



The *Fusarium* Mycotoxins
in Finnish Cereal Grains:
How to Control and
Manage the Risk

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In memory of my parents

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ABSTRACT

The central goal of grain cultivation is the production of high-quality food or feed-related raw materials for the processing industry. Management of *Fusarium* mycotoxins in Finnish cereal grains have a direct impact on human and animal health, and the confidence in a safe and healthy domestic cereals and cereal products.

Fusarium fungi and head blight have always emerged in Finland after rainy and poor summer weather conditions. During the 1960s and 1970s the spectrum of *Fusarium* species and the ability of the fungi to produce mycotoxins in domestic grain were subject to extensive investigation. The summer of 1987 was again very rainy and cold, and there was abundant and even visible occurrence of *Fusarium* head blight in grains. A decade passed, and another very rainy and cold summer was encountered in 1998. The last straw of the risk of mycotoxins in magnitude was the summers 2012 and 2013. Even up to a quarter and a fifth of domestic oats in grain trading, respectively, were not accepted for food use because of DON concentrations exceeded the EU limit.

The aims of the present study were to produce updated information of *Fusarium* species, and to define the changes in *Fusarium* mycotoxins in Finnish cereal grains in the years 1987-2014. Another important aims were to determine the basis of the toxin contents and agronomic factors behind the studied samples how to control and manage the *Fusarium* mycotoxin risk, and to predict by modeling the magnitude of the mycotoxin risk.

According to the results, the most common *Fusarium* species in Finnish cereal grains were *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. sporotrichioides* and *F. langsethiae*. When compared to previous studies from the 1970s and 1980s to the present day in Finland, a clear conclusion was drawn that during these years *F. graminearum*, *F. sporotrichioides* and *F. langsethiae* have come strongly into the picture. The number of exceptionally high DON concentrations and also the contents and positive findings of T-2 and HT-2 toxins have increased in Finland.

The following important control and management factors were emphasized: pay attention to the quality of seed and seed dressing; rotation - repeated cultivation of cereals is not recommended; careful timing of harvest and harvest drying - moisture content < 14 %; introduction of rapid test methods and sorting technology at farm level, and last but not least, minimize the risks of toxins by cultivation. Industrial sorting and dehulling reduced the DON, T-2+HT-2 and 3-AcDON levels in oat samples by 75–91%, 87 %, and 67–91%, respectively.

In the near future, increased collaboration among farmers, researchers, the grain processing industry and consumers is needed. Especially, there is a significant need to increase the competitiveness and cost-effectiveness of grain farming in the specialization of national and international markets, and make producers committed to the production of quality grains, novel utilization of by-products and recycling of nutrients. Among the cereals investigated, oats is the most susceptible to *Fusarium* infestation and the production of *Fusarium* mycotoxins in Finland. The market is eagerly looking for new high-yielding varieties capable of preventing *Fusarium* infestation and having low levels of mycotoxins.

SUOMENKIELINEN ABSTRAKTI

Viljan alkutuotannon tärkein tavoite on korkealaatuisen raaka-aineen tuottaminen elintarvike- ja rehuteollisuuden, kotieläintuotannon sekä muiden loppukäyttäjien tarpeisiin. Viljojen hometoksiinien hallinnalla varmistetaan kuluttajien ja eläinten hyvinvointi sekä luottamus turvalliseen ja terveelliseen kotimaiseen viljaan ja viljatuotteisiin.

Fusarium -sienet ja punahomeet ovat Suomessa nousseet esiin aina sateisten ja kosteiden sääolosuhteiden jälkeen. 1960- ja 1970 -luvulla havaittiin, että punahomeet heikentävät jyvien kehitystä, alentavat itävyyttä, aiheuttavat tyvitauteja ja tähkäfusarioosia. Vuosi 1987 oli hyvin sateinen ja kylmä kesä ja punahometta esiintyi viljoissa runsaasti. Kului vuosikymmen, ja koettiin jälleen erittäin sateinen ja kylmä kesä vuonna 1998. Sateinen kesä 2012 oli myös hyvin poikkeuksellinen. Toksiinitasot kohosivat ja kaurasadosta neljännes ei viljan vastaanotossa täyttänyt elintarvikeviljalle säädettyjä suurimpia sallittuja DON-pitoisuuksia. Vuosi 2013 oli lähes yhtä huono hometoksiinien osalta; viidennes kauraeristä ei täyttänyt DONin osalta elintarvikeviljalle säädettyjä enimmäispitoisuuksia. Tämä nosti riskin hallinnan työkalut erityiseen tarkkailuun.

Tämän tutkimuksen tavoitteena oli tuottaa ajantasainen tieto suomalaisen viljan *Fusarium* -sienten lajikirjosta, sekä selvittää viljojen hometoksiinien pitoisuuksien muutokset aikavälillä 1987–2014. Lisäksi erityisenä tavoitteena oli tutkia miten hometoksiinien muodostumista viljoissa voidaan estää ja hallita eri viljelyteknisillä toimenpiteillä ja mallintamalla.

Yleisimmät *Fusarium* -sienet suomalaisessa viljassa olivat *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. sporotrichioides* ja *F. langsethiae*. Kun verrataan näitä tuloksia *Fusarium* -lajikirjoon 1970-luvulla ja 1980-luvulla, niin selkeästi *F. graminearum*, *F. sporotrichioides* ja *F. langsethiae* ovat nousseet vahvoiksi *Fusarium* -toksiinien tuottajiksi 2000 -luvulla. Nämä muutokset näkyvät myös korkeiden DON -toksinipitoisuuksien määrän - ja T-2+HT-2 toksiinien pitoisuustasojen kasvussa sekä positiivisten löydösten lisääntymisessä.

Tärkeimmät riskinhallinnan työkalut ovat siemenen kunnostus ja peittäus, viljelykierto, puintiajankohdan valinta, ja puidun sadon nopea ja huolellinen kuivatus alle 14 %:iin; pikamittausten ja lajittelun käyttöönotto tilatasolla sekä panostaminen elinvoimaisen ja satoisan kasvuston aikaansaamiseksi. Lajittelulla ja kuorinnalla kauranäytteiden DON-, T-2+HT-2- ja 3-AcDON-pitoisuudet alenivat 75–91 %, 87 % ja 67–91 % vastaavassa järjestyksessä.

Merkittävä tarve on lisätä viljan viljelijöiden kilpailukykyä ja kustannustehokkuutta erikoistuvilla kansallisilla ja kansainvälisillä markkinoilla sekä sitouttaa viljelijät laatuviljan tuotantoon, sivuvirtojen uuteen hyödyntämiseen ja ravinteiden kierrätykseen. Kaikista tutkituista viljanäytteistä kaura oli herkin *Fusarium* -sienten tartunnalle ja *Fusarium* -toksiinien muodostumiselle. Tulevaisuuden viljamarkkinat odottavat herkeämättä uusia satoisia *Fusarium* -kestäviä lajikkeita.

LIST OF ABBREVIATIONS

15-AcDON	15-acetyldeoxynivalenol
3-AcDON	3-acetyldeoxynivalenol
AAPRESID	Argentina de productores en siembra directa
AFLP	amplified fragment length polymorphism
AFs	aflatoxins
ARfD	acute reference dose
ATA	alimentary toxic aleukia
BEA	beauvericin
BHA	butyl hydroxyanisole
CAP	common agricultural policy
CMS	choline metabolizing strains
CWRS	Canada western red spring
DAS	diacetoxyscirpenol
DON	deoxynivalenol
DON-3G	DON-3- β -D-glucopyranoside
DPS	dried blood spot
EFSA	European food safety authority
ELISA	enzyme-linked immunosorbent assay
ENNS	enniatis
F-2	6-(10-hydroxy-6-oxo-trans-1-undeceny)- β -resorcylic acid lactone
FAO	food and agriculture organization
FB	fumonisin b
FB1	fumonisin b1
FDK	<i>fusarium</i> -damaged kernels
FGSC	<i>fusarium</i> graminearum species complex
FHB	<i>fusarium</i> head blight
FIab	CAP subsidy areas A and B in Finland
Fic	CAP subsidy area C in Finland
FSMP	Finnish safety monitoring programme
F-X	fusarenone X
GAP	good agricultural practices
GC-MS	gas chromatography - mass spectrometry
GLM	general linear model
GMP	good manufacturing practices
GS	zadoks growth stages
HACCP	hazard analysis and critical control point
HGCA	home grown cereal authority
HPLC-FLD	liquid chromatograph-fluorescence detector
HT-2	HT-2 toxin
IARC	international agency for research on cancer
IPCS	international programme on chemical safety
IPM	integrated pest management

JECFA	joint FAO/WHO expert committee on food additives
LC-MS	liquid chromatography – mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MB (LB-UB)	mean and 95 th percentile presented as the middle bound estimate (lower bound estimate; upper bound estimate)
MCPA	2-methyl-4-chlorophenoxyacetic acid
aw	water activity
MON	moniliformin
MRL	maximum residue levels
NEO	neosolaniol
NIR	near-infrared reflectance
NIT	near-infrared transmittance
NIV	nivalenol
OCOi-Bu	isovaleryl group
OTA	ochratoxin A
P95	95 th percentile
P99,5	99,5 th percentile
PDA	potato dextrose agar
PP	propyl paraben
QTL	quantitative trait locus
R ²	coefficient of determination
RFLP	restriction fragment length polymorphism
2'R-OTA	2'R-ochratoxin A (thermal degradation product of ochratoxin A)
SAGPyA	secretaría de agricultura, pesca y alimentos
T-2	T-2 toxin
TDI	tolerable daily intake
TMTRI	TM = TagMan-QPCR and TRI comes from the word trichothecenes
TRILAN	TRI comes from the word trichothecenes and LAN from <i>F.</i> <i>langsethiae</i>
t-TDI	temporary tolerable daily intake
UNEP	United Nations environment programme
USDA	U.S. department of agriculture
VYR	finnish cereal committee
WHO	World Health Organization
WMO	World Meteorological Organization
ZON	zearalenone

LIST OF ORIGINAL PUBLICATIONS

- I. Hietaniemi, V., Kumpulainen, J. Contents of *Fusarium* toxins in Finnish and imported grains and feeds. *Food Additives and Contaminants* 1991, 8(2), 171–181.
- II. Hietaniemi, V., Kontturi, M., Rämö, S., Eurola, M., Kangas, A., Niskanen, M., Saastamoinen, M. Contents of trichothecenes in oats during official variety, organic cultivation and nitrogen fertilization trials in Finland. *Agricultural and Food Science*, 2004, 13(1-2), 54–67.
- III. Lindblad, M., Börjesson, T., Hietaniemi, Veli, Elen, O. Statistical analysis of agronomical factors and weather conditions influencing deoxynivalenol levels in oats in Scandinavia. *Food Additives and Contaminants: Part A: Chemistry Analysis Control Exposure & Risk Assessment*, 2012, 29(10), 1566–1571.
- IV. Hietaniemi, V, Rämö, S., Yli-Mattila, T., Jestoi, M., Peltonen, S., Kartio, M., Sieviläinen, E., Koivisto, T. & Parikka, P. Updated survey of *Fusarium* species and toxins in Finnish cereal grains. *Food Additives & Contaminants: Part A*, 2016, 33(5), 831–848.

1 INTRODUCTION

A measure of wisdom is the ability to learn from history. In 1891 Latta, Arthur, and Huston mentioned the prevalence of scab on a field where wheat had been grown continuously and on an adjacent field where wheat and corn had been grown alternately for 11 years. This seems to be the first recorded observations on the relation of previous cropping to the occurrence of wheat scab. In 1909, Selby and Manns also recorded observations on the relation of the previous cropping to the production of wheat scab. Some years later Bolley emphasized the importance of rotating wheat with some other unrelated crop such as clover, alfalfa, grasses, potatoes, flax, and corn. In 1918, Hoffer, Johnson, and Atanasoff found that wheat-scab infection may arise from *Gibberella* spores growing on old diseased cornstalks and that corn root rot can be produced under laboratory conditions by *Gibberella* spores isolated from scabbed wheat. In 1919, Holbert, Trost, and Hoffer reported on the occurrence of wheat scab as affected by crop rotations (Koehler, 1924).

Fusarium species survives in crop residues, and managing surface stubble through crop rotation, and tillage can be effective. Consequently, ploughing crop residues may reduce Fusarium Head Blight (FHB) and deoxynivalenol (DON) (McMullen et al., 1997; Dill-Macky and Jones, 2000), especially where wheat is planted after maize. Maize provides the best substrate for ascospore production, in conditions of warm weather and high precipitation and humidity (Sutton, 1982). However, Miller et al. (1998), Schaafsma et al. (2001) and Snijders (2004) indicated that the susceptibility of cultivars could be more important than tillage practice concerning disease incidence (Blandino et al., 2006).

Many research results (Blandino et al., 2006; Landschoot et al., 2013) from previous decades emphasize that crop rotation system, variety, fungicide treatment, weed management, host resistance and soil tillage are probably the most important agricultural factors governing the occurrence of FHB and structure of the FHB population (Fernando et al., 1997; Miller et al., 1998; Schaafsma et al., 2001; Bai and Shaner, 2004; Champeil et al., 2004b; Pereyra et al., 2004a; Bayer et al., 2006; Koch et al., 2006; Schaafsma and Hooker, 2007; Pereyra and Dill-Macky, 2008; Chandelier et al., 2011; Vogelgsang et al., 2011; Landschoot et al., 2012).

Balmas et al. (2000) and Moretti et al. (2002) emphasized that rainy or wet periods between heading and soft dough favor infection and the spread of FHB disease. Hooker et al. (2002) highlighted the importance of the climatic trend close to flowering, also through the development of a model in which the weather conditions in this period have been proved to influence the variations in the concentration of DON by 73%. A wide range of variation in the presence of DON was also shown by several surveys of mycotoxins in wheat, and wheat-based foods carried out in northern Italy (Delogu et al., 2005; Pascale et al., 2001; Blandino et al., 2006). Changes in climate may influence predisposition of hosts to contamination by altering crop development and by affecting insects that create wounds on which mycotoxin-producers proliferate (Cotty & Jaime-Garcia, 2007). Furthermore, DON

content is related to the virulence of fungal isolates (Hestbjerg et al., 2002; Mesterházy, 2002) and influenced by the resistance of the host (Mesterházy, 2002; Miedaner et al., 2003; Wilde & Miedaner, 2006; van der Burgt et al., 2011).

Fusarium fungi and head blight have emerged in Finland after rainy and humid summer weather conditions. During the 1960s and 1970s, the spectrum of *Fusarium* species and the ability of these fungi to produce mycotoxins in domestic grain were subject to extensive investigation. *Fusarium* fungi were found to impair the development of cereal grains, reduce the germinative capacity, cause stem base diseases and ear fusariosis. At the beginning of the 1980s in Finland, the National Veterinary and Food Research Institute (EELA, presently the Finnish Food Safety Authority Evira) actively invested in mycotoxin research and development of methods for chromatographic determination of mycotoxin concentrations. The summer of 1987 was again very rainy and cold, and there was the abundant and even visible occurrence of *Fusarium* head blight in grains. At this time, the study of toxin concentrations was intensified further. From 1987, MTT Agrifood Research Finland (presently Natural Resources Institute Finland (LUKE)) started to put considerable effort into the analysis of mycotoxins.

A decade passed, and after a warm and dry summer in 1997, another very rainy and cold summer was encountered in 1998. Fortunately, the nationally supported research on mycotoxins in grain, and valuable reference material obtained at the end of the 1980s, had already taken strong steps toward a better understanding of the relationship between climate and mycotoxin levels. Surprisingly, the research results indicated that mycotoxin levels were not strongly elevated in 1998. The linear correlation between wet weather conditions and high mycotoxin levels was found to be poor. More mycotoxins were produced in 1997 and 1999 than in 1998 despite the fact that mould fungi were present in abundance in grains in 1998. It was stated that problems also occur during dry and warm summers. It was furthermore noted that particular attention should be paid to the mycotoxin levels in cereal grains and especially in oats. The conclusions were, that without thorough research and monitoring, *Fusarium* fungi and mycotoxins will inevitably continue to cause stem base diseases in cereals, impair the development of grains, lower crop yields and economic productivity. Mycotoxins may ultimately put the safe use of food-grade or feed grain at risk.

Current research indicates that the management of mycotoxin risk will eventually secure the well-being of consumers and animals as well as the confidence in safe and healthy domestic grain raw material and cereal products. While acute poisonings due to mycotoxins are rare, prolonged exposure is harmful. The following adverse effects of *Fusarium* mycotoxins have been observed in humans: nausea, central nervous system symptoms and disturbances in heart function (Champeil et al., 2004a). In animal experiments, the toxin DON has been shown upon single dosage to cause lack of appetite and vomiting. Upon prolonged exposure, *Fusarium* toxins cause reduced yield, impairment of resistance and fertility disturbances to animals. Zearalenone is a hormone-like compound that can cause abortions, especially in pigs (Landschoot et al., 2013).

In the literature review following, the risk of *Fusarium* fungi, control and management of the mycotoxin risk pre-harvest and post-harvest will be discussed in more detail. Also, mycotoxins may generate technological problems, such as adverse effects on baking quality, malting of beer, and on fermentation (Prange et al., 2005). In the experimental part, the results of up-dated survey of *Fusarium* species and toxins in Finnish cereal grains during 1987-2014 are described. In the same part, different procedures have been described how *Fusarium* toxins in cereals can be prevented and manage regarding various agronomic factors and modeling.

2 THE RISK OF FUSARIUM HEAD (OR EAR) BLIGHT AND THE TOXINS PRODUCED BY FUNGI

Fusarium Head Blight is a devastating disease affecting a broad range of small grain cereals. FHB remains endemic in Europe and North America. According to McKay (1957), a severe head blight outbreak in Ireland in 1942, decreased yield in wheat by between 21% and 55%. The second outbreak happened 1954 and the yield reductions in wheat and oat crops were up to 50%. Results of a large-scale field survey of wheat crops in the Atlantic Provinces of Canada during 1980 (Martin and Johnston, 1982) found that FHB was responsible for between 30% and 70% yield loss. For example, tolerance levels in Canada for Fusarium-damaged kernels (FDK) are very low due to processing problems and potential food safety concerns. For example, FDK greater than 0.25% by weight downgrades the Canada Western Red Spring (CWRS) class of wheat from CWRS #1 to CWRS #2. An FDK value of over 1% downgrades it to CWRS #3, and over 2% to CWRS #4 (Canadian Grain Commission, 2007). For malting barley, the tolerance for FDK is nil for Super Select and 0.2% for Select, whereas FDK for feed barley is 1%. These low tolerance levels represent significant economic losses to producers in affected areas (Fernandez et al., 2009).

Pirgozliev et al. (2003) reported that Fusarium head blight epidemics in wheat and barley occurred in southern Idaho in 1982 and 1984. The yield losses were estimated to be as high as 50% (Michuta-Grimm and Foster, 1989). According to Saylor (1998) and Windels (2000), in several US states in the 1990s, a lot of grain was lost, equivalent to 2.6–3 billion dollars. Hard red spring wheat crops were the worst affected with ca. 52% production losses, while soft red wheat and durum wheat experienced 38% and 10% production losses, respectively.

In China, the largest area affected by FHB during 1951–1985 was located in the mid and lower regions of the Yangtze River valley. Research carried out during the period recorded 19 FHB outbreaks in cereal grains. Grain yields of wheat were reduced by 5–15% in years when moderate epidemics of FHB were registered and up to 40% in years when disease outbreaks were severe (Zhuping, 1994). During head blight epidemics in the Northern Argentinean Pampas areas, yield losses between 10% and 50% were recorded (Moschini et al., 2001; Pirgozliev et al. 2003). Surveys in the 2000s on the occurrence of DON in cereals collected in Italy have indicated widespread DON contamination in wheat samples grown in northern and central Italy with high incidence. The highest concentrations of DON reported up to 15 mg/kg (Lops et al., 1998; Pascale et al., 2000; Pascale et al., 2002; Haidukowski et al., 2005).

In Europe, the incidence of the disease and mycotoxin production appears to have increased during 1985–2015 (Pettersson, 1991; Parry et al., 1995; Salas et al., 1999; Edwards et al., 2009, 2009a, 2009b, 2009c; Hietaniemi et al., 2016). The increased occurrence of FHB may be mainly explained by changing climatic conditions, which favoured infection of cereals (Bai and Shaner, 1994; van der Fels-Klerx et al., 2012; van der Fels-Klerx et al., 2012; Olesen et al., 2012). Nevertheless, it seems that the increase in the

crop areas of wheat, barley, and maize associated with short rotations and with agriculture practices that favour retention of crop residues have increased the survival of the FHB agents (Ioos et al., 2005).

