predictor of DON content**, as DON can accumulate in the absence of visual symptoms and not all species or isolates, causing typical FHB symptoms, produce DON (e.g. *Microdochium* species).

Because DON and other trichothecenes are also toxic to the plant itself, wheat has developed a broad array of detoxification processes, leading to the formation of so-called "**masked mycotoxins**". Masked mycotoxins are an emerging health issue as these conjugated forms remain latently present in the plant tissue, ready to be released when exposed to enzymes in the animal/human digestive system or upon food processing.

5 HOW TO PREVENT OR CONTROL MOULD INFECTION AND MYCOTOXIN PRODUCTION?

Because of their enormous impact on public health and agricultural practices, it is highly **important to continuously prevent and control the incidence of mould infections and the associated mycotoxin production**. Measures should be taken throughout the complete cereal production chain, from the field (to reduce mycotoxins associated with field moulds such as *Fusarium*, *Alternaria* and *Claviceps*) up to the finished product (to reduce storage spoilers as *Aspergillus* and *Penicillium*). Therefore, the prevention and control of mould infections start in the field with **good agricultural practices** (GAP). The main idea behind GAP, with respect to diseases, is to **reduce the amount of inoculum** and to **prevent the dispersal of inoculum**. Another aspect of mould prevention and control is the **continuous monitoring of the crop**, in this way control measures can be taken in time. Especially during the flowering period, wheat is very vulnerable to infection with Fusarium head blight. Below, a number of good agricultural practices are given, mainly focusing on *Fusarium* species in wheat, but the principles also greatly apply to other crops and their associated moulds.

Once the grain is harvested, measures need to be taken to maintain the grain quality during storage, transport and further processing. Hence, some **good practice** recommendations are given for **collection, handling, storage, dispatch and transport** operations. Also the importance of regularly monitoring mycotoxin levels throughout the production chain will be addressed.

6 GOOD AGRICULTURAL PRACTICES

6.1 CHOICE OF VARIETY

FARM

CHOICE OF VARIETY

- Use varieties recommended for a certain region

- Choose a variety with host resistance for FHB

To make the crop less vulnerable to infections by moulds, it is very important to limit all stress factors that can influence the growth and development of the plants. The soil, climatic conditions and agronomic practices normally used can be selective factors for choosing the best hybrid or variety for a certain region. Hence, only varieties that are recommended for a particular region should be grown in that particular region. Another factor that can be taken into account when choosing a variety is its resistance to certain diseases. Whenever available, the farmer should choose varieties that exhibit a certain host resistance and can be moderately resistant against a specific type of fungal disease like Fusarium head blight.

6.2 CROP ROTATION

FARM

CROP ROTATION

Rotating *Fusarium* host crops with non-host crops:

HIGH RISK **Maize → Wheat**

LOW RISK **Potato → Wheat**

It has been proven that the alteration of crops which are hosts for *Fusarium* species (e.g. grasses, maize, wheat and other cereals) with crops which are no hosts (e.g. potatoes, beets, onions, beans, clover, alfalfa, vegetables and chicory) can reduce the risk of FHB infection and DON production. By rotating host crops with non-host crops, the production of infectious material is interrupted and the survival chance of inoculum is reduced. In this way, the inoculum level on the field can be kept under control which results in a lower infection pressure when host crops are grown.

Good examples of crop rotation are wheat/legume rotations. Including maize in the rotation should be avoided, since maize is very susceptible to *Fusarium* infection. Also the planting of consecutive small grain crops should be avoided, unless risk assessments indicate only low infection pressure. It should be indicated that *Fusarium* species are able to survive by growing on decaying host crop residues.

6.3 CROP PLANNING

FARM

CROP PLANNING

- Avoid high temperatures and drought stress during seed development and maturation

- Avoid wet periods during early flowering

- Maintain recommended plant spacing to avoid overcrowding (increased humidity)

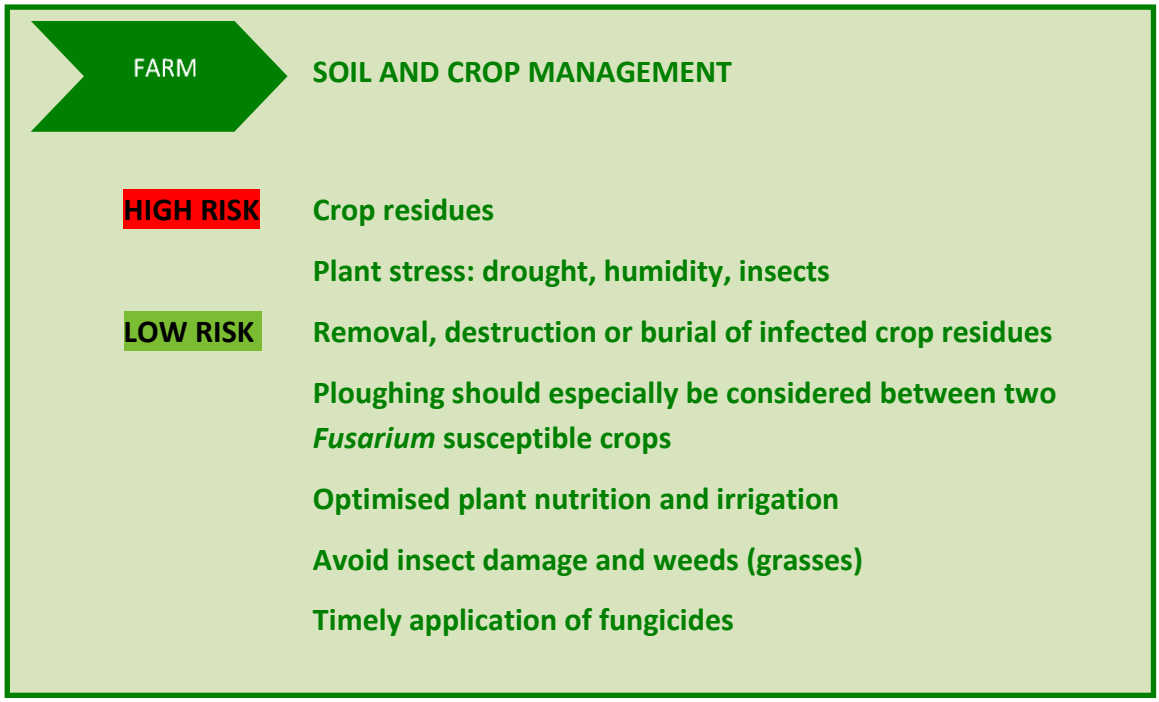
HIGH RISK **Delaying harvest of infected crops may increase mycotoxin content**

LOW RISK **Planning to harvest the crop at low moisture content and full maturity**

It is known that certain climatic conditions favours the development of moulds in the crop. For example, climatic conditions that extend the ripening in the field before harvest as well as drought stress are two environmental risk factors for a *Fusarium* infection in the field. Hence, crop planting should be carefully planned to avoid high temperatures and drought stress during seed development and maturation. If possible, wet periods during early flowering should be avoided as well. Otherwise, planning to harvest the crop at low moisture content and full maturity may minimise mycotoxin contamination, unless the crop is likely to be subjected to extreme heat, rainfall or drought conditions. In these cases, delaying the harvest of a crop, already infested with *Fusarium*, may result in a significant rise of mycotoxin levels in the grains.

Also special attention should be paid to plant spacing. By maintaining the recommended row and intra-plant spacing for the species or varieties, overcrowding can be avoided. Overcrowding or a high canopy density can result in longer periods of humid conditions after rain or dew, enhancing the sporulation of moulds. With respect to plant spacing, seed and breeding companies can provide useful information.

6.4 SOIL AND CROP MANAGEMENT



Any practice resulting in the **removal, destruction or burial of infected crop residues** is considered as good soil cultivation. The deeper the soil is inverted, for example by ploughing, the less plausible *Fusarium* moulds can grow on the following crop. Ploughing should especially be considered between two *Fusarium* susceptible crops. Whenever possible, the seed bed for each crop should be prepared by ploughing under or removing old seed heads, stalks and other harvest residues. All these residues may have served or may potentially serve as substrate for mycotoxin-producing moulds.

It is also very important to minimise plant stress where possible. Plant stress can be caused by many factors, like drought, cold, nutrient deficiencies and adverse reactions to materials applied to the crop. Irrigation can minimise drought stress. Spray irrigation or excess irrigation during the flowering period should be avoided.

The application of fertilizers can minimise plant stress, especially during seed development, by assuring an adequate soil pH and plant nutrition. Nutrient supply should be adapted or optimised to the needs of the plants and soil conditions. The rational use of fertilizers and plant growth regulators should avoid excessive plant growth and lodging of the crop. Lodged grain is known to be susceptible to increased *Fusarium* infection.