FHB affects the nutritional, baking and malting qualities of cereal grains (Mielke and Meyer, 1990; Dexter et al., 1997; Ioos et al., 2005). Bechtel et al. (1985) found that *F. graminearum* was capable of destroying starch granules, storage proteins and cell walls during the invasion of wheat grains. Dexter et al. (1997) showed that Canadian hard red spring wheat grain samples that contained *Fusarium*-damaged grains exhibited weak dough properties and poor baking quality. When Nightingale et al. (1999) studied the effects of fungal proteases on wheat storage proteins, they found that *F. graminearum* and *F. avenaceum* may produce proteolytic enzymes. These enzymes hydrolyse endosperm proteins during dough mixing and fermentation. The result is a weaker dough and decreased loaf volume. In barley, infection of grains with *Fusarium* spp. reduces malt quality and yield. *Fusarium* infection in malt cause uncontrolled foaming of beer during the malting process (Narziss et al., 1990; Schwarz et al., 2001, 2002; Pirgozliev et al., 2003).

A better understanding of the factors affecting pathogen inoculum, crop infection and production of mycotoxins is critical for devising highly effective strategies to reduce inoculum levels, disease development, and mycotoxins produced by them (Fernandez et al., 2009). However, none of these management methods, such as host resistance, variety, crop rotation, tillage, and fungicide application used alone has been entirely valid (Paul et al., 2008; Hooker et al., 2002).

2.1 The nature of *Fusarium* head blight

Wagacha & Muthomi (2007) reported that the genus *Fusarium* contains over 20 species (De Hoog et al., 2000). According to the latest publications (Gräfenhan et al., 2011; O'Donnell et al., 2012), trichothecene-producing *Fusarium* species form a well-supported clade which is more closely related to trichothecene-nonproducing *F. avenaceum*/*F. arthrosporioides*/*F. tricinctum*/*F. acuminatum*/*F. torulosum* species complex (Yli-Mattila et al., 2006) rather than that to fumonisin-producing *Fusarium* species. Within the trichothecene-producing clade the most important species complexes are DON/NIV-producing *F. graminearum* species complex and T-2/HT-2-toxin producing *F. sporotrichioides*-*F. langsethiae* species complex (Yli-Mattila & Gackaeva, 2016).

Several taxonomic manuals and keys for identification of *Fusarium* fungi have been developed. For example, Wollenweber and Reinking (1935), Snyder and Hansen (1940, 1941, 1945), Booth (1971), Gerlach and Nirenberg (1982), Nelson et al. (1983) and Marasas et al. (1984) have developed such keys and manuals. According to Wagacha & Muthomi (2007) identification of *Fusarium* species is difficult due to significant variation in morphological and nonmorphological characteristics. Separation of *Fusarium* species is based on primary and secondary characteristics. Primary characteristics include presence or absence of microconidia and their shape, whether or not microconidia are borne in chains,

the shape of the macroconidia and the type of micro conidiophores (Windels, 1991). As a rule DON-producing species are able to produce only macroconidia (*Fusarium graminearum* species complex (FGSC), *F. culmorum*), while the T-2/HT-2 toxin-producing species form a large number of microconidia (*F. sporotrichioides*, *F. langsethiae* and *F. sibiricum*). The main nivalenol (NIV)-producing species (*F. poae*) forms many microconidia and only very rarely a few macroconidia, whereas other NIV-producing species (*F. cerealis* and NIV-producing chemotypes of *F. graminearum* and *F. culmorum*) form only macroconidia. Macroconidia-forming species are also more aggressive pathogens than those species, which mainly produce microconidia (Jestoi et al., 2008; Imathiu et al., 2010; Divon et al., 2012).

Secondary characteristics include presence or absence of chlamyospores and their configuration and position and presence or absence of sclerotia or sporodochia (Wagacha and Muthomi, 2007). An initial inoculum may result in the infection of seedlings, resulting in the development of seedling blight and root rot. Later, during anthesis and the early seed development period, airborne conidia or ascospores may infect the ears of cereal plants, and consequently, these results in the development of FHB (Nelson et al., 1983; Burgess et al., 1988; Xu, 2003; Wagacha and Muthomi, 2007).

According to Landschoot et al. (2013), *Fusarium* head blight is a disease complex that means that it may be caused by individual species or a combination of related species. Warm and humid weather, frequent rainfall and heavy dew favour spore germination (Xu, 2003) and ear infection (Wagacha and Muthomi, 2007). Moschini et al. (2001), Schaafsma et al. (2001), Hooker et al. (2002) and Klem et al. (2007) observed a significant correlation between weather conditions during anthesis on FHB incidence and mycotoxin content at harvest. Intense rainfall during the period of anthesis disperses *Fusarium* inoculum from crop residues and promotes FHB infection. However, considerable infection is still possible at the milky growth stage and prolonged periods of warm, humid conditions are conducive for mould growth and eventually secondary infections (Parry et al., 1995; Hooker et al., 2002; Wagacha and Muthomi, 2007). Furthermore, also during the vegetative growth stage weather conditions contribute to disease pressure (Kriss et al., 2010; Landschoot et al., 2012; Landschoot et al., 2013).

Wagacha and Muthomi (2007) reported that wheat, maize, barley and oats residues have long been noted as the primary sources of inoculum in FHB epidemics. *F. culmorum* and *F. graminearum* are the most significant and widespread agents of FHB in cereal grains in Europe, Canada, USA, and almost worldwide. *F. culmorum* and *F. graminearum* belong to the section discolour (Nelson et al., 1983). Members of the discolour section are often referred to as the cereal fusaria (Booth, 1975). Based on the review paper by Wagacha and Muthomi (2007), cereal fusaria do not form microconidia, except under certain cultural conditions, and are distinguished by the morphology of the macroconidia. Macroconidia are comparatively thick-walled, distinctly septate, fusiform to falcate with a beaked or fusoid apical cell. Chlamyospores are usually present and may form either from hyphae or the cells of the macroconidia. On potato dextrose agar, the growth of chlamyospores is rapid,

with dense aerial mycelium. The mycelium is white but often yellow to tan. Orange to red-brown sporodochia appears as the culture ages. The underside is carmine red (Figure 1). The fungus is relatively stable in culture, but mutants may occur (Wagacha and Muthomi, 2007).

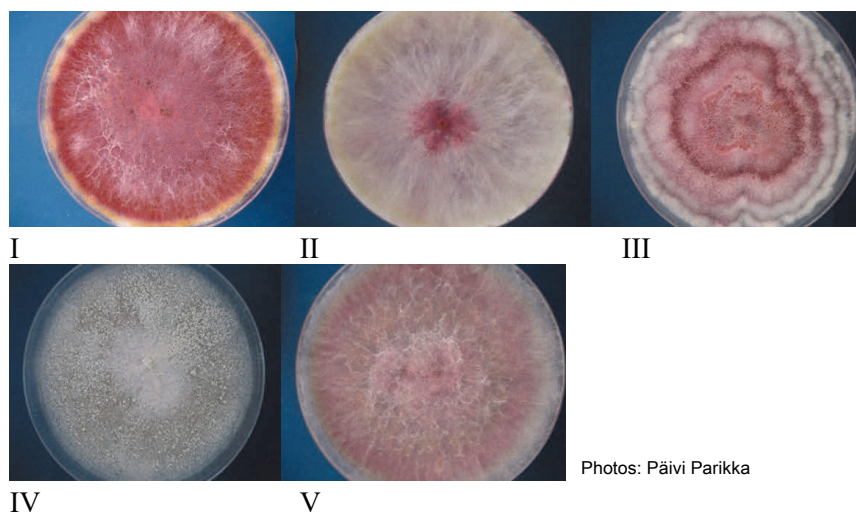


Figure 1. The growth of *F. culmorum* (I), *F. graminearum* (II), *F. avenaceum* (III), *F. langsethiae* (IV) and *F. sporotrichioides* (V) on potato dextrose agar (PDA) medium in dark.

Under suitable environmental conditions, *F. culmorum* is capable of causing severe disease and damage for growing crop (Lacey et al., 1999). The pathogen is a soil-inhabiting fungus that is a competitive saprophyte and facultative parasite. According to Wagacha and Muthomi (2007) its populations in wheat field soil have been shown to vary greatly during the season, increasing greatly in dry conditions that favour its pathogenic activity on stem bases (Goswami and Kistler, 2004; Bateman and Murray, 2001; Bateman et al., 1998; Vigier et al., 1997; Wagacha and Muthomi, 2007). Also, Magan and Lacey (1984) found that of a range of field fungi, *F. culmorum* was the only one able to compete with and dominate other fungi, particularly at a high water activity ($a_w > 0.95$). On the other hand, *F. graminearum* appears to have a competitive advantage over other species under cooler conditions (Marin et al., 1998b; Velluti et al., 2000). Marin et al. (1998b) suggested that *F. graminearum* has a competitive advantage over *F. moniliforme* and *F. proliferatum* at 15 °C, while at 25–30 °C these species coexisted in the same niche (Doohan et al., 2003).

F. graminearum has an additional epidemiological advantage because it regularly forms abundant perithecia (*Gibberella zeae*), resulting in production of ascospores. Ascospores are forcibly discharged into the air, which significantly increases the dispersal distance from the colonized residue where perithecia form. The discharge of inoculum is triggered by a drop in air temperature accompanied by a rise in relative humidity (Paulitz and Seaman, 1994;

Paulitz, 1996). Ascospore release occurs over a range of temperatures (10–30 °C) and this explains why the optimal temperature observed for ascospore dispersal was 16 °C (Sutton, 1982). However, ascospore release is inhibited by rain or continuously high relative humidity (>80%), and Gilbert and Tekauz (2000) postulated that there is a threshold humidity beyond which release slows or stops (Doohan et al. 2003). Ascospores and conidia cause significant infections (Fernando et al., 1997; Scholz and Steffenson, 2001; Xu, 2003).

Unlike *F. graminearum*, *F. culmorum* is not known to produce ascospores. *F. culmorum* produces asexual spores (conidia), which are the primary mode of dispersal. The conidia are dispersed onto the cereals heads either by rain splash or wind (Fernando et al., 1997; Jenkinson and Parry, 1994; Wagacha and Muthomi, 2007). According to Horberg (2002), laboratory research showed that the maximum dispersal height and distance of *F. culmorum* and *F. poae* are 60 cm and 70 cm, respectively (Horberg, 2002). Furthermore, the splash dispersal patterns are indistinguishable for the two species. Thus, only a slight proportion of conidia on the crop debris at soil level can reach the ears by rain splash. It has been speculated that symptomless infections on green leaves, such as the flag leaf, may provide an important bridge between conidia on crop debris and ears. Isolation of apparently healthy and diseased leaves indicated that *Fusarium* species associated with FHB survive parasitically and saprophytically on leaves throughout the season (Ali and Francl, 2001; Xu, 2003).

Sutton (1982) showed that *F. graminearum* inoculum is formed under warm rather than cool conditions. In the case of sexual reproduction, the optimal temperatures for *F. graminearum* perithecial and ascospore production were 29 and 25–28 °C at high water activity, respectively. According to Xu (2003) minimum and optimum temperatures for ascospore production are about 7–10 and 15–20 °C, respectively. Ascospore production appears to be critically dependent on soil moisture, too. When the soil moisture content is below 30%, ascospore production is not possible. When it is greater than 80%, ascospore production is at its maximum.

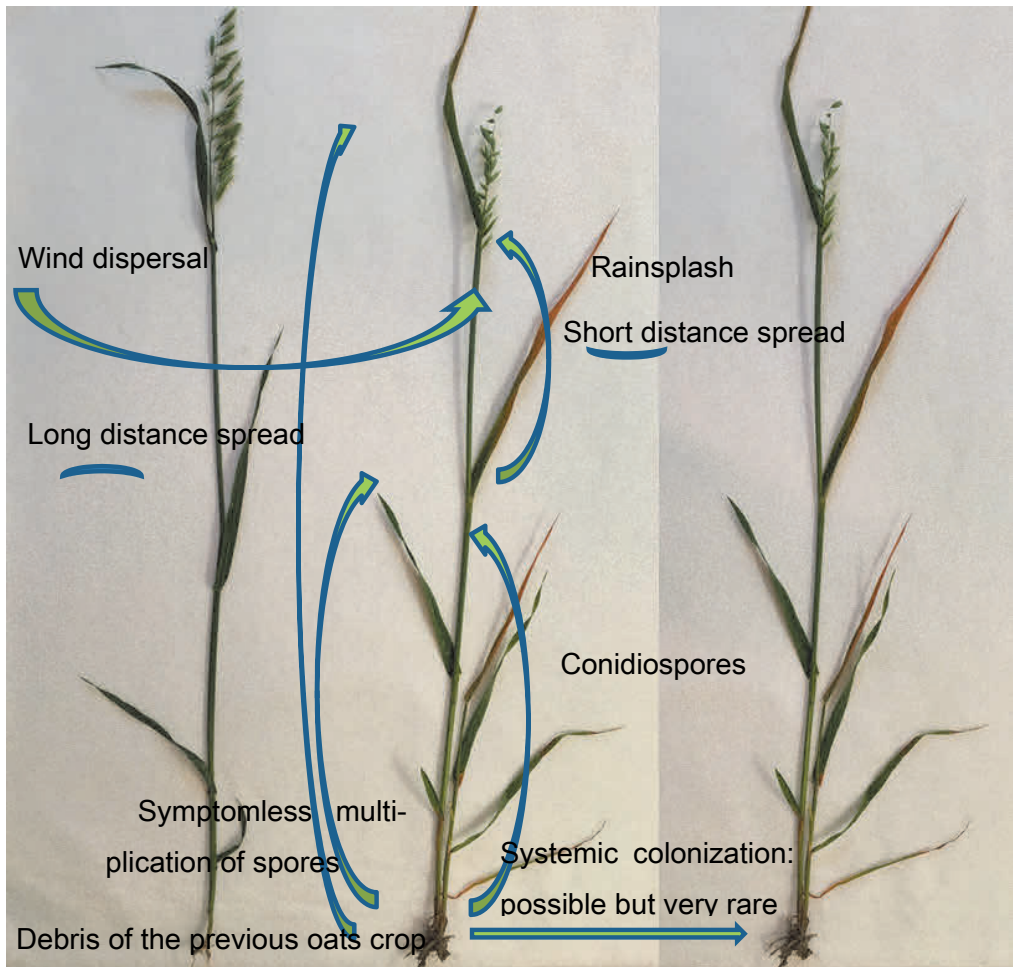


Photo: Jerry Hietaniemi

Figure 2. Description of the epidemiology of oats head blight caused by *Fusarium graminearum*.

Macroconidia of *F. graminearum* are produced at an optimal temperature of 28–32 °C and their production are severely inhibited below 16 and above 36 °C (Tschanz et al., 1976). On wheat spikelets, Andersen (1948) showed that millions of conidia of *F. graminearum* were produced on moist wheat heads at 20–30 °C, and lesser numbers at 15 °C. Macroconidia appeared within five days at 20 °C and within three days at 25–30 °C. Exposure of spikelets to moisture reduced conidial formation time to 1–2 days, with conidial numbers increasing with increasing humidity (Figure 2). Inoculum increased during rainy periods but the timing of this increase was variable. These results suggest that while rainfall may be needed for perithecial and ascospore formation and maturation, it may not trigger the release of ascospores (Xu, 2003).

According to Yli-Mattila & Gagkaeva (2016), the type A trichothecene-producing species can be divided into two main groups, which are visible in phylogenetic trees (Proctor et al., 2009; Yli-Mattila et al., 2011a). The first group consists of species (*F. sporotrichioides*, *F. sibiricum*, *F. langsethiae* and *F. armeniacum*), which are able to produce large amounts of T-2 and HT-2 toxins. The rest of the type A trichothecene-producing species (*F. poae*, *F. kyushuense*, *F. venenatum* and *F. sambucinum*) are not able to produce large amounts of T-2 and HT-2 toxins. Also interesting is that Burkin et al. (2008) recently identified a new *Fusarium* species among the *Fusarium* fungi isolates originating from Siberia and morphologically resembling *F. poae* isolates which produce high amounts of T-2 toxin. After detailed investigations these isolates were described as a new species, *F. sibiricum* (Yli-Mattila et al., 2011a), which produces high levels of T-2 and HT-2 toxins. The identification is based on molecular, morphological and metabolite characters, but it is difficult to identify *F. poae*, *F. langsethiae* and *F. sibiricum* based on only morphological characters.

According to Osborne and Stein (2007), the causal agents of FHB survive over winter as mycelia in non-decomposed, infected crop residues from the previous year. The following production of ascospores in the perithecia locates on the surface of the pathogen-infested residues. The soil itself and contaminated seeds are also sources of inoculum. However, their role in the production of inoculum is not as significant as with residues (Khongla and Sutton, 1988). Residues of soybean, sunflower, grasses, pasture plants, and some broad-leaved weeds have been reported to be sources of inoculum for *Fusarium* spp., too (Dill-Macky and Salas, 2001; Pereyra and Dill-Macky, 2008). According to Dill-Macky and Jones (2000), it is also strongly supported in many studies that if wheat is grown on the same field in back-to-back years, the risk to FHB and production of mycotoxins in the grain is greater. These pathogens survive longer on residues such as stem nodes, which do not degrade quickly (Blandino et al., 2010; Xu, 2003; Pereyra and Dill-Macky, 2008; Fernandez et al., 2009; Liu et al., 2011; Audenaert et al., 2013).

In northern and in southern Europe, a FHB is typically dominated by the DON-producing species *F. culmorum* and *F. graminearum*, respectively. However, *F. graminearum* is steadily spreading northwards. The distribution of T-2+HT-2-producing species such as *F. sporotrichioides* and *F. langsethiae* is largely limited to Europe, although this may, in part, be due to the lack of analysis in other regions of the world. Other species implicated are *Fusarium poae* and *Fusarium avenaceum* which both produce toxins and related *Microdochium nivale* varieties which do not produce toxins (Sutton, 1982; Magan et al., 2002; Wagacha and Muthomi, 2007).

Wagacha and Muthomi (2007) reported that *F. culmorum* has been identified as the most important species in western Germany (Muthomi et al., 2000) and in the Rhineland region of Germany together with *F. avenaceum* (Lieneman, 2002). Based on the studies by Kosiak et al. (2003) *F. culmorum* was among the four most frequently isolated *Fusarium* spp. from wheat, barley, and oats in Norway. Others were *F. avenaceum*, *F. poae* and *F. tricinctum*. A study by Clear and Patrick (2006) ranked *F. culmorum*, besides *F. graminearum* and *F.*

avenaceum, among the three most dominant *Fusarium* species in cereal grains in Canada. However, in the new Millennium studies in some countries where *F. culmorum* has been previously predominant have reported *F. graminearum* to be predominating (Jennings et al., 2004a,b; Hietaniemi et al., 2016). Also, Waalwijk et al. (2003) reported replacement of *F. culmorum* by *F. graminearum* as the predominant trichothecene-producing ear blight pathogen in the Netherlands. *F. graminearum* has also been since 2000 more commonly on wheat grain in Germany (Obst and Fuchs, 2000) where harmful levels of deoxynivalenol (DON) have been reported (Placinta et al., 1999; Wagacha and Muthoni, 2007). Shah et al. (2005) found *F. graminearum* to be the most important species in Italy during 1999-2002. This trend could be indicative of a change in warmer weather, a genetic change in the *F. graminearum* population or changes in cropping practices (Bateman, 2005). The trend could also reflect a changing trend of the dominance of *Fusarium* spp. in different parts of the world (Wagacha and Muthomi, 2007).

Species related to *F. sporotrichioides* produce T-2 and HT-2 toxins. New T-2/HT-2 toxin-producing *Fusarium* species (*F. langsethiae* and *F. sibiricum*) have been recently found in northern Europe and Asia (Yli-Mattila et al. 2011) and a similar isolate was also found in Iran (Kachuei et al., 2009). *F. langsethiae* is mainly distributed in Europe, while *F. sibiricum* is mainly distributed in Siberia and Russian Far East (Yli-Mattila et al., 2011). *F. poae* is an intermediate between trichothecene type A and B producers. It can produce in vitro DAS, which is a type A trichothecene, but it is also the main NIV-producer in northern Europe (Yli-Mattila et al., 2008; Pettersson, 1991; Yli-Mattila et al., 2004) and northern Japan (Sugiura et al., 1993). NIV, unlike DON, occurs more frequently after dry and warm growing seasons (Pettersson et al., 1995).

The role and importance of mycotoxins in overall fungal metabolism has not yet been fully elucidated, but an increasing amount of evidence supports a recently proposed central “oxidative stress theory for mycotoxin biosynthesis” (Reverberi et al., 2010). Indeed, recent studies have indicated that the fungal response to oxidative stress is dependent on whether a DON-chemotype is present (Ponts et al., 2009), and have shown increased toxin production to be a response to oxidative stress (Audenaert et al., 2010). Also, it is known that DON acts as a virulence factor and is imperative in the spread of *F. graminearum* after initial infection of the wheat plant (Maier et al., 2006). The fungus interferes with the plant defense system at multiple levels, and it has been shown that DON hijacks the pathways leading to the typical oxidative burst and programmed cell death. Also, polyamines synthesized by the plant during its interaction with *F. graminearum* induce trichothecene biosynthesis (Desmond et al., 2008; Gardiner D. et al., 2010; Audenaert et al., 2013).