Damage, caused to the crop by insects, birds or rodents, can also result in a crop that is sensitive to *Fusarium* infection. As far as possible, insect damage can be minimised by taking preventive measures. If necessary, appropriate use of registered insecticides and fungicides can control insect damage and subsequent mould infection. Also, an adequate control of weeds, especially grasses which can host *Fusarium* species, is an important issue. Mechanical methods or registered herbicides can be used. The use of pesticides should be fit in an integrated pest management programme.

Especially, the timely application of fungicides is crucial to control *Fusarium* infection and should be based on weather conditions and crop development. *Fusarium* infection usually occurs under humid conditions during the flowering period. The crop should be monitored for and potentially treated against *Fusarium* infection in this period. Fungicides must be applied between three days before and three days after infection, covering all heads (Figure 3). The effectiveness of fungicide application can be improved by using the appropriate type of nozzles (e.g. double fan nozzles) or the use of adjuvants.

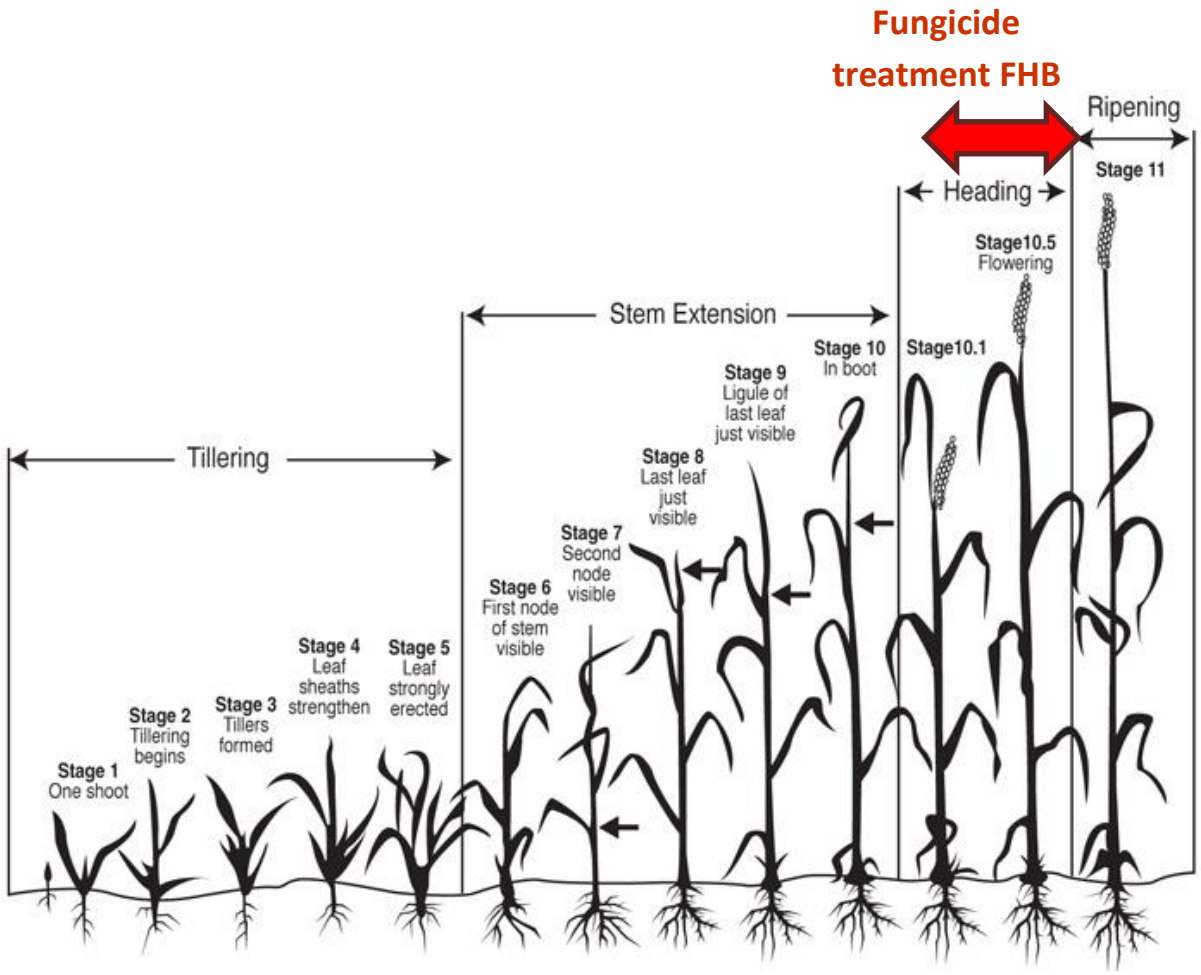


Figure 3: Visualisation of the optimum timing for fungicide treatment against FHB in wheat.

6.5 HARVESTING

FARM — **HARVESTING**

HIGH RISK	**Moisture content is too high (> 15%)**
	FHB infected parts of the field
	Shrivelled, damaged or lodged grain
LOW RISK	**Pre-harvest DON score**
	Separation for food/feed
	Difference damp/clean/dry

Harvest is the first stage in the production chain where moisture content and grain quality can be managed in an efficient way. The moisture content and quality of the harvest-ripe grain can vary considerably across the field and should be monitored at different spots in the field or in each load of the harvested grain during harvest operations. Monitoring of grain quality and more specifically DON content can be easily performed in the field before harvest, by sampling and pre-harvest testing for mycotoxins. Pre-harvest testing for mycotoxins is a very good way to estimate the damage caused by FHB, especially when visual symptoms were absent. However, in the period between the pre-harvest testing and harvest the mycotoxin content can still increase due to bad weather conditions. Also good harvesting practices are necessary to avoid further contamination of mycotoxins.

At harvest, a separation of FHB infected material should be conducted where possible. Also lodged grain can be a source of high levels of *Fusarium* infection. If practicable, the grain can be separated based on market quality requirements, such as bread making or animal feed, and ex-field quality, such as lodged, damp, clean or dry. Also mechanical damage and contact with soil should be avoided during harvesting. Small, shrivelled grain may have higher amounts of mycotoxins as well. By correct setting of the combine or cleaning after harvest, shrivelled and damaged grain can be removed and contact with soil can be avoided.

The most important factor at harvest is the final moisture content of the grain. The moisture content is of paramount importance for the microbial stability and quality maintenance after harvest. Depending on the weather conditions during the harvest period, it may be necessary to further dry the grain in order to obtain the appropriate moisture content.

6.6 INTERACTION BETWEEN WEATHER CONDITIONS AND AGRICULTURAL PRACTICES: CONSEQUENCES FOR DON CONTENT

The experimental field study tested the quantitative effect of agricultural practices and weather conditions on FHB and DON content and the interaction between factors. The examined parameters included the previous crop (susceptible or not to FHB), susceptibility of the chosen wheat variety, rainfall at flowering and tillage method (ploughing or not). With the obtained information from these field experiments, a decision tree could be build (Figure 4). The first splitting variable is **rainfall during flowering**. This emphasizes that weather conditions at flowering are the most important factor determining the DON content. If it rains during flowering, the previous crop type seems the most important factor. Conversely, if it does not rain during flowering, the susceptibility of the wheat variety has the greatest influence on the DON content. According to the decision tree, the risk for DON contamination is lowest when it did not rain during flowering, a resistant variety was used and the previous crop was not host for *Fusarium*. On the contrary, the highest level of DON can be expected when it rained during flowering, the previous crop was host, a susceptible variety was used and the field was not ploughed before sowing. This decision tree should allow farmers to know when they should be alert and take action against FHB.

The study also highlighted that intensive maize-wheat rotations can result in DON contents up to eight times higher than the EU limits for human consumption. It was shown that a crop rotation with non-host crops for *Fusarium* species combined with inversed tillage and a moderately resistant wheat variety significantly reduces the FHB incidence and associated mycotoxin contamination. However, the quantitative effect of agricultural factors should be framed within the context of disease pressure, which is in the first place determined by weather conditions (during flowering).