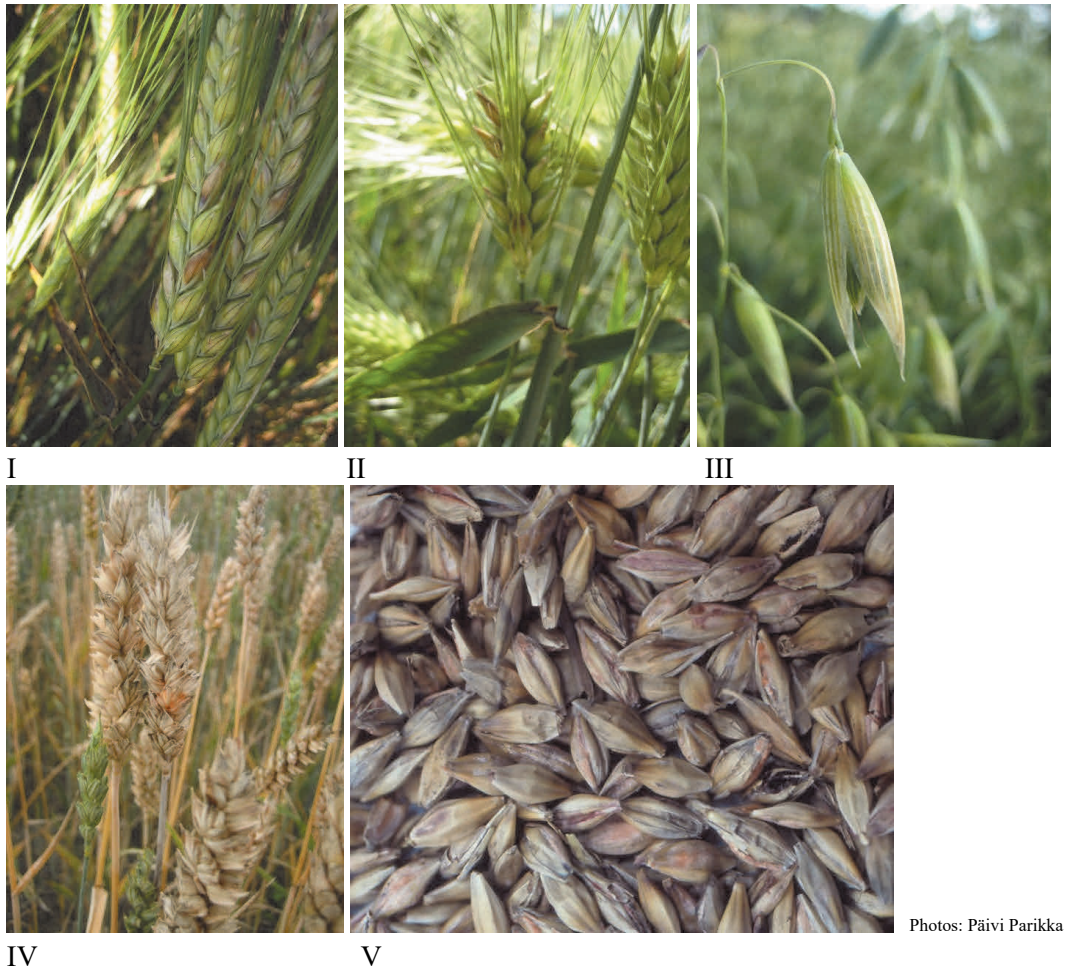
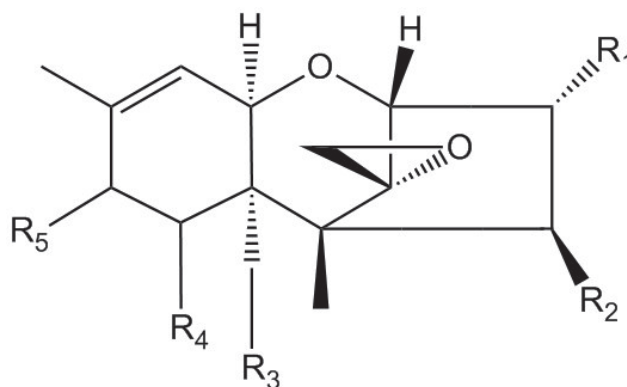


Figure 3. FHB infestation I-V: The development of symptoms in spikelets, brown colour of kernels, reddish mould; symptoms are more visible in the case of barley and wheat than oats.

2.2 Chemistry of *Fusarium* Toxins

Fusarium toxin production in grain begins in the field and can continue throughout storage. The most important classes of *Fusarium* mycotoxins, based on their harmful effects on human and animal health, are the trichothecenes, fumonisins, moniliformin and zearalenone (ZON) (D’Mello et al., 1999). Trichothecenes are the largest group of mycotoxins. Chemically, trichothecenes are a large group of sesquiterpene epoxides and are characterized by the presence (type B trichothecenes) or absence (type A trichothecenes) of a keto group at the C-8 position (Figure 4). The trichothecenes, including deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), nivalenol (NIV), fusarenone X (F-X), diacetoxyscirpenol (DAS), T-2 and HT-2 toxins are common mycotoxins of cereals (Magan and Olsen, 2004; Jennings et al., 2000) and occur

naturally worldwide on cereal grains (Dalcero et al., 1997; Müller et al., 1997; Park et al., 1996; Ryu et al., 1996; Kim et al., 1993; Fujisawa et al., 1992; Abbas et al., 1988). Consumption of these toxins is a real problem for humans and farm animals (Eriksen and Alexander, 1998; Rotter et al., 1996; Wagacha & Muthomi, 2007).



Trichothecene		R1	R2	R3	R4	R5
Type A	T-2 toxin	OH	OAc	OAc	H	OCOi-Bu
	HT-2 toxin	OH	OH	OAc	H	OCOi-Bu
	Diacetoxyscirpenol	OH	OAc	OAc	H	H
Type B	Deoxynivalenol (DON)	OH	H	OH	OH	=O
	3-AcDON	OAc	H	OH	OH	=O
	15-AcDON	OH	H	OAc	OH	=O
	Nivalenol	OH	OH	OH	OH	=O
	Fusarenon-X	OH	OAc	OH	OH	=O

Figure 4. Molecular structures of trichothecenes A and B type.

ZON (Figure 5), also known as F-2 toxin, 6-(10-hydroxy-6-oxo-trans-1-undeceny)- β -resorcylic acid lactone, is a very heat-stable compound, despite its large lactone ring (Ryu et al., 1999). ZON is estrogenic, and the action on the hypothalamus and pituitary glands appears to be the same as estrogen (Cheeke & Shull, 1985; Martins & Martins, 2002; Doohan et al., 2003).

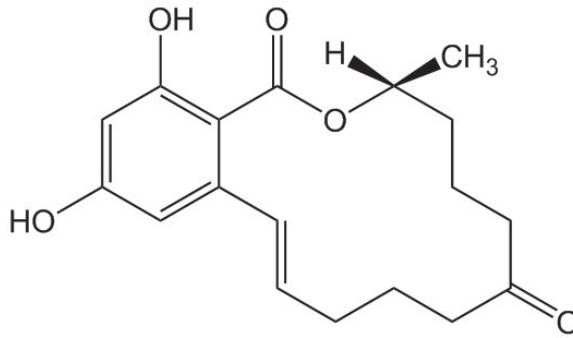


Figure 5. Molecular structure of zearalenone.

F. graminearum, *F. culmorum*, *F. poae*, *F. oxysporum*, *F. sporotrichioides*, *F. langsethiae*, *F. sibiricum* and *F. equiseti* are producers of trichothecenes and ZON (D’Mello and Macdonald, 1997; D’Mello et al., 1999, Yli-Mattila et al., 2011). *F. sporotrichioides* and *F. langsethiae* predominately produce type A trichothecenes, which includes the T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol. *F. culmorum*, *F. graminearum* and *F. poae* predominately produce type B trichothecenes, including DON (also known as vomitoxin), its derivatives 3-AcDON and 15-AcDON and nivalenol (Table 1).

Table 1. The major *Fusarium* toxin producers, classes of *Fusarium* mycotoxin and optimal production conditions in cereal grains (Doohan et al., 2003).

Species	Matrix	Toxin	Optimum production conditions	References
<i>F. culmorum</i> , <i>F. graminearum</i>	Oats, barley, wheat, maize, rice	Type B trichothecenes: DON, 3-AcDON, 15-AcDON, NIV	Warm and humid (25-28 °C, aw = 0.97)	Greenhalgh et al., 1983; Lori et al., 1990; Beattice et al., 1998; Homdork et al., 2000
<i>F. langsethiae</i> , <i>F. sporotrichioides</i> , <i>F. poae</i>	Oats, barley, wheat, maize, rice	Type A trichothecenes: T-2 toxin, HT-2 toxin, and DAS	Moderately warm and humid (20-25 °C, aw = 0.990)	Rabie et al., 1986; Miller, 1994; Mateo et al., 2002
<i>F. culmorum</i> , <i>F. graminearum</i>	Oats, barley, wheat, maize, rice	ZON	Warm (17-28 °C), or temperature cycles (e.g. 25-28 °C for 14-15 days; 12-15 °C for 20-28 days) and humid (aw = 0.97 or 90 % RH)	Lori et al., 1990; Jimenez et al., 1996; Ryu and Bullerman, 1999; Homdork et al., 2000; Martins & Martins, 2002

Doohan et al. (2003) showed that production of trichothecenes by *F. culmorum* and *F. graminearum* favoured by warm and humid conditions. Hope et al. (2005) showed that growth of *F. culmorum* occurred at water activity (aw) greater than 0.90 while DON production was optimum at 25 °C. Llorens et al. (2004) reported optimum temperature values of 28, 20 and 15 °C for DON, NIV, and 3-AcDON, respectively, for *F. culmorum* and *F. graminearum*. *F. culmorum* produces no DON at below 0.90 aw. According to

Versonder et al. (1982) minimum temperatures for DON production is 11 °C, dependent on the time of incubation. Also, Llorens et al. (2006) reported 20 °C to be the optimum temperature for ZON production for *F. culmorum*. According to Hope and Magan (2003), *F. culmorum* grown on wheat-based media had differential temperature and aw optima for DON and NIV suggesting that production by this fungus may respond differently to temperature and aw stress. For example, *F. culmorum* may produce NIV under sub-optimal conditions for improving competitiveness. However, while it produces less NIV than DON, the former metabolite is more toxic than the latter (Hope et al., 2005). Regarding ZON production by fungi, several studies have found that highest ZON levels were produced in *F. graminearum* and *F. oxysporum*-infected maize at aw 0.97 and by changing the incubation temperatures from 25 to 28 °C for 14–15 days, followed by 12–15 °C for 20–28 days (Jiménez et al., 1996; Ryu and Bullerman, 1999; Martins and Martins, 2002; Doohan et al., 2003; Wagacha & Muthomi, 2007).

The biosynthesis of *Fusarium* trichothecenes has been studied in *F. sporotrichioides*, which produces T-2 toxin, and in *F. culmorum*, which produces DON and 3-AcDON (McCormick, 2003). Several genes involved in the biosynthesis of trichothecenes have been described. Based on the studies of Fekete et al. (1997) and Hohn and Desjardins (1992) the Tri5 gene encodes the trichodiene synthase, which catalyzes the first step in the biosynthesis of trichothecenes. Proctor et al. (1995) reported that the Tri6 gene encodes a protein that regulates the trichothecene biosynthesis genes and has been sequenced in *F. sporotrichioides* (Matsumoto et al., 2004), *F. graminearum* (Matsumoto et al., 2004; Brown et al., 2001; Lee et al., 2001), and *F. cerealis* (Matsumoto et al., 2004). It has been shown for several *Fusarium* species that the Tri5 (Hohn and Desjardins, 1992; Hohn and Beremand, 1989) and Tri6 (Matsumoto et al., 2004) genes were present in single copy. Functioning of Tri13 and Tri7 genes are required for the production of NIV and 4-acetyl-nivalenol, respectively. A study by Chandler et al. (2003) genes Tri13 and Tri7 from the trichothecene biosynthetic gene cluster convert DON to NIV (Tri13) and NIV to 4-acetyl-NIV (Tri7). Mutations have been identified in isolates that can produce DON but are unable to convert it to NIV. In such isolates of *F. culmorum* according to Jennings et al. (2004a), the Tri7 gene is deleted entirely. Presence or absence, as well as the functionality of any of the fore-mentioned genes, determine the final toxin, produced by a particular *Fusarium* species isolate (Hestbjerg et al., 2002; Wagacha & Muthomi, 2007).

The ability of plant cells to metabolize mycotoxins was firstly described for DON in maize suspension culture (Sewald et al., 1992). The modulation of the DON molecule by plant cells is especially based on the glucosylation of DON to DON-3-β-D-glucopyranoside (DON-3G). This glucose conjugate exhibited a dramatically reduced ability to inhibit protein synthesis of wheat ribosomes in vitro (Poppenberger et al., 2003). Interestingly, these glucosylation reactions are not limited to laboratory experiments with plant cell cultures. Recently, an accumulating amount of data demonstrates the occurrence of DON-3G in naturally infected maize and small-grain cereals (Berthiller et al., 2005, 2009; De Boevre et al., 2012; Sasanya et al., 2008; Skrbic et al., 2011). The ratio DON-3G/DON

varied in relation to years and genotypes but reached levels up to 29% (Berthiller et al., 2009) and even to 70% (De Boevre et al., 2012). Berthiller et al. (2009) found DON-3G concentrations up to 1070 µg/kg. It is also known that, after processing, levels of contamination can differ, for example in flour. However, an increasing number of people prefer cereal products that are less or not processed and, hence, it is advisable to further assess the exposure towards these contaminants by considering cereal and cereal-based foods (Audenaert et al., 2013).

In search of an explanation for this actual conversion of toxins to glucosylated derivatives, Miller and Greenhalgh (1988) were among the first to speculate that formation of a less toxic DON conjugate might be responsible for partial FHB resistance of wheat. Lemmens et al. (2005) demonstrated that the ability of wheat lines to convert DON to DON-3G was linked to a quantitative trait locus (QTL), namely Qfhs.ndsu-3B which had previously been reported to be associated with FHB resistance against spreading of *Fusarium* infection and has been designated as “Fhb1” resistance. They hypothesized that this QTL encodes for a DON-glucosyltransferase, or regulates the expression thereof (Audenaert et al., 2013).

In contrast to the overwhelming amount of information on DON-glucosylation, information on other mycotoxins in this regard is limited. Lemmens et al. (2005) stated the possibility that the glucosyltransferase function is only effective against *Fusarium* strains that produce DON or structurally highly similar trichothecenes. This hypothesis would fundamentally change the perception of resistance against FHB in wheat plants, which has always been considered to be species-independent (Parry, Jenkinson & Mcleod, 1995). Lemmens et al. (2008) investigated the situation for nivalenol (NIV) and were not able to conclusively prove a link between Fhb1-mediated glucosylation and plant resistance against *F. graminearum* infection. However, according to Audenaert et al. (2013), it is very attractive to speculate on the importance of DON glucosylation in the field, where plants are met with highly diverse populations of different *Fusarium* species with divergent chemotypes. Interestingly, the toxins for which Fhb1-mediated glucosylation has been investigated, NIV and DON, are both type B trichothecenes (characterized by a carbonyl function at C-8) while several fairly prevalent *Fusarium* species such as *F. poae* also produce type A trichothecenes such as diacetoxyscirpenol and neosolaniol (NEO). Reports on the phytotoxicity and availability for glucosylation of these toxins are very limited to non-existent.

Based on the results of Kimura et al. (2006) 3-O-acetylation of the trichothecene ring is related to self-protection of the producers. In their work they cloned the Tri101 gene encoding trichothecene 3-O-acetyltransferase (Kimura et al., 1998). Although 3-AcDON is still highly toxic to plants (Wakulinski, 1998; Bruins et al., 1993; Wang and Miller, 1988; Eudes et al., 2000) at low concentrations, its toxicity appears to be connected to C-3 deacetylation inside the cell. Thus, transgenic expression of Tri101, which consistently eliminates C-3 deacetylated trichothecenes within plant cells are predicted to protect cereal grains from the phytotoxic effect of trichothecenes and to reduce disease severity.

Phytotoxicity of DON and other trichothecenes has been shown and therefore it makes sense that wheat has evolved a broad array of detoxification processes among which conjugation to probably less (phyto) toxic compounds is the most important. According to Berthiller et al. (2013) the detoxification process and the concomitant formation of ‘masked mycotoxins’ is an emerging health issue as these conjugated forms remain latently present in the plant tissue, ready to be released upon exposure to enzymes in the animal/human digestive system or upon food processing. For example, the accumulation of masked mycotoxins during germination of wheat and barley in the brewing process illustrates this food safety issue (Maul et al., 2012; Audenaert et al., 2013).

2.3 Toxicity of *Fusarium* toxins

If grain contaminated with *Fusarium* toxins is used for human consumption or feed for an animal, a range of adverse toxicosis as well as other health disorders is observed. In the middle of the last century, in Russia, the consumption of food prepared from over-wintered cereals, contaminated with *F. poae* and *F. sporotrichioides*, caused human poisoning known as Alimentary Toxic Aleukia (ATA). Symptoms of ATA include fever, necrotic angina, leukopenia, haemorrhaging and exhaustion of bone marrow (Joffe, 1978; Mirocha, 1984; Beardall & Miller, 1994). Joffe & Yagen (1977) reported that strains of fungi isolated from the grains at the time were later shown to produce T-2 and related toxins. These were toxins of *F. sporotrichioides*, which grows on wet grain left in the field and to some extent on the glumes of small grains (Miller, 1994; Miller et al., 1998).

In China, 53 outbreaks of human food poisoning were associated with scabby and mouldy cereals occurred between 1960 and 1991 (Luo, 1992). Huang (1992) reported that in the Anhui Province of China in 1991, approximately 130,000 people were affected by gastrointestinal disorders, accompanied by abdominal pain, nausea, vomiting, fatigue, and fever. Analysis of eight wheat and two barley samples showed that DON was present in all samples at levels ranging from 0.016 to 51.45 mg/kg. NIV was also determined in all these eight wheat samples and one of the barley samples (0.001–6.93 mg/kg). Furthermore, both barley samples and six wheat samples contained ZON at concentrations of between 0.046 and 0.3 mg/kg (Li et al., 1999). In 1998 and 1999, Li et al. (2002) analyzed wheat samples were taken from harvest crops in the Henan Province of China, from which cases of human toxicosis was reported (Luo et al., 1987). Thirty samples out of the 31 determined (97%) from the Puyang area of this province contained DON, and 21 of them (70%) exceeded the Chinese advisory limit of 1 mg/DON kg grain (Pirgozliev et al., 2003). Similarly, in India in 1987, a gastrointestinal disorder outbreak in the Kashmir Valley was associated with the ingestion of *Fusarium* mycotoxins (Bhat et al., 1989).

Rotter et al. (1995, 1996) stated that the effect of DON-contaminated feed grain is dependent on the animals involved and on the severity and time of exposure to contaminated grain. According to Trenholm et al. (1984), pigs show the greatest sensitivity to DON, while ruminants and poultry appear to show higher tolerance to the toxin (Mirocha

& Christensen, 1974). The oestrogenic compound ZON causes various reproductive disorders in young pigs ranging from vulva vaginitis and vaginal prolapses to enlargement of the uterus and atrophy of the ovaries (Mirocha et al., 1971). Consumption of feed grain contaminated with ZON by pregnant sows resulted in an increase in stillborn pigs and small litters (Miller et al., 1973). T-2 toxin reduces feed consumption and weight gain in chickens due to severe oral lesions (Kubena et al., 1994). The toxin has also been connected to coagulopathy (Doerr et al., 1981) and altered feathering (Wyatt et al., 1975; Pirgozliev et al., 2003).

Mycotoxins will cause weakened performance, sickness or even death in humans and animals when ingested, inhaled or absorbed through the skin (Wagacha et al., 2008 and Capriotti et al., 2010). Due to toxic effects on humans and animals, the risk assessment of mycotoxins is of high relevance (Kuiper-Goodman, 2000). The International Agency for Research on Cancer (IARC) has classified aflatoxins (AFs) as carcinogenic to humans, while ochratoxin A (OTA) and fumonisin B (FB) were classified as possibly carcinogenic. Trichothecenes and zearalenone were classified as non-carcinogenic but cause other adverse effects (IARC). Factors concerning mycotoxin contamination of food and feed worldwide include environmental, socio-economic and food production. Environmental conditions especially high humidity and variations in temperature favour fungal proliferation resulting in contamination of food and feed. According to Wagacha et al. (2008), the socio-economic status of the majority of inhabitants of sub-Saharan Africa predisposes them to consumption of mycotoxin-contaminated products either directly or at various points in the food chain.