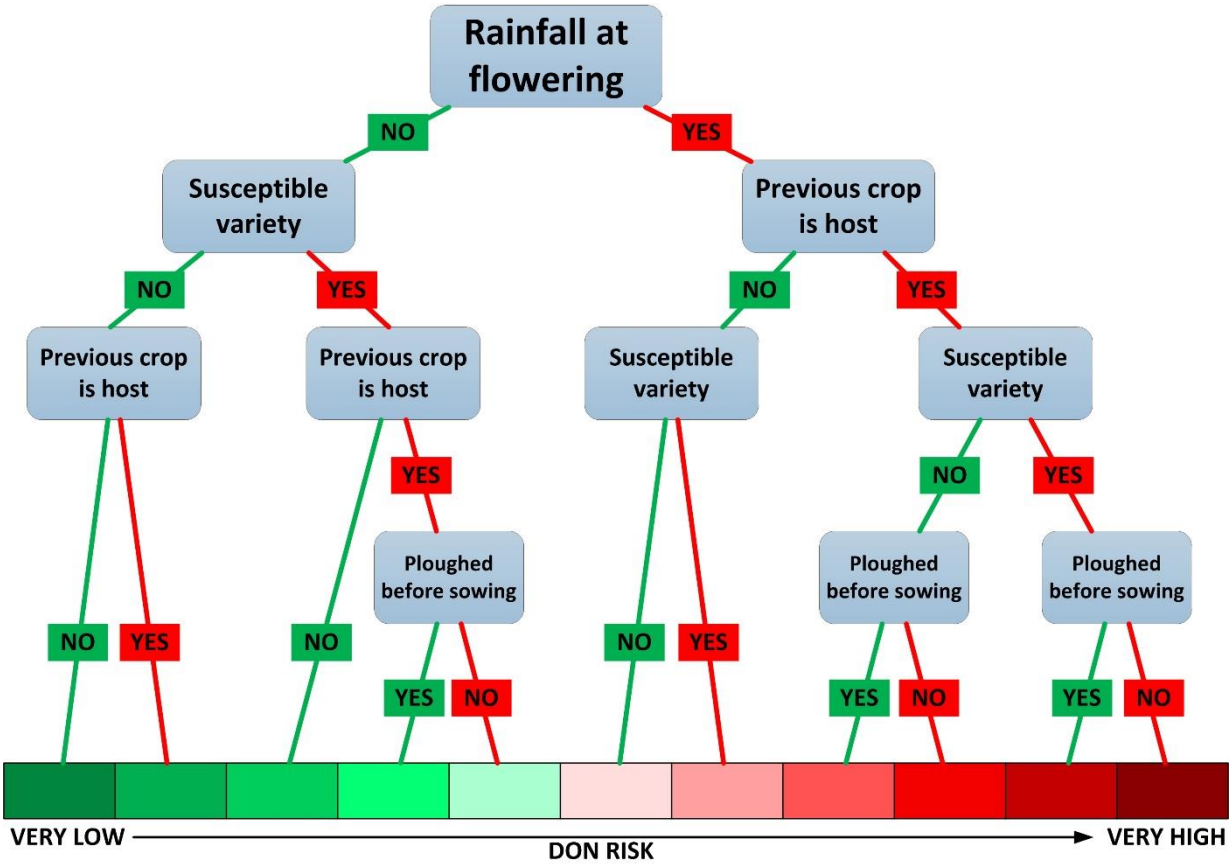


Figure 4: Decision tree for DON risk showing the relative importance of the weather conditions during flowering (rainfall or not), the host crop (a susceptible host crop (maize, wheat) or non-susceptible host crop (potatoes, sugar beets, etc.), the tillage method (ploughing or minimal tillage method) and the susceptibility of the grown wheat variety (a susceptible variety for FHB or not). The coloured blocks at the bottom denote the levels of DON content that can be expected if the conditions above are fulfilled.

7 GOOD PRACTICE RECOMMENDATIONS FOR COLLECTION/RECEIPT OPERATIONS[1]

After harvest, it is important that the quality of the grain is monitored and that grain damage, quality loss and microbial spoilage are minimised as far as possible, in particular with respect to mould growth and mycotoxin production. Therefore, general and specific good hygiene and quality control measures should be taken to avoid the spread of inoculum by contaminated combines, trailers, augers, grain stores and all kind of equipment that have not been cleaned and contain leftover contaminated grain from the previous harvest. A rigorous hygiene programme at the beginning of and during harvest, during storage and transport is hence of great importance.

[1] All general good hygiene guidelines for collection are available in the 'European Good Hygiene Practices Guide for the collection, storage, trading and transport of cereals, oilseeds and protein crops', only those with respect to mycotoxins are given here.

7.1 GENERAL GOOD HYGIENE PRACTICES

General good hygiene practices start in fact during harvesting. To avoid mycotoxin contamination and dispersal of mycotoxin-producing moulds, combines and harvest transporters should be **cleaned before and after every harvest operation**. Farmers and contractors should be reminded of their obligation to keep their agricultural trailer or truck clean, **both at the inside and outside**. If other products than cereals or oilseeds have been transported with the truck, the truck should be cleaned. Farmers and contractors should pursue the good practices recommendations for field crops, particularly the rules on cleaning and succession of transport.

Also at the collection or storage facility, some hygienic rules should be respected. The surroundings of the collection facilities (including lawns, concrete areas, pits, etc.) should be properly maintained. Rain and run-off water can drain well. Traps for rodents are placed in the areas surrounding grain storage and waste storage locations.

7.2 QUALITY MONITORING AT THE INTAKE LEVEL

COLLECTION

QUALITY MONITORING

- sampling plan

- moisture content

- mycotoxin analysis

PRE-SELECTION BASED ON QUALITY

before allocating into a certain storage facility (silo):

- avoid mixing of parties of highly different quality

- segregate based on moisture content, protein content, hectolitre weight, crop data, presence of insects...

At the delivery of a cereal batch, its quality level need to be determined. The analysis of moisture content is hereby crucial regarding microbial spoilage, including mould growth and mycotoxin formation. To determine the quality level of the cereal batch, a number

of measures are taken, among which sampling, olfactory and visual inspection of the delivered batch (e.g. mould growth, insects, etc.) and analysis of moisture and impurity contents. The operator must set the criteria used for **quality determination, classification and allocation** of the received products. These criteria determine the type of technological analyses that need to be performed on the received products to characterise them.

These specific quality criteria are also used to perform a pre-selection, already at the intake level and before allocating into a certain storage facility (silo). The grain can be separated based on the moisture content, protein content, hectolitre weight, crop data, presence of insects, etc. This pre-selection will aid in reducing the risk of taking in large batches of grain with elevated mycotoxin levels as well as in avoiding the mixing of batches with highly different quality. An additional sorting based on density or gravity separation can also efficiently reduce the amount of mould spores and associated mycotoxins. In this way, broken, damaged, shrivelled or infected kernels are removed and the amount of DON, ZEN and fumonisins can be significantly reduced. Cleaning of the external surface of grains can also minimise the dispersal of *Fusarium* spores from infected grains to healthy ones.

Essential elements in quality monitoring are the **sampling and mycotoxin analysis** of the cereal batch (Commission Regulation 401/2006). Mycotoxin analysis should be conducted at several stages, from harvest until dispatching: at pre-harvest stage, at receipt, during pre-storage or drying, during storage and at dispatching/delivery. It should be emphasized that batches containing too high levels of mycotoxins cannot be placed on market as such, nor after mixture with other foodstuffs or used as an ingredient in other food products.

7.2.1 USE OF THE RAPID BIOSENSOR FOR DON

The Rapid Biosensor is based on a continuous dust sampling of the wheat at intake, followed by an extraction and detection by an immunosensor.

The sampling system should be placed close to the intake point where a fast movement of wheat takes place. The movement of the cereals in closed transportation tubes results in dust production. The dust is removed by an aspiration system connected to a cyclone for air separation. The dust is automatically extracted and the DON in the extract is measured. Increased DON concentration in dust from 5000 ppb and up may be an indication of contaminated wheat. Further segregation, sampling and LC-MS analysis is recommended.

7.3 TRACEABILITY

COLLECTION

TRACEABILITY

- Track and trace

- Allocated as food or feed

Important for traders!!

The traceability of the marketing of cereals is not an easy task. The complexity arises from the fact that small batches with varying qualities are taken in, allocated to different storage facilities and finally mixed with other deliveries. However, in case a food or feed safety issue occurs, it must be possible to track and trace these goods. In this perspective, traceability should be seen as a risk management instrument that allows the food and feed business operators as well as the competent authorities to proceed with precise and targeted withdrawals and recalls (in accordance with Commission Regulation 178/2002).

7.4 DRYING

COLLECTION

DRYING

HIGH RISK **Store grains with high moisture content (> 15%)**

LOW RISK **Drying → moisture below ± 15 %**

Method depending on moisture content at harvesting

At harvest or immediately afterwards, the moisture content of the crop is determined. To avoid mould growth during storage, the moisture content of cereal grain should be lower than that required for mould growth. For wheat, the moisture content should be less than 15%, whereas for maize it should be less than 14%. When a batch of grain delivered by the farmer is too damp, the necessary measures must be taken to dry and safeguard the grain from decay during storage. The grain should be dried as soon as possible after harvest. The period between harvest and drying, when the wet, freshly harvested grain is kept piled, should be minimised, since mould growth can already occur within a few days, possibly accompanied by heating. During this period the wet grain should be sufficiently aerated to avoid overheating prior to drying. This issue also implies that harvest should be planned according to the capacity of the dryers.