The resulting implications include immune-suppression, impaired growth, various cancers, and death depending on the type, period and amount of exposure. According to Richard (2007), most of these diseases occur after consumption of mycotoxin-contaminated cereal grain or products made from such grains. However, other routes of exposure exist, too, for example via air. The diagnosis of mycotoxicoses may prove to be difficult because of the similarity of signs of disease to those caused by other agents. Therefore, diagnosis of mycotoxicoses is dependent upon adequate testing for mycotoxins involving sampling, sample preparation, and analysis.

2.4 Regulation

Various scientific factors such as the availability of toxicological data, survey data, knowledge about the distribution of mycotoxins in commodities, and analytical methodology play roles in the decision-making process of setting limits for mycotoxins (Reiter et al., 2009). Economic and political factors such as commercial interests and sufficiency of the food supply have their effects as well (van Egmond HP, 2002). The European Union (http://europa.eu/legislation_summaries/foodsafety/contamination_environmental_factors/121290_en.htm) has established maximum residue levels (MRL) for DON and ZON in unprocessed cereals (Commission Regulation (EC) No 1881/2006). MRL for DON in unprocessed oats, durum wheat, and maize is 1750 µg/kg and in other cereal

1250 µg/kg. MRL for ZON in maize is 200 µg/kg and in other cereals 100 µg/kg. On 27 March 2013, the European Commission issued a recommendation concerning the occurrence of T-2 and HT-2 toxins in grain and grain products. The highest permitted levels for unprocessed grains are T-2 and HT-2 toxins (summed value), 1 000 µg/kg for oats, 200 µg/kg for barley, and 100 µg/kg for wheat and rye. The limit values apply to all food-grade grain sold in the food industry.

Guidance values (mg/kg) of DON for products intended for animal feed relative to a feedingstuff with a moisture content of 12 % are as follows (Commission Recommendation 2006/576/EC): Cereals and cereal products with the exception of maize by-products 8 mg/kg; maize by-products 12 mg/kg; complementary and complete feedingstuffs 5 mg/kg with the exception of complementary and complete feedingstuffs for pigs 0.9 mg/kg and complementary and complete feedingstuffs for calves (< 4 months), lambs and kids 2 mg/kg. Guidance values for ZON in feed materials are as follows: Cereals and cereal products with the exception of maize by-products 2 mg/kg; maize by-products 3 mg/kg; complementary and complete feedingstuffs for piglets and gilts (young sows) 0.1 mg/kg; complementary and complete feedingstuffs for sows and fattening pigs 0.25 mg/kg; complementary and complete feedingstuffs for calves, dairy cattle, sheep (including lamb) and goats (including kids) 0.5 mg/kg. Throughout the food and feed analysis chain, methods have to be precise and reproducible (Capriotti et al., 2010).

According to Commission Recommendation 2006/576/EC, maximum levels of *Fusarium* toxins should be set for unprocessed cereals placed on the market for first-stage processing. Cleaning, sorting and drying procedures are not considered as first-stage processing in so far as no physical action is exerted on the grain kernel itself. Scouring is to be considered as first-stage processing. Since the degree to which *Fusarium* toxins in unprocessed cereals are removed by cleaning and processing may vary, it is appropriate to set maximum levels for final consumer cereal products as well as for major food ingredients derived from cereals to have enforceable legislation in the interest of ensuring public health protection.

Tolerable daily intake (TDI) and temporary tolerable daily intake (t-TDI) values are provided for DON (TDI = 1 µg/kg b.w.), NIV (t-TDI = 0.7 µg/kg b.w.), T-2 and HT-2 toxin (combined t-TDI = 0.06 µg/kg b.w.), and ZON (0.2 µg/kg b.w.) (EFSA Journal, 2013a; EFSA Journal, 2013b; EFSA Journal, 2011a; EFSA Journal, 2011b). In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) extended TDI for DON to the group of DON and its acetyl derivatives 3-AcDON and 15-AcDON and also derived an Acute Reference Dose (ARfD) at 8 µg/kg b.w. This DON group TDI value protects the human population from an unacceptable exposure to 3-acetyl deoxynivalenol and 15-acetyl deoxynivalenol. The same applies to nivalenol for which to some extent cooccurrence with deoxynivalenol can also be observed. Regarding, other trichothecenes, such as fusarenon-X, T2-triol, and diacetoxyscirpenol, the limited information available indicates that they do not occur widely and the levels found are generally low.

Commission Recommendation 2006/583/EC of 17 August 2006 on the prevention and reduction of *Fusarium* toxins in cereals and cereal products contains general principles for the prevention and reduction of *Fusarium* toxin contamination (trichothecenes, zearalenone, and fumonisins) in cereals to be implemented by the development of national codes of practice based on these principles. Climatic conditions during the growing season, in particular at flowering, have a major effect on the *Fusarium* toxin content. However, Good Agricultural Practices (GAP), whereby the risk factors are minimized, can prevent, to a certain degree, the contamination by *Fusarium* fungi.

2.5 Sampling and questionnaire

2.5.1 Sampling

Sampling is a critical part in the precision of the determination of the levels of mycotoxins, which are very heterogeneously distributed in a grain batch. Therefore, sample variation is often the largest error in determining concentrations of mycotoxins in food commodities (IARC Sci Publ., 2012). Sampling variability is large because a small percentage of kernels are contaminated and the level of contamination on a single seed can be very significant. The worldwide safety evaluation of mycotoxins requires sampling plans that give acceptably accurate values for the levels of contamination in specific batches or lots of a commodity.

Commission Regulation (EC) No 401/2006 of 23 February 2006 laid down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs as follows: Sampling shall be performed by an authorized person as designated by the Member State. Each lot which is to be examined shall be sampled separately. By the particular sampling provisions for the different mycotoxins, large lots shall be subdivided into sublots to be sampled separately. As far as possible incremental samples shall be taken at various places distributed throughout the lot or subplot. The aggregate sample shall be made up by combining the incremental samples. The replicate samples for enforcement, trade (defence) and reference (referee) purposes shall be taken from the homogenized aggregate sample, unless such procedure conflicts with Member States' rules as regards the rights of the food business operator. Each sample shall be placed in a clean, inert container offering adequate protection from contamination and against damage in transit. All necessary precautions shall be taken to avoid any change in the composition of the sample, which might arise during transportation or storage. Each sample taken for official use shall be sealed at the place of sampling and identified following the rules of the Member State. A record shall be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst. The weight of the incremental sample shall be about 100 grams unless otherwise defined in the regulation annexes.

General survey of the method of sampling for cereals and cereal products (Commission Regulation (EC) No 401/2006; Table 2).

Table 2. Commission Regulation (EC) No 401/2006 for sampling of cereals and cereal products.

Food group	Lot weight (tonnes)	Weight or number of sublots	Number of incremental samples	Total sample weight (kg)
Cereal and cereal products	≥ 1500	500 tonnes	100	10
	> 300 and < 1500	3 sublots	100	10
	≥ 50 and ≤ 300	100 tonnes	100	10
	< 50	-	3-100 (*)	1-10

(*) Depending on the lot weight

Specific sampling protocols for mycotoxins should be followed, which regulate the number and size of incremental samples as well as the size of the aggregate sample to be taken for research purposes. For meaningful data to be generated from surveillance studies, country-based and regionally representative samples should be collected from selected batches of food and feed (Whitaker, 2003). There is need for efficient, cost-effective sampling and analytical methods that can be used for detection analysis of mycotoxins in grain trading, at farm, and in food and feed industry (Wagacha et al., 2008; Da Silva et al., 2008).

2.5.2 Questionnaire and mycotoxin risk assessment sheet

In the monitoring studies together with sampling guidelines, a standardized questionnaire is used to administer to farmers. For example, in the Finnish mycotoxin survey, farmers were asked to provide information about the following: the quality of seed, variety of the grain, growing area and agronomic variables such as sowing date, the type of soil, plant rotation, nitrogen fertilization, plant protection procedures during the growing season, growth period, harvesting-related moisture, harvest quantities, harvest date, the method of harvest drying and sorting.

In the United Kingdom (www. <http://stage.hgca.com/media/418930/is40-risk-assessment-for-fusarium-mycotoxins-in-wheat.pdf>) a questionnaire and “Risk assessment” sheet have been developed for farmers to estimate the risk of *Fusarium* mycotoxins in wheat (Table 3).

Table 3. Mycotoxin Risk Assessment Sheet in the United Kingdom.

Factor	Details	Risk	Field name	Field name ...
Region	High risk area	4		
	Moderate risk area	2		
	Low risk area	-2		
	Very low risk area	-4		
Previous crop	Maize	6		
	Other	0		
Cultivation	Direct drilled	4		
	Standard non-inversion tillage	3		
	Intensive non-inversion tillage	2		
	Plough (soil inversion)	0		
Wheat variety	RL Resistance rating 1–5	1		
	RL Resistance rating 6–9	0		
	RL Resistance rating unknown	1		
	Your pre-flowering score			
T3 fungicide	Under 50 % dose rate of approved fungicide	0		
	50–74 % dose rate of approved fungicide	-2		
	75 % or above dose rate of approved fungicide	-3		
Rainfall at flowering (GS 59–69)	More than 80 mm	9		
	40–80 mm	6		
	10–40 mm	3		
	Less than 10 mm	0		
Rainfall pre-harvest (GS 87 to harvest)	More than 120 mm	12		
	80–120 mm	9		
	40–80 mm	6		
	20–40 mm	3		
	Less than 20 mm	0		
	Your final score			

2.5.3 Methods of analysis

Mycotoxins are present in food at very low concentrations, parts per billion (ppb or $\mu\text{g}/\text{kg}$) or parts per million (ppm or mg/kg) levels. However, mycotoxins may cause toxic effects at such low levels. Thus, regulatory limits are set accordingly for each mycotoxin in various commodities or foods to protect public health. The development of reliable methods for detection and quantification of mycotoxins is essential in research, surveillance, and regulation. As mycotoxins tend to remain not only in raw agricultural commodities but their products manufactured in the downstream processes, the development, and application of the reliable method is a very challenging task in considering the diverse physical and chemical nature of the raw and processed products as well as the properties of mycotoxins of interest (Jung Lee & Ryu, 2015).

In the last few decades, significant improvements have happened in the development of methods for identification, detection, and quantification of mycotoxins. These methods are based on chromatographic separations (Figure 6) such as gas chromatography-mass spectrometry (GC-MS), HPLC, UPLC, and liquid chromatography-mass spectrometry (LC-MS). Because most of these chromatographic methods require expertise and expensive instrumentation, rapid methods such as enzyme-linked immunosorbent assay (ELISA) and immunochemical techniques (e.g. lateral flow test) have become methods of choice for routine analyses in the field. For grain traders and farmers rapid tests offer sensitive and rapid analyses with simplicity and low cost (Jung Lee & Ryu, 2015).



Photo: Veli Hietaniemi

Figure 6. Equipment used for the determination of mycotoxins by GC-MS-technique at the Natural Resources Institute Finland.

Advances in detection and quantification of mycotoxins is most visible in HPLC, UPLC and LC-MS offering high sensitivity, accuracy, and efficiency. In particular, LC/MS has become a universal approach for mycotoxin analysis and confirmation (Zöllner and Mayer-Helm, 2006). It provides higher sensitivity and selectivity than HPLC coupled with UV or fluorescence. It is also possible to identify and characterize mycotoxin metabolites or degradation products. Thus, an increasing number of researchers have used LC-MS for metabolism and toxicokinetic studies as well as for identification and quantification of reaction products formed during food processing (Warth et al., 2012; Bittner et al., 2015). Moreover, it can provide a platform to quantify multiple mycotoxins in a single analysis (Al-Taher et al., 2013). The method of multianalysis is particularly desirable because mycotoxins may occur in various combinations produced by a single or several fungal species in food matrices (Njumbe Ediage et al., 2011).

ELISA has been used widely for rapid screening and monitoring of mycotoxin contamination in raw material and final products (Paepens et al., 2004). Commercial ELISA kits are available for the most of the major mycotoxins in various food matrices (Schneider et al., 2004). Without clean-up or concentration steps, ELISA has emerged as a rapid test with its high sensitivity, low cost, and ease of application that may also be used in field conditions (Zheng et al., 2005). The ELISA is based on the reaction between antigens and antibodies, but antibodies often show cross-reactivity with compounds similar to the target mycotoxins (Lee et al., 2014). The presence of components structurally related to mycotoxins can interfere with antigen–antibody binding and may lead to erroneous measurements. Also, ‘matrix effect’ or ‘matrix interference’ may cause under- or overestimation of mycotoxins concentrations. As food matrices contain various naturally occurring antigens that are a mixture of macromolecules with several epitopes, HPLC or UPLC analysis is often employed for confirmation of mycotoxins. Hence, it is important to apply validated ELISA methods for targeted mycotoxin in specific commodity (Jung Lee & Ryu, 2015).

Also, some ‘sensors’ have been developed for the rapid and low-cost determination of mycotoxins in various food matrices. These sensor technologies are based on the bio- and/or chemical reactions of the mycotoxin or other metabolites produced by fungi. For instance, electronic noses adsorb volatile organic compounds of low molecular weight, which are released by many fungi as products of secondary metabolism. According to Olsson et al. (2002) and Logrieco et al. (2005) electronic noses measure the concentration of these compounds with a variety of transduction systems based on electrical-, optical-, or mass-transduction, such as with metal oxide sensors or surface acoustic wave sensors. Similarly, electronic tongue instruments have also been developed for liquid samples (Söderström et al., 2003). Optical analysis methods, such as Fourier Transform mid-infrared spectroscopy (Kos et al., 2003) and near-infrared transmittance spectroscopy (Pettersson and Åberg, 2003), may also provide fast and non-destructive detection of mycotoxins. According to Jung Lee & Ryu (2015) the significant restrictions for most of these analyses are the large matrix dependence and the lack or limitation of appropriate calibration materials. These techniques respond to a broad range of compounds the sensor array generates patterns of responses that can be distinguished for different samples.

It should be noted that biomonitoring is important in estimating human exposure to mycotoxins and for quantitative risk assessments. For example, the dried blood spot (DBS) technique in which the blood samples are blotted and dried on filter paper on site, can easily be stored for a long time (over years) and analyzed in the laboratory by HPLC or LC/MS (Török et al., 2002). Recently, Cramer and others (2015) detected ochratoxin A (OTA) and its degradation product 2’R-OTA in blood from coffee drinkers using the DBS method.

2.6 *Fusarium* toxins: occurrence and exposure

According to the European Food Safety Authority (European Food Safety Authority, 2013) a total of 26,613 analytical results covering food, feed and unprocessed grains of undefined end-use, collected by 21 European countries and Norway between 2007 and 2012 were included in the review: Deoxynivalenol in food and feed: occurrence and exposure. According to the report 47 % of the samples were coming from random sampling, 51 % from selective sampling, which may be based on a risk analysis, and 2 % from suspect sampling to investigate a suspicion of non-conformity.

Based on the results of the report of the European Food Safety Authority (2013) DON was found in 43.5 %, 75.2 %, and 44.6 % of unprocessed grains in food, feed, and undefined end-use samples, respectively. The highest levels and the number of positive findings out of the analyzed samples were found in maize, wheat, and oat grains and derived food and feed products (Table 4). Contents of DON were much higher in wheat bran than the other wheat milling products. DON levels in processed cereals were significantly lower than those in unprocessed grains and grain milling products. However, feed contained higher levels of DON than unprocessed grains of undefined end-use and foods. DON levels were higher in compound feed for poultry than in compound feed for other animal species. The level of DON exceeded maximum levels in 0.8 % of the food samples and guidance values in 1.7 % of the feed samples (European Food Safety Authority, 2013).

Table 4. Contents of DON ($\mu\text{g}/\text{kg}$) in cereal grains for human consumption (EFSA, 2013).

Food group	N ^(a)	Concentration $\mu\text{g}/\text{kg}$	
		Mean MB (LB - UB) ^(b)	P95 MB (LB - UB) ^(b)
Grains for human consumption	2936	111.8 (99.4; 124.1)	520
Barley grain	209	49.6 (31.2; 68.1)	170
Maize grain	136	237.9 (231.5; 244.2)	1453
Oats, grain	203	209 (203.4; 214.6)	738
Rye grain	615	38.1 (20.8; 55.3)	137.1
Wheat grain	1357	154.3 (143.8; 164.8)	660
Wheat germ	12	414.2 (411.3; 417.1)	-
Wheat grain	1064	162.8 (152.3; 173.3)	682
Wheat grain, durum	46	341.3 (326.2; 356.5)	-
Wheat grain, soft	141	98.4 (93.6; 103.3)	447
Bulgur wheat	94	17.4 (0; 34.9)	25 (0; 50)

(a) N: number of samples, (b) MB (LB-UB): mean and 95th percentile presented as the middle bound estimate (lower bound estimate; upper bound estimate). When the middle, lower and upper bound estimates are equal, only one estimate is given.

The European Food Safety Authority (2013) also reported that the DON derivatives (3-AcDON, 15-AcDON) were seldom found and at lower levels than DON. DON was also present in almost all the cases, when 3-AcDON and/or 15-AcDON were determined. The average percentage contribution of 3-AcDON to the sum of DON and its derivatives was less than 2 % at the lower-bound estimate and around 13–20 % at the upper-bound estimate. The percentage contribution of 15-AcDON to the sum of DON and its derivatives was up to 10–15 % at both lower and upper bound estimates for maize grains. Only from one member state data of DON-3-G were available. DON-3-G was found almost always together with DON in around 5 % of the samples, and represented on average 5.6 % of the lower-bound sum of DON and DON-3-G. According to Nagl et al. (2012) DON-3-G may be metabolized in the gastrointestinal tract of humans and animals to DON.

According to the European Food Safety Authority (2013), based on the dietary intake estimates, infants, toddlers and other children were the most exposed groups regarding chronic exposure. Chronic dietary exposure of children to DON (upper-bound) was estimated to be on average between 0.54 and 1.02 $\mu\text{g}/\text{kg}$ b.w. per day and at the 95th percentile between 0.95 and 1.86 $\mu\text{g}/\text{kg}$ b.w. per day. Chronic dietary exposure of adolescents, adults, elderly and very elderly to DON (upper-bound) was estimated to be on average between 0.22 and 0.58 $\mu\text{g}/\text{kg}$ b.w. per day and at the 95th percentile between 0.43 and 1.08 $\mu\text{g}/\text{kg}$ b.w. per day depending on the population group (European Food Safety Authority, 2013). The main contributor to the total chronic exposure was “bread and rolls” representing between 30.9 and 72.3% of the total exposure.

3-AcDON and 15-AcDON represented less than 2.2% of the lower-bound estimate of the chronic human exposure to the sum of DON, 3-AcDON, and 15-AcDON. However, when considering the upper-bound, they were found to represent up to 63.4% of the total exposure, showing the uncertainty around their real contribution to the total exposure. DON-3-G was not taken into account in the exposure assessment because of the lack of analysis data. However, an overestimation of the DON exposure is expected considering the methodology used and the conservative assumptions made to assess dietary exposure.

The chronic exposure of animals was estimated at the upper-bound between 3.9 and 43.3 $\mu\text{g}/\text{kg}$ b.w. per day, and the acute exposure levels between 11.6 and 137.9 $\mu\text{g}/\text{kg}$ b.w. Poultry were found to have the highest level of exposure, followed by pigs, companion animals and fish.

The European Food Safety Authority (2013) stated that in order to improve the accuracy of the assessment of food contamination levels and exposure to DON throughout Europe, it would be important to harmonize further the sampling strategy such as number of samples, food covered, targeting design, and the performance of the analytical methods used for the monitoring programmes. Further data should be collected on DON-3-G, 3-AcDON and 15-AcDON to better characterize their potential contribution to the total exposure to DON. It is also recommended to measure DON in those foods identified as main contributors to the total exposure, but for which the estimations of the contamination levels were not robust, such as oat flour, porridge and composite foods. Collecting more accurate data on the different feeding systems used in Europe would also improve the quality of the animal exposure assessment to contaminants.

Studies of the occurrence and exposure of *Fusarium* toxins have been concentrated on deoxynivalenol because a major part of various monitoring surveys and exposure studies has been focused on DON. However, Leblanc et al. (2005) reported from the first French total diet study, in which they attempted to assess the exposure to HT-2 toxin in France, that only one out of 235 composite samples was above the LOQ of 0.080 mg/kg. Therefore they did not carry out any exposure assessment study for this mycotoxin. In contrast, Cano-Sancho et al. (2012) conducted a large dietary intake study of the main foodstuffs in Catalonia in Spain related to T-2 and HT-2 contamination for all population age groups. Based on the results of Cano-Sancho et al. (2012) the population group most exposed to the toxin T-2 and HT-2 is expected to be children; the child population group could even exceed the safety limits of 0.06 $\mu\text{g}/\text{kg}$ bw/day.