8 GOOD HYGIENE PRACTICE RECOMMENDATIONS FOR STORAGE OPERATIONS[2]

STORAGE

GOOD HYGIENE PRACTICES DURING STORAGE

Quality control at intake

Avoid contamination of stored products by the environment and cross-contamination between stored products

Avoid moisture

LOW RISK **Good storage conditions: cool, dry and ventilated**

Pest control

Adequate cleaning and maintenance of premises

8.1 LAYOUT OF THE STORAGE AND HANDLING PREMISES

[2] All general good hygiene guidelines for storage are available in the 'European Good Hygiene Practices Guide for the collection, storage, trading and transport of cereals, oilseeds and protein crops', only those with respect to mycotoxins are given here.

During storage, the quality of the stored goods should be assured. This means that contamination risks from moulds, insects, animals and other non-food substances should be prevented. With regard to moulds and the associated mycotoxins, the most important **source of spoilage is the presence of water**. Therefore, the premises and their infrastructure need to be kept in good repair. The roofing in particular should be kept watertight to prevent the stored products from being altered by water, which can cause mould and attract insects. Other sources of moisture, like condensation on cold surfaces or leaking water pipes, should be avoided. In case of bagged commodities, the bags must be clean, dry and stacked on pallets.

8.2 STORAGE IN WAREHOUSES AND SILOS

8.2.1 STORAGE CONDITIONS

The storage conditions like **temperature and humidity** (mainly water activity) have a profound influence on the microbial stability of the grain. The prevailing temperature during storage has an effect on the mould growth and activity. When cereal grains are stored in a silo or a warehouse, a large volume of air is immobilised. The temperature in the centre of the grain volume remains closely to the harvest temperature, while the cereal grains at the outside are in contact with the storage walls. The outside zones cool faster when the outside temperature decreases during autumn. Condensation and wet spots may occur, in this way favouring mould growth. As a result of increased mould growth, the temperature in the contaminated zone will rise rapidly. A temperature rise of 2-3°C may indicate mould growth or insect infestation. Odour also can indicate that the grain is heating. In addition, the majority of moulds have growth optima between 25 and 30°C.

Temperature and humidity should be controlled and recorded regularly at different places and heights in the silo or warehouse. **Cooling and drying** operations combined with **ventilation** are necessary to maintain proper and uniform temperature levels and to minimise mould infestation. Ventilation occurs by sending a mass of air into the storage facility. This operation removes heat and restores the balance between grain temperature and air temperature. The optimisation of ventilation largely depends on the operator's know-how. These rules should be kept in mind:

- Air mass at least equivalent to the mass of grain
- The grain is ventilated with air that is cooler than the grain (min. temperature difference of 5°C)
- The evacuation of hot air is facilitated to prevent dew points from forming.

In case of infection, the attacked portions of the grain should be separated and a sample should be sent for analysis. After separation, the temperature is lowered and the remaining grain is aerated. Infected grain may not be used for food or feed production.

8.2.2 PEST CONTROL

Grain storage facilities are very attractive to insects and rodents, increasing the contamination risk and spoilage of the stored goods. Next to direct damage to the stored products, moisture can accumulate from their activities, providing ideal conditions for mould growth and mycotoxin accumulation. Some insects are vectors of mycotoxin producing moulds as well (e.g. *Aspergillus flavus* by nitidulid beetles).

Therefore, the storage and handling premises and galleries should be cleaned regularly and treated with appropriate fungicides, insecticides, rodenticides or alternative methods. Special attention should be paid to cleaning when storage facilities are emptied. The grain itself should be protected against moulds and insects by using all measures available at the site (cleaning, storage control, ventilation, fumigation, rational use of storage pesticides etc.). However, the use of pesticides should be strictly limited and should comply with legislation.

Regarding fumigation, phosphine is an effective fumigant for disinfection and protection of cereals against insects and mould invasion, after the banning of methyl bromide. Essential oils offer an alternative to phosphine to control insect pests and mould infections.

8.2.3 CLEANING AND MAINTENANCE OF WAREHOUSES, SILOS AND INSTALLATIONS

Grains infested by mould, residues from the bottom of bins that have not been cleaned, insects or moulds in premises where dust has accumulated due to poor cleaning or the inability to clean due to the design of the premises, are all sources of contamination.