De Boevre et al. (2013) studied human exposure from cereal-based food products ($n = 174$) in Belgium. Fibre-enriched bread, bran-enriched bread, breakfast cereals, popcorn and oatmeal were collected in Belgian supermarkets according to a structured sampling plan and analysed during the period 2010–2011. From the samples, contents of DON, 3-AcDON, 15-AcDON, ZON, α -zearalenol, β -zearalenol, T-2, HT-2, and their respective masked forms, including, DON-3-G, zearalenone-4-glucoside, α -zearalenol-4-glucoside, β -zearalenol-4-glucoside and zearalenone-4-sulfate were analysed. According to a probabilistic exposure analysis, the mean (and P95) mycotoxin intake for the sum of the DON-equivalents, ZON-

equivalents, and the sum of HT-2- and T-2-toxin for all cereal-based foods was 0.1162 (0.4047, P95), 0.0447 (0.1568, P95) and 0.0258 (0.0924, P95) $\mu\text{g}/\text{kg}$ body weight per day, respectively. These values were below the tolerable daily intake (TDI) levels for DON, ZON and T-2+HT-2 (1.0, 0.25 and 0.1 $\mu\text{g}/\text{kg}$ body weight per day, respectively). According to De Boevre et al. (2013) fibre-enriched bread, bran-enriched bread and all cereal-based foods had T-2 and HT-2 intakes (P99.5) of 0.7052, 0.1335 and 0.1950 $\mu\text{g}/\text{kg}$ b.w. per day, respectively. The authors stated that the toxin levels found in the analysed foods cause on a daily scale a high exposure to the Belgian population.

Many studies regarding T-2 and HT-2 toxins in cereals in Nordic countries, UK and Central Europe confirm that more comprehensive dietary intake studies of these toxins are needed (Pettersson et al., 2008; Barrier-Guillot et al., 2008; Edwards et al., 2009, 2009b; Hietaniemi et al., 2016). In a 2002–2005 study of oats in the United Kingdom by Edwards (2009), high incidences and high mean concentrations of HT-2 and T-2 were found. Edwards reported that levels of HT-2+T-2 were higher than those previously reported for any cereal worldwide. Also, regression analysis shows that there were strong correlations between HT-2 and other type A trichothecenes (T-2, T-2 triol and neosalaniol), which would indicate that they are produced by the same *Fusarium* isolates as part of the same synthetic pathway and, therefore, are co-contaminants. Year and region had a significant effect on HT-2+T-2 concentration. Overall, according to Hietaniemi et al. (2016), Edwards et al. (2009, 2009b) and Pettersson et al. (2008), results from Nordic countries and the United Kingdom suggest that the occurrence of these highly toxic compounds has increased dramatically over the last decade, especially in oats. Also, Barrier-Guillot et al. (2008) reported high concentrations of HT-2 and T-2 in French barley.

3 CONTROL AND MANAGE THE RISK

Production of high-quality raw material for the needs of the food and feed industry, livestock production and other end users is the main objective of the primary production of cereals. Total quality management is a significant competitive factor for domestic grain raw materials. It is a central task for quality management to ascertain and document the quality of raw grain material suitable for the intended use for the grain supply chain processing it and to increase cost-effectiveness, productivity, and consumer safety.

Quality management of grain supply chains ‘primary production – food and cosmetic industry – consumer’ or ‘primary production – livestock production – food industry – consumer’ can be significantly improved by introducing more measurement technology, guidance on use, and logistics management to the process of harvesting, drying and storing of grain. Along with the management of the chain, specialized sections of the chain will also provide more possibilities to increase the export of cereals and processed grains, e.g. organic oats, pure oats, baby food and batches of varieties. At this stage, versatile grain quality analysis, documentation, and storage of application-specific quality adopt a significant role and require the development of existing systems. At present, a major proportion of harvested grain is already being stored in farm silos, and single plot-specific accounting reveals cultivation history. Maintaining and improving the level of competence of rural entrepreneurs and other operators is the main objective of rural development. For an agricultural enterprise, the know-how and competence of the entrepreneur constitute the most important success factors.

According to Wagacha and Muthomi (2007), the available control and management procedures for FHB may be classified as a short and long term. The short-term control and management procedures include the use of fungicides, biocontrol, and cultural practices while the use of resistant genotypes is the most promising long-term management option. The primary goal is to prevent the infestation of *Fusarium* species in areas where it does not yet occur. If the infestation has been identified in an area, it is important to reduce inoculum available for dispersal, prevent dispersal of inoculum and infection of spikelets. Therefore, early detection, control and management of trichothecene-producing *Fusarium* spp. are critical to prevent toxins entering the food chain.

3.1 Control and manage the risk pre-harvest

Because of the continued importance of *Fusarium* mycobiota, the spread of *Fusarium* species and mycotoxins produced by them, strategies need to be designed to stop or reduce the rate of spread, and to decrease the damage it causes in areas where it is already well established. Understanding the impact of agronomic practices on disease and inoculum levels should form part of comprehensive strategies aimed at controlling FHB. A comprehensive strategy should also include the role of *Fusarium* infection of crop roots,

crowns and crop residues as sources and reservoirs of fungal inoculum and their potential carryover from one growing season to the next (Fernandez et al., 2009; Hofgaard et al., 2016).



Photo: Veli Hietaniemi

Figure 7. Start of the growing season in 2014 in Finland. Thinking about right pre-harvest farming decisions for a healthy and safe crop.

The most important preventive measures for both FHB and foliar diseases are: to minimize the pathogen inocula in the field by using crop rotation (Champeil et al., 2004b; Krupinsky et al., 2004), to reduce previous crop residues through soil tillage (Maiorano et al., 2008) or to use resistant varieties (Loyce et al., 2008; Tóth et al., 2008). Limited soil tillage or no-tillage increases the frequency of FHB, whereas deep tillage, such as ploughing, decreases it (Miller et al., 1998). Maiorano et al. (2008) reported a close relationship between DON contamination in wheat grains and the quantity of maize crop residues on the soil surface at anthesis. Moreover, FHB severity and DON content are clearly affected by the interaction of previous crop residues, and tillage practice applied (Dill-Macky and Jones, 2000; Champeil et al., 2004; Koch et al., 2006; Maiorano et al., 2008; Blandino et al., 2012). According to Xu (2003) the primary reservoir of inoculum is debris from the previous crop. FHB epidemics are supported by cropping systems that leave a high amount of crop debris on the soil surface (Pereyra and Dill-Macky, 2008; Blandino et al., 2010), and pathogens survive longer on residues that do not degrade easily, such as stem nodes or stalks (Sutton, 1982). However, in climatic conditions conducive to fungal diseases, those preventive agronomic factors mentioned above are perhaps not sufficient, and direct control through the use of fungicide application is necessary (McMullen et al., 2008; Blandino et al., 2011; Blandino et al., 2012).

Miller et al. (1998) and Schaafsma et al. (2001) found that no significant effects were observed in minimum tillage or nontillage treatments on FHB or DON. A study by Blandino et al. (2010) indicate there is still a lack of knowledge regarding the effect of crop residue density on DON contamination in wheat grains. More precise information on the influence of crop debris density is crucial to interpreting the effect of tillage practices.

As far as variety susceptibility to FHB and DON is concerned, breeding progress in cereals, using conventional methods, molecular markers or through transgenic approaches, have been discussed in great detail in several reviews (Hollins et al., 2003; Snijders, 2004). At present, fully FHB-resistant wheat cultivars do not exist. Therefore disease control relies on the use of commercial cultivars with moderate or low resistance (Mesterházy et al., 2005). Wheat varieties more resistant to FHB have been shown to reduce DON production to almost zero in recent studies (Tóth et al., 2008; Blandino et al., 2012).

According to Edwards (2004) combining control methods can be expected to be more efficient, especially if the climatic conditions are favourable for FHB infection (Edwards, 2004). Therefore, GAP requires an integrated approach that addresses all the possible risk factors to prevent DON contamination (Pirgozliev et al., 2003). Moreover, although information is available on the basic effect of individual agricultural practices on *Fusarium* infection and DON contamination in wheat, according to Blandino et al. (2012) only a few studies have been conducted to quantify the relative importance of each of these factors compared to the others or to verify their interactions and combined effects.

3.1.1 High-quality seed and resistant cultivars

Seed conditioning, dressing and the use of certified seed have been prioritized high in the management of toxin risk. In general, the most important quality indicators of sowing seeds include varietal properties, germinative capacity, and thousand grain weight. Often there is no information available on the *Fusarium* infestation and mycotoxin levels of a seed batch. Batch-specific assays for *Fusarium* fungi and mycotoxins should be taken into use in seed management. In Finland for dressing and against seedling blight and other diseases the following active ingredients have been used: carboxin, imazalil, fludioxonil, triadimenol, fuberidazole, syproconazole, triticonazole and prothioconazole.

According to Aldred & Magan (2004) *Fusarium* species-resistant cultivars should be the most economic, environment-friendly and efficient method of disease control. Two types of resistance to FHB of wheat have been reported; resistance to primary infection and resistance to the spread of the disease within a spike (Schroeder and Christensen, 1963). Head blight resistance in wheat is not specific for either *F. graminearum* or *F. culmorum*. Resistance components include resistance to penetration, resistance to colonization and mechanisms that influence kernel DON content. The resistance to *Fusarium* in wheat is a quantitative trait with relative high heritability and controlled by a few genes with major effect. A major QTL for head blight resistance from the Chinese variety Sumai 3 has been identified and verified by several research groups via molecular marker analysis. Research

is now directed at identifying additional QTLs to make the accumulation of resistance genes in elite wheat lines possible. The policy of official variety list trials may affect the head blight resistant level of future wheat varieties by excluding candidate varieties that are too susceptible to *Fusarium* (Snijders, 2004; Aldred & Magan, 2004).

Miller et al. (1985) found that resistant cereal lines inoculated with *F. graminearum* contained lower concentrations of DON in the grain than susceptible cultivars. Snijders and Krechting (1992) suggested that it could be possible that a resistance mechanism that neutralizes DON production exists and DON may play a role in FHB pathogenesis. According to Wagacha & Muthomi (2007) it is now generally agreed that FHB resistance is controlled by a polygenic system. Effects of the dominance of genes probably influence FHB resistance. Also, additive effects appear to be important, and resistance genes can be accumulated (Bai et al., 1999; Snijders, 1990). Kang and Buchenauer (2000) reported that FHB resistant cultivars were able to develop active defense reactions during infection and spreading of *F. culmorum* in host tissues. The researchers also reported lower accumulation of DON in the tissues of infected spikes of resistant wheat cultivars (Wagacha & Muthomi, 2007).

According to Mesterházy (2002), wheat varieties most resistant to FHB were shown to reduce DON production to near zero. In fact, resistance seemed to depend mostly on inhibition of toxin production directly, since the most aggressive disease-causing fungal strains were also those producing the highest levels of DON. Mesterházy suggested that an increased availability of such resistant varieties, coupled with the use of appropriate fungicides, was the core of an integrated approach to mycotoxin control associated with *Fusarium*.

In the studies of Mesterházy, the pathogenicity of species representing geographically separated species/lineages of the *F. graminearum* species complex and *F. culmorum* was examined on various wheat cultivars with highly different levels of resistance to FHB. The various wheat genotypes exhibited similar reactions against the different isolates of *F. graminearum sensu stricto* and *F. culmorum*, similar to isolates of other *Fusarium* species as described by Mesterházy 1995, 2002, and Mesterházy et al. (2005). Tóth et al. (2008) also found that the wheat cultivars behave very similarly to all members of the *Fusarium graminearum* species complex and the lineages of *F. culmorum*. The mostly very close and highly significant correlations show this clearly. Therefore, for resistance breeding, one pathogenic isolate of any *Fusarium* species is sufficient, since the resistance is not specific, wheat recognizes only virulence differences (Gang et al., 1998; Mesterházy et al., 2005; Tóth et al., 2008).

Studies in the 1990s and 2000s on chromosomal location of FHB resistance genes/loci in the wheat genome have used Restriction Fragment Length Polymorphism (RFLP) and Amplified Fragment Length Polymorphism (AFLP) methods and recombinant inbred lines as a mapping population. A review by Wagacha and Muthomi (2007) reported that quantitative trait loci for resistance to FHB have been preliminarily mapped on the following chromosomes: 1B, 2AL, 3 BS, 3A, 5A and 6B (Buertsmaier et al., 2002;

Anderson et al, 2001; Ruckenbauer et al., 2001; Bai et al., 1999, Waldron et al., 1999). Sources of resistance have been found in China, South America and Czech Republic (Mesterházy et al., 1999; Mesterházy, 1995; Snijders, 1990). Currently, there are no wheat cultivars with at high level of resistance to FHB although some cultivars have partial resistance that limit yield loss and mycotoxins contamination (Pereyra and Dill-Macky, 2004). Wisniewska and Kowalczyk (2005) reported a breeding line with useable resistance to *F. culmorum* and other *Fusarium* spp. (Wagacha & Muthomi, 2007).

This non-specificity could be demonstrated for all traits examined including ear and kernel infection, yield loss and toxin contamination. Accordingly, it seems that the resistance is not restricted to the visual FHB field reactions, but determines to a large extent the response to toxin contamination, *Fusarium*-damage kernels (FDK) and yield loss, too. The European *F. culmorum* isolates from wheat had the highest FHB and FDK values, caused the highest yield losses and trichothecene accumulation. Of the *F. graminearum* species complex, *F. graminearum*, *F. cortaderiae* and *F. acaciae-mearnsii* were the most pathogenic and *F. graminearum* caused the highest DON/NIV accumulation (Goswami & Kistler, 2005). It is noteworthy that the most resistant genotypes were symptomless or exhibited only very moderate infection in response to all of the *Fusarium* species and isolates tested. According to Toth et al. (2008) relatively straightforward breeding technology has been carried out in many parts of the world, and this explains why the resistant materials behave similarly in very different regions of the globe.

There is not a single toxin that is responsible for the pathogenicity of all *Fusarium* species (Mesterházy et al., 2005; Toth et al., 2008). For this reason, other common traits that could be the basis of the common resistance to different *Fusarium* species should be identified. However, for DON-producing isolates, the amount of toxin produced is proportional to virulence. There is no correlation between grain toxin content and aggressiveness if the toxin content is calculated relatively to the fungal biomass.

Some *F. graminearum* isolates can produce DON, 3-AcDON and 15-AcDON and NIV (Sugiura et al., 1990; Szécsi and Bartók, 1995; Ward et al., 2002; Szécsi et al., 2005; Tóth et al., 2005). According to Abramson et al. (1993), *F. poae* and *F. sporotrichioides* can produce DON, too. However, the new members of the *F. graminearum* species complex do not seem to have an importance for wheat breeding as the resistance is similarly effective against all of them. This agrees well with the various studies of Mesterházy regarding common resistance (Mesterházy, 1983, 1995, 2002; Mesterházy et al., 2005). As the mechanism of resistance is not specific to a *Fusarium* species, the resistance of cultivars should be durable. Accordingly, resistant genotypes can be cultivated successfully in areas where different *Fusarium* species are dominant or co-occur. The resistance level is more important to regulate toxin contamination than aggressiveness. Even the most aggressive isolates can produce only a very limited amount of disease and toxin on the genotypes with high resistance (Mesterházy et al., 2005; Toth et al., 2008).

3.1.1.1 Resistance mechanism - glucosylation

According to Lemmens et al. (2008), Fhb1 (syn. QTL Qfhs.ndsu-3B) is one of the most prevalent resistance sources in resistance breeding against *Fusarium*, and has always been associated with highly efficient resistance against disease spreading in the wheat ears, so-called type II resistance. This gene has also been shown to be highly important for the detoxification of DON (Lemmens et al., 2005); consequently it is possible that breeding practices over the last decades have inadvertently incorporated capacity for glucosylation in modern wheat varieties. This characteristic has however never been screened in commercial wheat cultivars on a large scale, nor is its importance in disease containment known in the Belgian setting (Audenaert et al., 2013).

Assuming that glucosylation is a key component and characteristic of Fhb1 resistance (Lemmens et al., 2005), the study of Audenaert et al. (2013) offers the first proof that several Belgian commercial wheat cultivars do possess this resistance gene to some extent. This approach leans heavily on the use of glucosylation as a “symptom” of Fhb1 resistance. However, in this assessment, it would be necessary to use genetic markers to make sure the presence of the known Fhb1 QTL. The high correlation between DON glucosylation and disease index, however, does support the hypothesis that indeed efficiency of DON glucosylation plays a significant role in commercial cultivars.

Based on the results of Audenaert et al. (2013) DON levels in the field were logically lower than those encountered during the experiments in the laboratory. The high correlation between disease index and glucosylation that was observed under laboratory conditions did not hold up in the field. According to a 10-year study by Landschoot et al. (2012) on Belgian winter wheat it was not possible to determine a clear correlation between disease severity and DON incidence. These authors argued that DON content is a function of many other factors than just visible disease severity. Therefore, the lack of a correlation between disease index and DON-3G is a logical consequence.

Efficacy of Fhb1 against toxins other than DON and the closely related nivalenol (NIV) has not yet been described, while potential type A trichothecene producers such as *F. poae* are in some years quite important within the *Fusarium* population. However, it is evident from studies of Audenaert et al. (2013) that in *F. graminearum*-dominated populations, significant amounts of DON can be glucosylated to DON-3G in commercial wheat cultivars, which in a controlled environment was confirmed to have a profound effect on *Fusarium* disease limitation. There are also other possible metabolites of DON such as DON-glutathione which could be part of the resistance mechanism (Gardiner S.A. et al., 2010).

Blandino et al. (2012) concluded that wheat variety susceptibility plays a more important role for low or medium disease pressure, whereas under high disease pressure, the difference between susceptible or moderately resistant varieties was not significant. Conversely, Chandelier et al. (2011) and Landschoot et al. (2012) concluded that under minor infection pressure the effect of wheat varieties was less visible than under high infection pressure. Based on the experimental results of the study of Landschoot et al.

(2013), they confirmed the findings of Chandelier et al. (2011) and their previous research. Under high infection pressure, the DON of the moderately resistant varieties was 44% lower compared to the susceptible varieties, whereas under low infection pressure a reduction of 35% was reached by growing a moderately resistant variety (Landschoot et al., 2013).

At present, no fully FHB-resistant wheat varieties exist. Therefore, the control of FHB relies on the use of commercial varieties with partial resistance (Mesterházy et al., 2005). Different resistance types are available, although to date only type I (resistance towards initial infection) and especially type II (resistance towards spread) are relevant for plant breeders (Bai et al., 2001; Bai and Shaner, 2004). However, the development of resistant cultivars is a crucial area (Schaafsma et al., 2001; Isebaert et al., 2009). It has been shown in many studies 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 (Schaafsma et al., 2001; Diamond and Cooke, 2002; Mesterházy, 2002; Aldred & Magan, 2004; Isebaert et al., 2009).

3.1.2 Field management

3.1.2.1 Crop rotation

Minimal crop rotation was significantly connected to increases of total *Fusarium*, *F. graminearum*, *F. langsethiae*, DON and HT-2 (Bernhoft et al., 2012). The results are in agreement with many reports regarding *Fusarium* infection and DON levels in cereal grains (Aldred and Magan, 2004; Edwards, 2004; Oldenburg, 2004; Beyer et al., 2006; Köpke et al., 2007). The factor 'Other cereal species last year' had a larger importance than 'Same cereal species last year'. Also, the factor 'Non-cereal crop last year' significantly decreased *F. langsethiae* and HT-2. The studies mentioned above indicate that continuous cropping of cereal species is a disadvantage concerning *Fusarium* infestation and mycotoxins. According to Bernhoft et al. (2010, 2012), the finding of largely the same *Fusarium* species on barley, oats, and wheat indicate that any of these cereal species may make the *Fusarium* inoculum correspondingly available for the crop produced the following year.

According to Maiorano et al. (2008) previous crop residues such as maize stalks and grain, and straw of barley, wheat, and other cereals are found to be the principal inoculum sources for *F. graminearum* and *F. culmorum*. The results obtained in these experiments also demonstrate that the density of the residues left by the preceding crop apparently affects FHB frequency and DON contamination. Teich and Hamilton (1985) and Bateman et al. (1998) stated earlier in the 1980s and 1990s that the inoculum potential of the *Fusarium* population could directly depend on the quantity of available crop debris (Blandino et al., 2010).

Bottalico and Perrone (2002) found higher DON contents in wheat following grain maize than in wheat following maize for silage (Landschoot et al., 2013). Several authors (Teich and Nelson, 1984; Schaafsma et al., 2001; Yi et al., 2001) have reported that the frequency of the disease on wheat is lower following soybean than following another wheat crop, or

worse still, maize. Dill-Macky and Jones (2000) recorded that soybean crops leave fewer residues than wheat crops, which in turn leave fewer residues than maize crops. The DON level in wheat following soybeans, averaged across tillage treatments, was 25% lower than in wheat following wheat and 50% of the level in wheat following corn. Also, based on the results of Dill-Macky and Salas (2001) burning crop residues reduces the inoculum potential of *F. graminearum* present in residues and hence the potential inoculums for FHB (Figure 8). Such observations underline that the influence of the preceding crop may not only be related to their nature, which can affect the composition of the pathogen complex throughout the following year (Steinkellner and Langer, 2004; Fernandez et al., 2008), but also to the density of the residues left on the soil surface (Blandino et al., 2010).



Photo: Veli Hietaniemi

Figure 8. Burning crop residues reduces the inoculum potential of *Fusarium* species present in crop residues (Dill-Macky & Salas, 2001).

3.1.2.2 Reduced tillage

Reduced tillage systems involve leaving all or part of the crop residue on the soil surface after harvesting to reduce soil erosion, conserve energy, increase soil moisture, and increase crop yields (Figure 9). Limited soil tillage operations that leave a part of crop residues unburied increase the number of *Fusarium* pathogens on the ground (Fernandez et al., 2008) and could provide an inoculum source and increase the frequency of head blight (Koch et al., 2006; Bockus and Shroyer, 1998). A range of alternative hosts, including maize, soybean, sorghum, wild oats and various common weeds, have been reported to be sources of *F. graminearum* inoculum (Sutton, 1982; Fernandez, 1991; Dill-Macky and Jones, 2000; Pereyra et al., 2004a). Therefore, cultural practices have an important effect on the development of FHB (Lori et al., 2009).

In Argentina, the wheat cropping area is near 6 million hectares, of which 55% is cultivated using no-till systems (Secretaría de Agricultura, Pesca y Alimentos, SAGPyA, 2006), ranking Argentina third in production of no-till wheat after the USA and Brasil (Asociación Argentina de Productores en Siembra Directa, AAPRESID, 2004). Until the 1980s, conventional tillage practices dominated Argentinean production. Since then, the area cultivated using conservative tillage practices has increased, and higher yields of crops are obtained with this system when compared to conventional tillage. Lori et al. (2009) reported that a fast change in the use of this technology in Argentina was possible due to good knowledge of growing techniques in the region which were available through research and development as well as farmers' experiences, giving immediate and considerable economic returns.

In Brazil, Almeida et al. (2007) and Lori et al. (2009) observed that reduction or removal of surface stubble mass, by tillage practices before planting, did not decrease FHB occurrence and DON mycotoxin produced by wheat. The authors also concluded that in an FHB epidemic year, the primary factor for disease development and consequently higher mycotoxin production is the environmental conditions prevailing during the heading stage of the host plants.

Conservation tillage was reported to be the primary cause of recent FHB epidemics in the upper Midwest of the United States (Dill-Macky and Jones, 2000) where the characteristic of the production system is the extensive use of corn in crop rotations. According to Blandino et al. (2010), the use of direct sowing should only be recommended in rotation with crops that are not alternative hosts of pathogens or which leave moderate amounts of debris on the soil surface and in environments with little risk of infection. Possible strategies, which need to be further investigated to reduce the risk of *Fusarium* survival and dispersal with conservative tillage practices, could be the application of fungicides or microorganisms with an antagonist action, such as *Microsphaeropsis*, *Trichoderma*, *Bacillus* and *Streptomyces* on crop residues (Yuen and Schoneweis, 2007; Blandino et al., 2010).

According to Fernandez et al. (2009), significant tillage effects for *F. culmorum* and *F. graminearum* were only observed in wheat planted after another cereal crop, where *F. graminearum* was favored by minimum-till, and *F. culmorum* was lowest under zero-till management. The *Fusarium* species isolated from crop residues varied depending on tillage method. *F. culmorum* in cereal residues had the lowest percentage occurrence under zero-till when the current crop was an oilseed. Under these same residue conditions, *F. graminearum* had the lowest percentage occurrence under conventional-till.



Photo: Veli Hietaniemi

Figure 9. Direct sowing of oats in spring 2013 in Sastamala, Finland.

3.1.2.3 Deep tillage

A reduction in the *Fusarium* inoculum from the residue to the cereal heads is limited by deep tillage, such as ploughing (Figure 10). Champeil et al. (2004a) reported that about 90% of the *Fusarium* population is located in the first 10 cm of soil and although these pathogens can survive for four years at a depth of 20–25 cm, they are only active and able to develop on plant debris in the first 5 cm of soil. Based on the results of Blandino et al. (2010), the amount of residues left in reduced tillage in the first 5–10 cm of soil significantly increased the contamination with DON compared to the ploughed plots. Blandino et al. (2010) suggest that the mechanical removal of crop residues from the soil surface before direct sowing might not be sufficient to control the disease. If weather conditions support inoculum production, the dispersal of spores would be guaranteed by the amount of debris incorporated in the first stratum of soil (Maiorano et al., 2008).

Khonga and Sutton (1988) demonstrated that *Fusarium* perithecia and macroconidia were not produced on residues that had been completely buried by ploughing. However, Miller et al. (1998) suggested that only a small amount of inoculum may be required when weather conditions are favourable for infection. On the other hand, daily minimum temperatures below 9 °C and maximum temperatures greater than 26 °C registered around anthesis may inhibit the growth of the fungus (Moschini & Fortugno, 1996; Lori et al., 2009; Blandino et al., 2010).

Lori et al. (2009) reported DON levels 1.4 times higher in plots with minimal tillage, whereas in Blandino et al. (2012) the mean DON levels in plots with no tillage was 4.3 times higher than in fields that were ploughed before sowing. According to Landschoot et al. (2013) at low disease pressure the average DON content of fields that were not ploughed before sowing was only slightly lower (7%) than in ploughed fields. In contrast, when the

disease pressure was high the DON contamination of the ploughed fields was 27% lower than the unploughed fields.



Photo: Veli Hietaniemi

Figure 10. Deep tillage in autumn is used quite a lot in Finland.

3.1.2.4 Nitrogen fertilization

Nitrogen is needed to provide plants with the required building blocks for growth and to resist or recover from disease injury. Plants suffering from a lack of nitrogen are typically weaker, grow slowly, age faster and become more susceptible to pathogens (Snocijers et al., 2000; Lori et al., 2009). However, agronomic practices such as nitrogen application can affect disease development. High contents of nitrogen often increase the susceptibility of plants to diseases (Agrios, 1997). However, the utilization of added inputs to obtain higher yields is part of sustainable systems in cereal production. Nitrogen dynamics and crop response to nitrogen fertilization under conservation tillage practices (as no-till) can be different from those under conventional tillage, modifying nitrogen accumulation and partition in the harvest and the efficiency in fertilizers used.

Huber and Watson (1974) observed that the form of nitrogen available to plants and pathogens also affects the severity of the disease. High soil nitrogen content promoted FHB in wheat, and additional nitrogen (ammonia) increased the disease in control plots (Teich, 1989; Ivashenko and Nazarovskaya, 1990; Martin et al., 1991). Martin et al. (1991) observed that increasing ammonium nitrate applications resulted in increases of *Fusarium*-infected grain in wheat, barley, and triticale. Comparative studies showed that applications of ammonium nitrate resulted in more FHB infected heads than applications of urea (Teich and Nelson, 1984; Teich, 1987). Applications of nitrolime to wheat plots reduced the incidence of FHB by 59% when compared to the plots treated with calcium ammonium nitrate (Yi et al., 2001). However, Lori et al. (2009) indicated there was no difference in head blight infection between regular and high fertilization levels. Similarly, other authors

found that nitrogen did not change the inherent susceptibility of wheat to *F. graminearum* (Teich and Hamilton, 1985; Fauzi and Paulitz, 1994; Lori et al., 2009).

Lemmens et al. (2004) showed that, at a nitrogen application rate in the range of 0–80 kg N per ha, the FHB infection, and the DON contamination of wheat grain significantly increased with increasing nitrogen rate. Also, Milev et al. (2008) reported FHB being promoted by addition of inorganic fertilizer (120 kg N and P per ha) compared with nonfertilized controls. However, using rates 0, 60 and 100 kg N per ha, Subedi et al. (2007) found inconsistent effects. Subedi et al. (2007) also applied 40 kg N at anthesis for the highest N rate. Studies by Hietaniemi et al. (2004), Váňová et al. (2008) and Yoshida et al. (2008) did not find any significant effects of N application on FHB or DON. Lower DON levels were found only at low nitrogen fertilization rates as described above (van der Burgt et al., 2011). Microclimatic differences during the flowering stage when infection occurs may have played a role in the inconsistent results (Osborne and Stein, 2007; Mesterházy, 2002). If weather conditions are very humid, and hence favourable for *Fusarium* development, even an open crop canopy will not dry sufficiently to slow down *Fusarium* development. Van der Burgt et al. (2011) also expect that such conditions would not show clear effects of nitrogen application rates and canopies differing in density.

In years with moderate levels of FHB, top dressing nitrogen in wheat increased grain-N contents, but simultaneously increased DON contents, decreased seed viability and in crops with relatively open canopies promotes weed growth (Timmermans et al., 2009, Weiner et al., 2001). That is why nitrogen top dressing increases FHB risk and is not advised in wheat crops. Field surveys in southwestern Ontario in Canada showed that fields with high weed densities had twice as many heads with FHB symptoms compared to weed-free areas (Teich and Nelson, 1984). The potential significance of weeds in the development of FHB epidemics has also been shown by Jenkinson and Parry (1994), who isolated *Fusarium* species, which proved to be pathogenic to wheat, from 14 species of common broad-leaved weeds. These researchers suggested that weeds provide an alternative source of inoculum for FHB epidemics, and that weed control may reduce inoculum availability (Pirgozliev et al., 2003).

3.1.2.5 Lodging

Lodged fields have been connected to an increase of total *Fusarium* and *F. graminearum*. According to Bernhoft et al. (2012), a significant correlation between lodged fields and use of mineral fertilizers was found. The results indicated that lodged fields were less connected to total *Fusarium* and *F. graminearum* than the use of mineral fertilizers. That is why an indirect connection between *Fusarium* and lodged fields via the use of mineral fertilizers producing tall and thick plants is plausible. Furthermore, Bernhoft et al. (2012) stated that the close contact between *Fusarium* on the ground and the cereal ears of lodged areas which have a lesser possibility of drying up after rainfall and morning dew might also play a role.

According to Nicholson et al. (2003) a study of 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. On the other hand, lodged fields were connected to decreased infection of *F. langsethiae*. Cereal ears close to the soil may receive less radiation from the sun. Accordingly, *F. langsethiae* infestation was linked to an elevated temperature in July (Bernhoft et al., 2012).

3.1.2.6 Organic farming

Bernhoft et al. (2012) reported that the mycotoxin problem has worsened in recent decades. Various trends in agriculture such as more humid growing seasons, insufficient of crop rotation, soil compaction by heavier machinery, and reduced soil tillage combined with herbicide spraying may explain this increase. The contents and versatility of mycotoxins produced are dependent on the *Fusarium* species and their ability to produce toxins, as well as the degree of the possible risk of mycotoxins concerning various cereal species (Foroud & Eudes, 2009; Bernhoft et al., 2010; Hofgaard et al., 2010).

According to the review paper of Brodal et al. (2016) the content of DON, T-2+HT-2 toxins, ZON, NIV, OTA and fumonisins in cereal grains have been studied intensively in organically and conventionally grown crops in temperate regions. Some of the studies have been based on data from controlled field trials, but most of them have been farm surveys and only some of them were food basket surveys. Almost half of the studies focused on DON in cereals. The majority of these studies found no significant influence of farming system on mycotoxin levels in cereal grains (Marx et al., 1995; Eltun, 1996; Berleth et al., 1998; Malmauret et al., 2002; Griesshaber et al., 2004; Champeil et al., 2004; Hietaniemi et al., 2004; Mäder et al., 2007; Hoogenboom et al., 2008; Edwards, 2009c; Kuzdraliński et al., 2013).

However, several studies showed less infestation of *Fusarium* species and lower mycotoxin content in organically than in conventionally produced cereals (Döll et al., 2002; Cirillo et al., 2003; Schneewis et al., 2005; Bakutis et al., 2006; Rossi et al., 2006; Pussemier et al., 2006; Köpke et al., 2007; Meister, 2009; Bernhoft et al., 2010; Edwards, 2009a, 2009b; Lacko-Bartosova & Kobida, 2011). Based on the Benbrook (2005) literature study, cereal kernels and food products derived from conventional production contain about 50% more mycotoxins than samples from the organic production system. Also, organically produced oats contained mainly lower levels of T-2+HT-2 toxins than conventionally produced oats. For example, Edwards (2009a, 2009b) compared the contents of T-2 and HT-2 toxins in wheat and barley with different production systems, and found that concentrations of these toxins were five times higher in oats and wheat samples derived from conventional farms. Three studies, one from Germany (Birzele et al., 2002), one from Poland (Perkowski et al., 2007) and one from the Czech Republic (Vanova et al., 2008), reported higher DON content in conventionally grown wheat produced without fungicide application compared to organically produced wheat. However, when fungicides, with effect against *Fusarium*, were used in conventional production, either lower DON content

was found in the conventionally than in the organically produced wheat (Perkowski et al., 2007), or no difference was seen in grain from the two farming systems (Birzele et al., 2002; Vanova et al., 2008).

Most studies on ZON reported no differences between farming systems. For the other mycotoxins such as NIV, DAS or 3-AcDON in cereals, mainly low levels and no differences between the two farming systems were reported. Brodal et al. (2016) reported that it cannot be concluded that either of the two farming systems increases the risk of mycotoxin contamination. Despite not using fungicides, an organic system appears generally able to maintain mycotoxin contamination at low levels. Many authors suggested that weather conditions, years, locations, tillage practice and crop rotation are more important for the development of *Fusarium* toxins than the type of farming (Bakutis et al., 2006; Bernhoft et al., 2010; Champeil et al., 2004; De Galarreta et al., 2015; Gimenez et al., 2012; Griesshaber et al., 2004; Hoogenboom et al., 2008; Meister, 2009; Munger et al., 2014; Quaranta et al., 2010).

It is known that organic farmers use crop rotation much more actively than conventional farmers. Based on the results of Bernhoft et al. (2012) the most striking difference between farming systems in Norway is found to be fertilization and pesticide treatments. The majority of conventional fields receive mineral fertilizers, only a few receive animal manure. Most of the organic fields receive no manure, possibly because the preceding crop is a green manure or clover ley. A few organic fields receive added mineral fertilizers. In contrast to Norway, in Finland animal manure is used quite a lot on the organic fields.

In Norway, according to Bernhoft et al. (2012) fungicides are largely used in conventional fields of wheat, to a lower extent on barley and minimal on oats. In Nordic countries the latter procedures are very much parallel, but the use of fungicides in barley and oat production is increasing all the time. The most commonly used fungicides in Norway were azoxystrobin combined with fenpropimorph or propiconazole combined with trifloxystrobin. Herbicides have not been employed in organic fields but used in most conventional fields irrespective of cereal species. The most common compounds have been tribenuron-methyl or MCPA (2-methyl-4-chlorophenoxyacetic acid) but also glyphosate have been used to a certain extent. Insecticides are not used in organic fields, but to some extent they have been used in conventional fields, particularly in wheat. The most commonly used compounds are alphacypermethrin or esfenvalerat. Chemical plant growth regulators have been used to some extent in conventional cereal production. The most common compounds are chlormequat chloride or etefon. Most organic producers use catch crops in the cereal fields, while only a few conventional producers use catch crops. In organic fields, fungicides, insecticides and chemical plant growth regulators are not used.

Studies by Bernhoft et al. (2012) suggest the use of mineral fertilizers was significantly connected to an increase of total *Fusarium* and *F. graminearum* in Norway. The results are in agreement with previous studies on the effect of nitrogen fertilizers on *Fusarium*, particularly *F. graminearum*, and DON, in cereal grains (Martin et al., 1991; Elen et al., 2000; Yi et al., 2001; Lemmens et al., 2004; Heier et al., 2005). Organic fertilizers seem to support *Fusarium*

to a lower extent. According to Bernhoft et al. (2012), both manure and other organic fertilizers were significantly connected to increased *F. graminearum* infestation, but to a lesser degree than mineral fertilizers. One possible explanation for the finding is that there may be particularly more *Fusarium* in the cereals with readily soluble nitrogen fertilizers. The nitrogen supply influences the chemical composition and cell wall structure of the plants (van Arendonk et al., 1997), and therefore the mold attack to the plants may be easier. Nitrogen fertilization increases the plants lushness and the humid microclimate in the fields, which may be optimal for *Fusarium* spread. Furthermore, nitrogen fertilization implies taller plants with heavier ears which increase the risk of lodging.

3.1.2.7 Physico-chemical factors of the soil

Besides the huge range of biotic soil factors influencing the *Fusarium* loads, physicochemical factors of the soil may also play a role. Particularly clay soil but also silty soil was connected to reduced infestation of *F. graminearum*. A range of reports indicates that soils rich in clay are more suppressive to *Fusarium* than coarser silty and particularly sandy soils (Amir and Alabouvette, 1993; Huang and Wong, 1998; Alabouvette, 1999; Knudsen et al., 1999; Shakhnazarova et al., 2000; Kurek & Jaroszuk-Scisel, 2003). An improved environment for *Fusarium* antagonistic microorganisms in clay soils is the proposed explanation (Bernhoft et al., 2012).

3.1.2.8 Long-range transport

Miller et al. (1998) reported that high concentrations of ascospores and macroconidia of *Fusarium*-species have also been trapped from the air during epidemics (Ayers et al., 1975; Martin, 1988; Paulitz, 1996; Tschanz et al., 1975). Inoculum for FHB can be dispersed from debris or other sources by air currents, insects and rain splash, land on the spike and cause disease (Fernandez and Fernandez, 1990; Miller, 1994). Damage caused by insects may be a point of entry for the FHB-causing species (Sutton, 1982; Warner and French, 1970; Windels et al., 1976).

3.1.3 Impact of weather conditions

According to Pugh et al. (1933) susceptibility to infection is the greatest from flowering to the early dough stage or Zadoks growth stages (GS) 60 to 83 (Zadoks et al., 1974; Tables 5 and 6). Infection is mainly dependent on the combination of rainfall, the duration of canopy wetness, and temperature conditions relative to the stage of wheat development. Pugh et al. (1933) found that wheat heads exposed to *F. graminearum* at 25°C for 36 h of continuous wetness were 18% infected, compared with 77% infected at 48 h of continuous wetness. Lacey et al. (1999) reported that they found minimal infection with a duration of wetness of less than 24 h. Temperatures of 30 to 32°C tend to reduce infection and fungal growth (Reid et al., 1999). Lacey et al. (1999) reported also reduced infection with temperatures of less than 9°C and greater than 26°C. Most of these studies have been conducted under controlled environmental conditions (Hooker et al., 2002).

Table 5. A decimal code for the growth stages of cereals: principal growth stages (Zadoks et al., 1974).

1-digit code	Description
0	Germination
1	Seedling growth
2	Tillering
3	Stem elongation
4	Booting
5	Inflorescence emergence
6	Anthesis
7	Milk development
8	Dough development
9	Ripening
T	Transplanting and recovery (rice only)

Table 6 presents a more detailed classification of the secondary growth stages by using a second digit, coded from 0 to 9, for each principal growth stage.

Table 6. A decimal code for the growth stages: secondary growth stages (Zadoks et al., 1974).