At least once a year or after collection, the handling premises and galleries should be cleaned to limit the accumulation of dust which favours the development of mould and attracts insects. Also the grain storage facilities (bins, compartments) need to be cleaned, particularly if the previous goods stored were contaminated (insects, mould). The used equipment is periodically cleaned, particularly before the storage of cereals and other grains (pits, dryers). Treatments are made according to Plant Protection Products manufacturers' instructions so that residues do not exceed authorised levels.

8.3 HANDLING OF WASTE

Cereal batches that cannot be placed on the market or be further processed because of exceeding levels of mycotoxins should be properly stored in a waste compartment, separately from the grain storage facilities. Also in the waste facilities good hygiene practices should be applied to avoid (cross-)contamination. Waste collection should be scheduled with appropriate frequency. An adequate pest control should be adapted. Especially insects that are vector of certain storage mould should be controlled, since these insects can easily move to the other facilities and contaminate the stored cereal grains.

8.4 CHEMICAL AND ANTIFUNGAL AGENTS

Weak acids are used as **preservatives** in food and feed to prevent fungal spoilage. The most common weak acid preservatives are sorbic acid, benzoic acid and propionic acid. Propionic acid and its salts are fungistatic and are sometimes used for preserving damp grain on the farm after harvest to avoid heating and mould growth prior to treatment. The preservatives should be applied with the correct equipment so that the whole batch is evenly covered.

The efficiency of these organic acids, however, is sometimes hampered by toxigenic moulds metabolizing the acids rendering them no longer active. Other control strategies comprise essential oils and antioxidants, but the implementation in practice is not easy due the high costs of the treatments.

9 GOOD HYGIENE PRACTICE RECOMMENDATIONS FOR DISPATCH/DELIVERY AND TRANSPORT OPERATIONS[3]

[3] All general good hygiene guidelines for transport are available in the 'European Good Hygiene Practices Guide for the collection, storage, trading and transport of cereals, oilseeds and protein crops', only those with respect to mycotoxins are given here.

DISTRIBUTION

GOOD HYGIENE PRACTICES DURING TRANSPORT

Clean and dry containers

No residues

Avoid rain

No pests and rodents

Most cereal grain intended for food or animal feed need to be transported for further processing. Often, transport operations can be considered as moving storage operations, especially in case of long-distance transports. Hence, similar rules as for good collection and storage practices apply to avoid (cross) contamination and safeguard food and feed safety at all times during transport, especially with respect to moulds and mycotoxin contamination. The transport contractor and the transporter are considered as responsible for ensuring that the equipment is conform food and feed safety requirements. The following general rules apply to all kinds of cereal transport (by road, sea, waterways and rail):

- Transport operator must be able to present a **logbook** listing their successive transport operations (type of goods, cleaning , etc.).
- To facilitate traceability, containers and transport loads should be **labelled** correctly.
- Rain and spray may not enter the loaded compartments during transport.
- Before loading the cereal products, all visible **residues** from the previous load must be **cleared** from the in- and outside of the vehicle, train hopper or boat. Especially cereal and crop residues are possible sources of mould infection and mycotoxin contamination.
- Before loading, compartments must be inspected that they:
  - Are **clean, watertight, dry, odourless and in good maintenance condition**.
  - Do not contain any sources of contamination (insects, moulds, rodents, residues from previous loads, etc.).
  - Are compatible with the loading and transport of the specific products.
  - Are suited to the required transport and protect the goods against moisture, insects and other possible sources of mycotoxins.
- Compartments that have been used to transport contaminated cereal products during the previous load must undergo a risk analysis and need to be cleaned and/or disinfected.

10 REFERENCE LIST

10.1 LEGISLATION AND RECOMMENDATIONS

Codex Alimentarius CAC/RCP 51-2003 Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals, including Annexes on Ochratoxin A, Zearalenone, Fumonisins and Tricothecenes

European Commission Directive 100/2003 on undesirable substances in animal feed

European Commission Recommendation 576/2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding

European Commission Recommendation 583/2006 on the prevention and reduction of Fusarium toxins in cereals and cereal products

European Commission Regulation 1881/2006 on the maximum levels for certain contaminants in foodstuffs

European Commission Regulation 401/2006 on the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

European Good Hygiene Practices Guide for the collection, storage, trading and transport of cereals, oilseeds and protein crops

10.2 WEBSITES

http://ec.europa.eu/food/food/chemicalsafety/contaminants/guidance-sampling-final.pdf: Guidance document for the sampling of cereals for mycotoxins

http://ec.europa.eu/food/food/chemicalsafety/contaminants/index_en.htm: patulin, ochratoxin A, Fusarium toxins, aflatoxins

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