2-digit code	General description	2-digit code	General description
	Germination		Inflorescence emergence
00	Dry seed	50	First spikelet of
01	Star of imbibition	51	inflorescence just visible
02		52	1/4 of inflorescence
03	Imbibition complete	53	emerged
04		54	1/2 of inflorescence
05	Radicle emerged from caryopsis	55	emerged
06		56	3/4 of inflorescence
07	Coleoptile emerged from caryopsis	57	emerged
08		58	Emergence of
09	Leaf just at coleoptile tip	59	inflorescence completed
	Seedling growth		Anthesis
10	First leaf through coleoptile	60	Beginning of
11	First leaf unfolded	61	anthesis
12	2 leaves unfolded	62	
13	3 leaves unfolded	63	
14	4 leaves unfolded	64	Anthesis half-
15	5 leaves unfolded	65	way
16	6 leaves unfolded	66	
17	7 leaves unfolded	67	
18	8 leaves unfolded	68	Anthesis
19	9 or more leaves unfolded	69	complete

Tillering		Milk development	
20	Main shoot only	70	
21	Main shoot and 1 tiller	71	Caryopsis water ripe
22	Main shoot and 2 tillers	72	
23	Main shoot and 3 tillers	73	Early milk
24	Main shoot and 4 tillers	74	
25	Main shoot and 5 tillers	75	Medium milk
26	Main shoot and 6 tillers	76	
27	Main shoot and 7 tillers	77	Late milk
28	Main shoot and 8 tillers	78	
29	Main shoot and 9 or more tillers	79	
Stem elongation		Dough development	
30	Pseudo stem erection	80	
31	1st node detectable	81	
32	2nd node detectable	82	
33	3rd node detectable	83	Early dough
34	4th node detectable	84	
35	5th node detectable	85	Soft dough
36	6th node detectable	86	
37	Flag leaf such visible	87	Hard dough
38		88	
39	Flag leaf ligule/collar just visible	89	
Booting		Ripening	
40		90	
41	Flag leaf sheath extending	91	Caryopsis hard (difficult to divide by thumb-nail)
42		92	Caryopsis hard (can no longer be dented by thumb-nail)
43	Boots just visible swollen	93	Caryopsis loosening in daytime
44		94	Over-ripe, straw dead and collapsing
45	Boots swollen	95	Seed dormant
46		96	Viable seed giving 50 % germination
47	Flag leaf sheath opening	97	Seed not dormant
48		98	Secondary dormancy induced
49	First awns visible	99	Secondary dormancy lost

According to Snijders (1990), rainfall has been shown to be an important factor in determining the occurrence of ear infection by *Fusarium*. Correlations with relative humidity and prevalence of infection in the previous season have also been shown to be crucial. Sutton (1982) found that unlike *F. culmorum*, *F. graminearum* produces wind-dispersed ascospores as well as splash-dispersed conidia. Susceptibility of wheat to *F. graminearum* infection is well known, and anthers have been associated with infection. Infection is considered to occur between anthesis and the soft dough stage (GS 85). However, Lacey et al. (1999) suggest that the period of susceptibility to *F. culmorum* is much more limited. Infection by *F. graminearum* requires relatively high temperatures (20–30°C) and 48–60 h surface wetness. Few infections occur with less than 24 h surface wetness. *F. culmorum* predominates in climates cooler than those most favourable for *F. graminearum*. A few infections of *F. culmorum* were able to occur without misting and the frequency increased with

increasing wet period to at least 72 h. On the other hand, Aldred & Magan (2004) reported that drought-damaged plants are more susceptible to infection. In addition, according to Hooker and Schaafsma (2003) in Canada, Detrixhe et al. (2003) in Belgium, and Rossi et al. (2003) in Italy agrometeorological 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 NIV production by *F. culmorum* in relation to water activity and temperature. Magan et al. (2003) found that the conditions under which both toxins were produced were far more restrictive than the conditions allowing growth of the fungus. Production occurred in the relatively narrow aw range (0.995–0.95) while growth persisted to 0.90 aw. However, the optimal conditions for DON and NIV production, 25 °C at 0.995 and 0.981 aw, respectively, were within the range for optimal for growth. These aw 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 (Aldred & Magan, 2004). Studies by Birzele et al. (2000) showed that DON was produced by *F. culmorum* at 17% moisture content (0.80–0.85 aw) in natural wheat grain. These conditions are marginal for germination of conidia of *F. culmorum* and most other Fusaria, and under which growth would not typically occur (Magan and Lacey, 1984b; Sanchis and Magan, 2004; Hope et al., 2005). Mycelial growth has not observed at <0.90 aw.

Based on the results of Martins & Martins (2002) the maximum levels of DON and ZON were obtained on the 35th day of infection, the ZON level being much higher than that for DON. After 35 days, the culture conditions that gave higher yields of deoxynivalenol were at 22 and 28 °C. At an incubation temperature of 28 °C 16 days, followed by 12 °C, for the same time, the production was low. The highest content of ZON was obtained at 28 °C for 16 days, followed by incubation at 12 °C at the 35th day. When the temperature was constant at 28 °C, the ZON production was lower than when incubated at 22 °C, at the 35th day. *F. graminearum* did not produce deoxynivalenol and zearalenone at 37 °C.

Precipitation during cereal flowering will increase *Fusarium* infestation in the mature grain (Köpke et al., 2007). Lacey et al. (1999) reported that contamination with deoxynivalenol was greatest after inoculation at about mid-anthesis, but small amounts were produced following inoculation at other times, at least to the late milk-early dough stages (GS 77-83), without the visible disease. In Nordic countries cereal flowering typically occurs at the end of June and at the beginning of July. According to Bernhoft et al. (2012) a strong correlation between precipitation in July and total *Fusarium* was found in Norway (Langseth and Elen, 1997).

However, *F. graminearum* was negatively correlated with July rainfall. *F. graminearum* infestation is generally favored by warm weather (Miller, 1994). In temperate areas as in Nordic countries, warm weather seldom occurs with precipitation. Thus, the results of elevated cereal DON levels in Norway in 1988–1996 after a rainy July may have been more caused by *F. culmorum*. This species may grow under cooler conditions than *F.*

graminearum (Miller, 1994), and was a more important source of DON in Norwegian cereals in the 1990s (Langseth and Elen, 1997; Kosiak et al., 2003). However, it has to be taken into account that the recent trend of decreased *F. culmorum* and increased *F. graminearum* is reported from many European countries (Bernhofs et al., 2012; Leplat et al., 2012; Xu et al., 2005; Hope et al., 2005; Hope and Magan, 2003).

In the study of Bernhofs et al. (2012), *F. langsethiae* was positively correlated with mean July temperature. Accordingly, Medina and Magan (2010) found a temperature optimum for *F. langsethiae* growth at 25°C, similarly as for *F. graminearum*. This temperature is much higher than the Nordic countries typical mean July temperature. As a result of global warming both *F. graminearum* and *F. langsethiae* may become more common in Nordic countries, increasing the problems associated with DON and T-2+HT-2 toxins, respectively.

Climatic conditions just before the harvest time are also critical concerning the risk of mycotoxins. Mean temperature and humidity during a two-week period before harvest have been emphasized. Also temperature changes may increase DON production (Ryu and Bullerman, 1999). Medina and Magan (2011) suggest that high water availability appears to be particularly important for toxin production from *F. langsethiae*, and far more critical than temperature. Wet weather also before harvest seems to be particularly bad for cereal contamination with T-2 and HT-2 toxin as well as of DON.

3.1.4 Pesticide treatments

Control of Fusarium Head Blight and mycotoxins using various fungicides has provided inconsistent results due to the complexity of causal organisms, timing of application and masking control of one *Fusarium* species by the subsequent growth of another species (Heier et al., 2005; Parry et al., 1995). According to Mesterházy et al. (2003), DON contamination was reduced by the fungicide to a greater extent in the low-risk agronomic and environmental conditions than in the high-risk ones. Mesterházy et al. (2003) achieved a higher efficacy when the fungicides were applied to a moderately resistant cultivar rather than to a susceptible one. McMullen et al. (2008) reported that a fungicide application reduced the DON content by 31% when the previous crop was wheat, while a reduction of 57% occurred when it was canola. Use of fungicides in the management of FHB, caused by fungi such as *F. culmorum* and *F. graminearum*, has been shown to be at its best 77% and 89% effective in reduction of disease severity and mycotoxins content, respectively (Haidukowski et al., 2004). In turn, lower levels, of at most 70% effectiveness, have been reported for fungicide control in field conditions for naturally infected wheat (Stack, 2000; Wagacha & Muthomi, 2007; Blandino et al., 2012).

Many studies (Blandino et al., 2006) have shown that FHB and DON concentration could be strongly influenced by fungicide treatments applied at mid-anthesis. The treatment with triazole fungicides carried out at mid-anthesis can lead to a reduction in incidence, a reduction in DON concentration in the grain, and an increase in yield. On the other hand, seed dressing or fungicide application at shooting were confirmed to be less effective.

3.1.4.1 Fungicides

There has been a significant focus on the development and use of fungicides to prevent and control infection of pathogenic *Fusarium* spp. and mycotoxins during growing season of small grain cereal crops (Liu et al., 2011). Many studies have shown that fungicides with triazole chemistry have been the most effective, providing the most promising results in percent reduction in FHB and DON (El-Allaf et al., 2001; Hershman and Draper, 2004; Hershman and Milus, 2002; Hershman and Milus, 2003; McMullen et al., 1999; Mesterházy and Bartók, 1997, 2001) relative to the untreated trials. Metconazole, prothioconazole, and tebuconazole have been reported to be the most effective fungicides for the control of *Fusarium* spp. and reducing the level of the main mycotoxins that occur in cereal grain (Pirgozliev et al., 2002; Klix et al., 2007; Paul et al., 2008). Based on the results of Paul et al. (2008) metconazole was the most effective treatment, with an efficacy of 45%, while the other active substance efficacies were 43% (prothioconazole), 23% (tebuconazole) and 12% (propiconazole). According to Koch et al. (2006) a tebuconazole treatment only slightly diminished the DON concentration by 14% when a medium-tolerant cultivar was cultivated after ploughing, while spraying a fungicide decreased the DON content by 71% when a susceptible variety was grown with minimum tillage conditions (Blandino et al., 2011, 2012).

According to Blandino et al. (2011) the prochloraz and epoxiconazole mixture application at heading confirms a clear effect on delaying flag leaf senescence, increasing grain yield and reducing FHB incidence and severity, and DON contamination. These effects were greater with higher ear and leaf disease pressure. As a consequence of fungicide application at heading in naturally-infected conditions, Blandino et al. (2006) and Ransom and McMullen (2008) observed an increase in grain yield of 11% and 5% in the lowest fungal pressure and of 27% and 44% in the highest one, respectively. In durum wheat cultivated in North Italy, the application of prochloraz and ciproconazole mixture at heading led to yield advantages between 8% and 40% (Blandino et al., 2009). On the other hand, based on the results of Milus & Parsons (1994) and Edwards et al. (2001) the application of azole fungicides for FHB control and DON grain accumulation significantly reduced FHB symptoms, but did not affect DON contamination.

According to Nicholson et al. (2003), the use of azoxystrobin showed a significant reduction in disease levels while increasing the levels of DON present in the grain. 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 a concomitant increase in toxin formation (Aldred & Magan, 2004). In contrast, Pirgozliev et al. (2008) reported in artificially inoculated trials that both metconazole and tebuconazole have reduced DON in grains by 50–60% more than applying azoxystrobin on its own, or in a mixture with either of the two triazole fungicides (Blandino et al., 2011). Pirgozliev et al. (2008) also emphasized the right timing of fungicide application (Figure 11).



Photo: Saara Liespuu

Figure 11. Right time (Z 64-65; anthesis halfway) of fungicide application for oats.

Dimmock and Gooding (2002) stated that the inclusion of strobilurin-fungicides in wheat FHB studies have been associated with an extended flag leaf life and increased grain yields and grain protein content. Strobilurins, which block electron transport in the mitochondrial respiratory chain, are also able to induce a much longer duration of the green flag leaf area compared to azoles (Ruske et al., 2003). Moreover, these fungicides have been shown to alter phytohormones level, to increase the activity of antioxidative enzymes and the net rate of photosynthesis (Oerke et al., 2001). Strobilurins have instead shown poor efficacy for the control of FHB caused by toxigenic *Fusarium* spp. (Pirgozliev et al., 2002). Also, in vitro and field studies strobilurins have revealed in some cases an increase in DON accumulation (Menniti et al., 2003). To assure lower mycotoxin contamination in wheat grain, the application of strobilurin fungicides is only recommended in a mixture with azoles (Pirgozliev et al., 2003; Blandino et al., 2011).

It should be noted that there are only a few studies carried out to examine the effect of anti-fungal agents on the growth of *F. langsethiae* strains or their ability to produce T-2 and HT-2 toxins (Magan et al., 2011; Medina & Magan, 2010; Kokkonen et al., 2010; Medina and Magan, 2011). Mateo et al. (2011) found that fenpropimorph, prochloraz, and tebuconazole were effective in controlling the growth of *F. langsethiae* and production of T-2 and HT-2. Prochloraz and tebuconazole were more effective than fenpropimorph against *F. langsethiae*. Fenpropimorph is more efficient against other species, such as *M. nivale* (Debieu et al., 2000). This anti-fungal agent belongs to themorpholine group of sterol biosynthesis inhibitors (Campagnac et al., 2009; Debieu et al., 1992; Marcireau et al., 1990) and is widely used to control pathogens, such as powdery mildew, rusts and leaf blotch diseases of cereals (Leroux, 2003). According to Mateo et al. (2011) strain, temperature, and type of fungicide significantly affected growth rate. These azole-based compounds were

more effective at 15 than at 20–25 °C. *F. langsethiae* has caused problems especially in oats in cooler climatic regions such as the UK, Ireland, and Scandinavia (Mateo et al., 2011; Edwards et al., 2009, 2009b; Hietaniemi et al., 2016).

Mankeviciene et al. (2008) reported that tebuconazole increased T-2 toxin levels in rye and winter triticale infested with *Fusarium* spp. compared with untreated cereals. DON production was also increased in barley crops treated with this fungicide (Malachova et al., 2010). Prothioconazole, quite a new triazole fungicide, was found to trigger DON biosynthesis when added at sub lethal doses in cultures of *F. graminearum* (Audenaert et al., 2010). Studies by Hershman & Draper (2004) and Paul et al. (2005) found that prothioconazole in combination with tebuconazole contributed to a significantly greater reduction in visual symptoms of FHB and DON contamination of grain than tebuconazole alone (Hershman and Draper, 2004; Paul et al., 2005). Therefore, it is necessary to perform additional studies on the influence that ecological factors and sub-inhibitory doses of the most commonly used agricultural antifungal agents have, not only on the development of fungal resistance but also on mycotoxin production (Haidukowski, M., 2005; Mateo et al., 2011).

Based on the results of Blandino et al. (2006) seed dressing with tebuconazole did not reduce the FHB incidence. A double treatment carried out at the end of shooting and then at anthesis did not show any substantial advantage over the single treatment at anthesis, for all the evaluated parameters. The trials treated with a mixture of triazoles and strobilurin, though offering 8% better yields compared to treatments with triazoles and showing a reduction of 80% symptomatology, led to a DON content which was often higher than the untreated control in the cooler and wetter conditions and environments.

In conclusion, according to Paul et al. (2008) prothioconazole+tebuconazole was the most effective fungicide for *Fusarium* disease, followed by metconazole (50%), prothioconazole (48%), tebuconazole (40%), and propiconazole (32%). For DON, metconazole was the most effective treatment, prothioconazole+tebuconazole and prothioconazole showed similar efficacy. Recent evaluations of integrated approaches for managing FHB and DON revealed that percent control increased substantially more when fungicide application was combined with residue management and cultivar resistance (Bayer et al., 2006; McMullen, 2007; Paul et al., 2007) than when any of these strategies were used alone.

3.1.4.2 Herbicides

Based on the results of Altman (1993) and Levesque & Rahe (1992) herbicides, including glyphosate, can inhibit or stimulate the growth of fungal pathogens, and can either increase or decrease disease development through direct or indirect means. Levesque and Rahe (1992) showed evidence that herbicides can have a direct effect on various components of the soil microflora, such as plant pathogens, antagonists, or mycorrhizae, which can potentially increase or decrease the incidence of plant disease. Pathogens able to infect

weeds can also enhance their inoculum potentials after weeds have been sprayed with herbicides, which could subsequently affect host crops (Fernandez et al., 2009).

The observation that *Fusarium* infections increased in fields previously sprayed with glyphosate agrees with reports of the association of glyphosate with *Fusarium* colonization of other crops. Several studies have shown a stimulatory effect of glyphosate on *Fusarium* populations (Kawate et al., 1997; Kremer et al., 2005; Levesque and Rahe, 1992; Rahe et al., 1990; Sanogo et al., 2001), including *F. avenaceum* and *F. culmorum* (Brown and Sharma, 1984; Levesque et al., 1987). Levesque et al. (1987) reported that glyphosate increased root colonization by *F. avenaceum* and *F. oxysporum* of various treated weeds, as well as increasing the propagule density of these fungal species in soil. Johal and Rahe (1984) and Rahe et al. (1990) showed that the glyphosate-induced root colonization by *Fusarium* spp. and other pathogens was the cause, and not the result, of plant death following application of certain doses of glyphosate, and that the efficacy of glyphosate depended on the synergistic action of these species and others in the soil.

Kawate et al. (1997) reported that *Fusarium* populations were greater in rhizosphere soil from glyphosate-treated than from untreated henbit (*Lamium amplexicaule* L.). They suggested that weed control with glyphosate in the spring might provide *Fusarium* pathogens an energy source for survival and proliferation. Glyphosate-treated couch grass was also rapidly colonized by *F. culmorum*, which subsequently caused damage to the following barley crop (Lynch and Penn, 1980). Brown and Sharma (1984) reported that flax plants treated with glyphosate were rapidly colonized by several species of fungi, including *F. culmorum*. According to Fernandez et al. (2009) glyphosate could also act directly on plants by inhibiting their phenolic metabolism which could potentially affect plant resistance, thus, causing them to be more susceptible to pathogenic organisms. Glyphosate was also shown to have a differential effect on fungi, thus potentially altering the outcome of competition between them (Wardle and Parkinson, 1992).

According to Bernhoft et al. (2012), relations between *Fusarium* infestation and the use of herbicides have not been studied so intensively compared to the various studies of fungicides for FHB. A study by Altman and Rovira (1989), glyphosate was shown to increase the incidence of *Fusarium* and other soil-borne pathogens more than two decades ago. On the other hand, Henriksen and Elen (2005) did not find an effect of glyphosate spraying on total *Fusarium* in wheat, barley or oats. However, Fernandez et al. (2009) reported a range of experiments on the effect of glyphosate on *Fusarium* infection in wheat and barley fields. Glyphosate treatment was consistently connected with higher FHB, particularly due to *F. graminearum* and *F. avenaceum*. Fernandez et al. (2009) suggested that the herbicide might cause changes in fungal communities via various mechanisms implying stimulation of *Fusarium* and impairment of other fungi as well as potentially impacting plant resistance.

3.1.5 Biological control agents

Biological control offers an additional strategy and can be used as part of an integrated management strategy for suppressing FHB. During the last twenty years, a number of studies have been carried out to screen a range of potential biocompetitive microorganisms to *Fusarium* pathogens of cereal ears (Dawson et al., 2002a, 2002b). These studies have shown that there are potential microorganisms which 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 some existing competitive microorganisms are effective at decreasing *Fusarium* fungi infestation and DON levels significantly. According to Aldred & Magan (2004) potential does exist in this area because 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 required protection. Potential for commercialization of these candidates has been examined intensively.

In vitro assays and trials in greenhouses and under field conditions have been shown that some bacteria within the genera *Bacillus* and *Pseudomonas* were able to reduce *F. graminearum* growth. Applications of the bacterial strain AS 43.4 (*Bacillus* spp.) isolated from wheat anthers decreased disease severity of FHB under glasshouse conditions by 67–95% and DON concentration in grain by 89–97% (Khan et al., 1999). Also, yeasts belonging to the genera *Rhodotorula*, *Sporobolomyces* and *Cryptococcus* were effective in controlling FHB (da Luz, 2000; Schisler et al., 2000; Wang et al., 2007). Several bacteria and fungi have been isolated, and some are being evaluated for commercial development as biopesticides (da Luz, 2000; Khan et al., 2004). However, according to Palazzini et al. (2009), one of the main limitations in the use of biopesticides is the limited tolerance to fluctuating environmental conditions and the difficulties in developing a stably formulated product. The level of water stress encountered by microorganisms in natural environments is an important physical parameter that influences their ability to grow and successfully compete for a determined specific habitat. There are some reports on physiological stress responses among biological control agents, most of them describing filamentous fungi or yeasts (Hallsworth and Magan, 1995, 1996; Frey and Magan, 1998; Teixidó et al., 1998; Pascual et al., 2000; Dunlap et al., 2007) and relatively little data related to bacteria (Bochow et al., 2001; Teixidó et al., 2005; Cañamas et al., 2007; Palazzini et al., 2009).

Betaine referred, referred to as glycine-betaine, is a commonly determined osmoprotectant. It is produced in large quantities by phototrophic bacteria in hypersaline environments. Betaine appears to be the most effective osmolyte accumulated in *B. subtilis* cells growing under osmotic stress conditions when its precursor, choline, is present. The high level of betaine allows *B. subtilis* cells to grow over a wide range of salinities (Whatmore et al., 1990; Holtman and Bremer, 2004). Betaine and also choline have been found in wheat flower tissues and have been implicated in stimulating the hyphal growth of the primary causal agent of FHB, *F. graminearum*. Choline metabolizing strains (CMS) from wheat anthers may, therefore, be a useful source of antagonists of *F. graminearum*.

According to Schisler et al. (2006), 123 of 738 microbial strains that were recovered from wheat anthers collected from plants grown in Illinois and Ohio in the USA were CMS as determined by growth in a liquid medium containing choline as a sole carbon and nitrogen source and a colorimetric, choline oxidase-based assay of culture filtrate. Thirty-one out of 123 CMS reduced FHB disease severity by at least 25% in greenhouse tests on wheat and 17 reduced FHB infection by at least 50%.

Based on the review by Wagacha and Muthomi (2007), there have also been efforts to identify biological antagonists, which could be used for example in integrated pest management (IPM) strategies. Isolates of *Clostrachys rosea* have been shown consistently to suppress sporulation of *F. culmorum* and *F. graminearum* on wheat straw and of *F. culmorum*, *F. graminearum*, *F. proliferatum* and *F. verticillioides* on maize stalks (Luongo et al., 2005). A strain of *F. equiseti* has been shown to consistently decreasing DON (470%) on wheat inoculated with *F. culmorum* with similar performance to the standard fungicide tebuconazole (Dawson et al., 2004). Diamond and Cooke (2003) reported a 60% reduction in FHB symptoms relative to the control treatment after 25 days on ears pre-inoculated with *Phoma betae* and challenged with *F. culmorum*. They further reported a significant increase in the number of grains per ear of wheat pre-inoculated with *Pythium ultimum* and *Phoma betae*. Two strains of *Pseudomonas fluorescens* have been reported to inhibit the growth of *F. culmorum* both in vivo and in vitro (Kurek et al., 2003; Wagacha & Muthomi, 2007).

According to Schisler et al. (2002) three (*Bacillus* strains 43.3 and 43.4 and *Cryptococcus* strain OH 182.9) out of seven FHB antagonists reduced disease severity by 48–95% and decreased DON quantity in grain by 83–98%. Unfortunately, under field conditions, the same antagonistic strains gave variable results. The *Bacillus* strains had no effect on either FHB severity or DON concentration in grain, while *Cryptococcus* strain OH 182.9 reduced FHB and DON by 50%. On the other hand, field studies by McMullen et al. (2002) showed that while the fungicide tebuconazole provided significant control of FHB, strain OH 182.9 had no effect on disease development. Pirgozliev et al. (2003) reported that unfortunately the discrepancy between the performance of biocontrol agents under environmentally controlled and field conditions is an issue that is commonly observed. There is a huge need for the development of commercial biocontrol products.

Only a few biocontrol products have been successfully commercialized. Palazzini et al. (2009) stated that one reason for this lack of commercial success is that biocontrol agents are living organisms affected by variable environmental conditions. Preadaptation to one particular stress condition can also render cells resistant to other stresses, a phenomenon known as cross-protection (Sanders et al., 1999). Furthermore, preparation of biocontrol agents, e.g. spray drying and storage, as well as field application protocols, may reduce the viability of the biocontrol agent (Costa et al., 2001; Teixidó et al., 2006; Yuen et al., 2007). Teixidó et al. (2006) showed that NaCl-stressed *P. agglomerans* cells survives the spray-drying process better than do the control cells. Thus, physiological improvement by osmotic treatments and the accumulation of compatible solutes could increase bacterial survival during the formulation process, storage and application stages. The physiological

improvement of biocontrol agents could be an effective strategy to enhance stress tolerance and biocontrol activity under fluctuating environmental conditions.

3.2 Control and manage the risk post-harvest

Poor post-harvest management can result in rapid quality loss in cereal grains as well as risk from mycotoxins. This is a particular problem in wet harvest years. The elimination of post-harvest fungal infection and production of mycotoxins during harvest drying and prolonged periods of grain storage can be managed in a controlled manner through good agricultural practices (GAP) and good manufacturing practices (GMP). By quality assurance, the drying procedure is optimized, and moisture levels in stored grain remain below risk-free levels. However, it is taken into account that spores of *Fusarium* species are ubiquitous in soils, on equipment, and inside storage structures despite thorough cleaning. Consequently, germination of mycotoxigenic species can occur within certain storage conditions if even a small amount of stored grain develops elevated temperature and moisture levels. Unfortunately, the size and design of large grain storage structures and the limited availability of technology often make precise monitoring of moisture and temperature impractical (Proposed Draft Revision of the Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals (CAC/RCP 51-2003), Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods, 2015).

3.2.1 Harvesting and storing of grain

Quality management of the grain supply chains ‘primary production – food and cosmetic industry – consumer’ or ‘primary production – livestock production – food industry – consumer’ can be developed further at the farm level by right timing of harvest (Figure 12), increasing measurement technology, and improving logistics management of the process of harvesting, drying and storing of grain.



Photo: Veli Hietaniemi

Figure 12. The crop is ready for harvest in 2014 in Sastamala, Finland.

Along with the management of the chain, specialized sections of the chain will also provide more possibilities to increase the export of cereals and processed grains, e.g. organic oats, pure oats, baby food and batches of varieties. At this stage, versatile grain quality analysis such as the near-infrared transmittance (NIT)/near-infrared reflectance (NIR) technique, documentation, and storage of application-specific quality adopt a significant role and require the development of existing systems. At present, a major proportion of harvested grain is already being stored in farm silos, and single plot-specific accounting reveals cultivation history.

Once the grain is dried and placed in a weather-proof and rodent-proof structure it can usually be stored for long periods without suffering quality loss from insect feeding or fungal deterioration leading to such conditions as lowered germination, increased free fatty acids and biochemical changes (Tipples, 1995). However, if the grain is stored in a large silo without aeration, it will be indirectly affected by the weather. The silo will retain the heat it had at harvest in the centre of the silo (Jays et al., 1994) and reflect external ambient temperatures near the periphery. In Canada and Nordic countries, grain can remain warm (15–20 °C) throughout the winter although outside temperatures can even be -20 to -30 °C. Air convection currents in silo carry moisture to the top-centre of the batch which can result in localized grain spoilage (Jayas, 1995; Sinha et al., 1973) and mycotoxin production (Abramson, 1991; Jayas & White, 2003).

Generally, grain stored at a moisture content equivalent to less than 0.70 aw (<14.6% moisture by weight) will not be subject to post-harvest fungal contamination and mycotoxin production. However, grain is often harvested at moisture levels far more than this and is often traded on a wet weight basis. Also, there are still some technological challenges

associated with bulk drying and storage of grain and instances of poor practice and negligence. According to Aldred and Magan (2004) 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 many actors 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 industry. 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 various 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 aw and maintaining the grain in this condition. According to Aldred and Magan (2004) the most important control measures relevant to storage stages may be as follows:

- Regular grain moisture measurement.
- Careful, efficient and fast drying of wet grain. Avoid delayed waiting period before drying.
- Good logistics systems.
- Modern warehousing and storage conditions.
- Facilities and equipment for fungal and mycotoxin determination or cooperation network for analysing these things.
- Operating system for grain drying and storage.

Although complicated, the post-harvest stages in the grain commodity chain, including drying, storage, transport, milling and baking, are far more conducive to a Hazard Analysis and Critical Control Point (HACCP) analysis than the pre-harvest stages. Unlike pre-harvest, these steps are characterized by the ability to apply definitive control measures and use versatile grain quality analysis, 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 (Aldred & Magan, 2004; Lacey et al., 1999).

3.2.2 Interactions of fungi during drying and storage

Surprisingly little research has been carried out concerning the spoilage fungi which interact with each other in the stored grain ecosystem and the effect of storage conditions on mycotoxin production. Aldred and Magan (2004) indicated that significant inter- and intra-specific interactions occur, depending on the species present and the prevailing environmental conditions. Most importantly, the dominance of particular 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 aw 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; Aldred and Magan, 2004).

It should also be taken into account 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 show high tolerance to the presence of some mycotoxins (Aldred & Magan, 2004).

3.2.3 Impact of sorting and dehulling – oats

According to Hietaniemi et al. (2008) industrial sorting and dehulling reduced the DON levels in oat samples by 75–91%. After sorting and dehulling, the concentrations of T-2 and HT-2 as well as NIV toxins were at or below the limit of detection (25 µg/kg). The process reduced the levels by at least 87% (Schwake-Anduschus et al., 2010). The 3-AcDON concentrations were reduced by 67–91%. Ground hull samples were also analyzed for trichothecene since industrial dehulling proved to be a highly efficient method for reducing mycotoxin levels in oats used as raw material. The levels of DON in the ground hull were approximately twice as high as in oats that had not been dehulled. If the ground hull is to be used in food or feed, it is critical to determine the mycotoxin levels. Hull waste from oats highly contaminated by mycotoxins is only suitable for non-food use, for example for energy purposes (Scudamore et al., 2007).

Besides traditional mechanical sorting based on the kernel size, optical sorting based on colour of kernels is used in the food and feed industry. Sophisticated optical sorters are provided with high-speed cameras. A very new sorter that recently entered the market uses near-infrared technology to look at the chemical structure and composition of kernels to pick out the *Fusarium* -infected kernels and set them aside for other purposes than food or feed. This technology can be used to sort durum wheat, soft wheat and malting barley on *Fusarium*, protein and vitreousness, at a speed of 25,000 kernels per second. A distinct advantage of the NIT -technique is that the sorter can look for *Fusarium* infected kernels without a visual manifestation. A disadvantage may be lower throughput and higher acquisitions costs compared to the mechanical and optical sorters.

3.2.4 Use of preservatives

The use of chemical preservatives in wheat-based food production only becomes necessary in the later processing stages, such as the use of propionates in bread. On the other hand, current pressure in the food industry is to reduce the use of chemical additives. However,

recent studies have 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, butyl hydroxyanisole (BHA), propyl paraben (PP), cinnamon oil and resveratrol gave a greater than 90% reduction in DON and NIV accumulation (Aldred & Magan, 2004). An antioxidant, resveratrol, in particular, showed a broad spectrum of mycotoxin control, although this is a relatively expensive product (Fanelli et al., 2003).

3.2.5 Processing – milling, bread making and malting process

3.2.5.1 Milling and bread making

In the case of wheat, primary processing will typically be milling to produce flour for bread making. The flour produced at the factory is susceptible to the same mycotoxin hazards as grain under similar environmental conditions. Therefore, the production, storage and transportation of flour require mostly the same type of management as is needed for grain.

However, due to the way the bread-making industry operates, the majority of flour produced will be in storage silos for a short period. 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 individual grain components during milling such as colour sorting could result in the reduction in toxin levels in contaminated grain. The bread making process itself does not appear to present any significant risk factors in relation to mycotoxin development (Aldred & Magan, 2004).

3.2.5.2 Malting process

Barley is one of the most important cereals in the world with an estimated global production above 143 million tons using 48 million hectares in 2016 (U.S. Department of Agriculture (USDA), 2016). The industrial use of barley grain has experienced continuous growth mainly due to its economic importance for malt production. The global malting industry accounts for an annual malt production capacity of 22 million tons where more than 90% is made from barley, the majority of which supplies the brewing industry. Raw barley accounts for up to 70% of total malt production costs (Food and Agriculture Organization (FAO), 2009) and in turn, barley malt contributes up to 40% of the costs of beer production. Therefore, it is important to reduce manufacturing costs and decrease raw material losses. The expected increase in globalization of the malting barley trade will be directly associated with an increased fungal infection and cross-contamination risk.

Fusarium species are a considerable threat to malt standard quality attributes as they interfere with the process. According to Oliveira et al. (2012), filamentous fungi can produce mycotoxins with carcinogenic and mutagenic influences transferable from grains to malt and other processed foods, such as beer (Champeil et al., 2004; Lancova et al., 2008;

Schwarz et al., 1995; Wolf-Hall & Schwarz, 2002). Fungal infection can be responsible for low aeration, as well as the secretion of undesirable enzymes, mycotoxins, hormones, and acids, which will affect barley metabolism and cause higher malting losses (Noots et al., 1999; Liddell et al., 2003; Oliveira et al., 2012). Based on the results of Noots et al. (1999) and Laitila et al. (2007), microorganisms found on barley are primarily mesophilic and psychotrophic depending on geographic and climatic conditions. This includes saprophytic and parasitic organisms that generate complex interactions facilitating grain colonization and exploiting its nutrient resources to proliferate both externally and internally. Complete elimination of mycotoxin-contaminated commodities may not be achievable (Codex Alimentarius, 2003), but a reduction is essential for the consumer.

According to Oliveira et al. (2012) the increased temperature during kilning in the malting process provides adverse conditions for fungal growth and elevation of DON levels. The fungus is viable up to the 50 °C stage of kilning (Vegi et al., 2011) and under heat stress produced excessive amounts of DON, as is supported by previous research (Champeil et al., 2004; Sarlin et al., 2005; Wolf-Hall, 2007). Recently, Vegi et al. (2011) studied the behaviour of high-quality, artificially inoculated and naturally infected barley grains, measuring the *F. graminearum* Tri5 gene and its production of DON during malting. The authors found differences in DON production and *Fusarium* growth (Tri5 DNA concentration) depending on the infection pattern (Vegi et al., 2011). Grains were inoculated after steeping, and the highest fungal and DON concentrations were measured shortly after the start of kilning (49°C-54°C). The depth of infection in affected grains and the capacity to colonize new grains are two major factors which contribute to *Fusarium* survival during malting.

Malting is a complex ecosystem where favourable conditions for microbial growth are present regarding nutrients, moisture, and temperature-enabling microorganisms to interact with the grains metabolically during the process. Accordingly, microorganisms will have a significant influence on malting performance and final malt quality (Laitila, 2007; Laitila et al., 2006, 2007; Noots et al., 1999; Raulio et al., 2009; Wolf-Hall, 2007). Microbes can also have beneficial effects during malting, such as the production of hydrolytic enzymes and hormones contributing to malt modification (Laitila, 2007; Laitila et al., 2011). Contents of DON in barley are strongly correlated with final concentrations in the malt and can influence wort colour (Schwarz et al., 2006; Oliveira et al., 2012).

3.2.6 Mycotoxin inactivation

One way to reduce the uptake of mycotoxins from contaminated feed is the use of mycotoxin binders. The aim of these additives is to inhibit the uptake of mycotoxins by an animal in vivo. The use of mycotoxin binding agents is occasionally recommended to farmers in order to protect animals against the harmful effects of mycotoxins occurring in contaminated feeds. These adsorbent materials are intended to act like a chemical capture

and adsorb mycotoxins in the gastrointestinal tract, thus preventing the uptake and subsequent distribution to target organs (Kolossova et al., 2009).

Five classes of mycotoxins are of major concern in animal husbandry: aflatoxins, trichothecenes, zearalenone, ochratoxins, and fumonisins. These toxins cause significant economic losses. Physical, chemical, and biological detoxification methods to decrease mycotoxin contents in food and feed have been used with varying success (Bhatnagar et al., 1991; Schatzmayr et al., 2006). Physical procedures are cleaning, mechanical sorting and separation as described previously in the text but also washing, density segregation, thermal inactivation, irradiation, ultrasound, and solvent extraction have been used. Also chemicals, like ammonium hydroxide to detoxify DON or calcium hydroxide monoethylamine to diminish the content of aflatoxin, T-2 and HT-2 toxin, DON, and ZON, have been tested. However, physical and chemical detoxification methods give often conflicting results or do not work reliably, and are too expensive (Avantaggaito et al., 2004, 2005). On the other hand, they may destroy or remove essential nutrients from the feedstuff and reduce feed use (Scott, 1991). Also, clay and zeolitic minerals have been used in animal nutrition to bind mycotoxins, but the binders are only very specific for aflatoxins and not other toxins. Among all aluminosilicates tested concerning mycotoxin adsorption, hydrated aluminosilicate (HSCAS) have been the most extensively studied. According to Avantaggaito et al. (2004), no adsorbent materials, with the exception of activated carbon, showed relevant ability in binding DON and NIV.

According to Schatzmayr et al. (2006) a novel strategy to control the problem of mycotoxicoses in animals is the application of microorganisms capable of biotransforming mycotoxins into nontoxic metabolites. The first microorganism with mycotoxin degradation activity was *Flavobacterium aurantiacum* with the ability to detoxify aflatoxins (Ciegler et al., 1996). Wegst and Lingens (1983) proved degradation of ochratoxin A by the aerobic bacterium *Phenylobacterium immobile*. *Gliocladium roseum* detoxified zearalenone by ring opening with subsequent decarboxylation in yields ranging between 80 and 90% (El-Sharkawy & Abul-Hajj, 1988). It is known, that the 12,13-epoxide ring is responsible for the toxic activity of trichothecenes. Breaking up this epoxide group causes a significant loss of toxicity. Several authors have described this de-epoxidation reaction of ruminal or intestinal flora (Kollarczik et al., 1994; He et al., 1992; Yoshizawa et al., 1983; Yoshizawa et al., 1994). Binder et al. (2000) were the first to isolate a pure bacterial strain, which was able to biotransform the epoxide group of trichothecenes into a diene (Fuchs et al., 2002).

According to Binder et al. (2000) deoxynivalenol is enzymatically converted by an epoxidase of *Eubacterium* BBSH 797 to the nontoxic metabolite deepoxy-deoxynivalenol (DOM-1). This strain has been isolated out of bovine rumen fluid. The mode of action was proven in vitro and also in vivo by applying trichothecenes, and the detoxifying strain *Eubacterium* BBSH 797 has been the first microbe used in a mycotoxin deactivation in feed additive. According to Fuchs et al. (2002) a treatment of T-2 toxin with BBSH 797 resulted to a partial hydrolysis to HT-2 toxin. No further degradation occurred. In the case of HT-2

toxin a treatment with BBSH 797 caused complete transformation of HT-2 into its deepoxy form.

Based on the results of Bruinink et al. (1999) and Schatzmayr et al. (2003) a novel yeast strain was isolated and characterized, which is capable of degrading ochratoxin A and zearalenone. Due to the yeast affiliation to the genus *Trichosporon* and to its main property to degrade OTA and ZON, this strain was named *Trichosporon mycotoxinivorans* (MTV, 115) (Molnar et al., 2004). The yeast can detoxify OTA by cleavage of the phenylalanine moiety from the isocoumarin derivate ochratoxin alpha (OT α). This metabolite has been described to be nontoxic or at least 500 times less toxic than the parent compound (Bruinink et al., 1999; Schatzmayr et al., 2003). The metabolization of ZON by *T. mycotoxinivorans* leads to a compound that is no longer estrogenic. This has been proven in an *in vitro* assay with breast cancer cells (Schatzmayr et al., 2003).

3.3 Forecasting mycotoxin risk

The primary objectives of developing a useful predictive model are to get accurate predictions for the degree of the *Fusarium* fungi and mycotoxin risk in growing cereal grains. Various *Fusarium* prediction models have been developed regarding disease symptoms, incidence, and mycotoxins mostly in wheat and maize (Table 7).

Table 7. *Fusarium* prediction models concerning disease symptoms, incidence, and mycotoxins mostly in wheat and maize (Xu, 2003).

Reference	Cereal grain	Mycotoxin or infection risk	Model and nickname	Country of operation
Rossi et al. (2001a)	Wheat, maize	FHB: sporulation, spore dispersal and infection	Risk assessment	Italy
Hooker et al. (2002)	Wheat	DON	Descriptive; DONcast	Canada, United States, Uruguay, France
Hooker and Schaafsma (2005)	Wheat	DON, fumonisin B1	Descriptive	Canada
Froment et al. (2011)	Wheat, maize	DON, ZON, fumonisins	Predictive; Qualimetre	France, Belgium
Maiorano et al. (2009), and Asselt et al. (2012)	Maize	Fumonisin	Predictive simulation	Italy, Netherlands
Torelli et al. (2012)	Maize	DON, ZON, fumonisins	Preliminary (neural network)	Italy
Van der Fels-Klerx et al. (2012)	Wheat	DON	Predictive	Netherlands & Scandinavia

According to Rossi et al. (2001a) system-based models are developed by dividing the whole process into several sub-processes such that the individual sub-processes can be predicted accurately from variables that can be easily obtained. Risk indices are then derived by linking individual sub-models sequentially. This model included three sub-processes: sporulation, spore dispersal, and infection. The model finally estimates daily infection risks (Xu, 2003).

Hooker et al. (2002) developed a predictive tool ‘DONcast’ to assist producers in decisions on whether or not to apply a fungicide, and for grain marketing decisions. Growers and crop advisors in Ontario in Canada have used it since 2000. DONcast was developed from data collected from over 750 farms across Ontario since 1996. Detailed agronomic and weather information were obtained for each DON sample, resulting in a very diverse, eclectic dataset representing combinations of agronomic and environmental variables. The high degree of diversity amongst the climatic and agronomic variables is highly desirable for multiple regression and subsequent robustness of the model for accurate predictions under a range of conditions in wheat-growing areas of the world.

For a prediction threshold of 1.0 mg/kg for example, predictions greater than 1.0 mg/kg would indicate favourable conditions for DON accumulation, and therefore, growers may use 1.0 mg/kg as a trigger to spray a fungicide for disease suppression and thus lower the DON in grain at harvest. Similarly, with predictions less than 1.0 mg/kg, growers may use this information in favour of a no-spray decision. Overall, DONcast has been accurate in 80 – 85% of the predictions in Ontario using a decision threshold of 1.0 mg/kg. Since 2000, it has been validated and calibrated in other regions of the world, including the United States, the prairie regions of Canada, and in Uruguay (Schaafsma et al., 2006) and France (Schaafsma & Hooker, 2007).

Hooker and Schaafsma (2005) in the descriptive model reported that year and variety had the largest impact on concentrations of both DON and Fumonisin B1 (FB1). The variety explained 25% of the variation in FB1 and DON; the year effect 19% and 12%, respectively. Pre-crops had a much smaller but still a notable effect. When interpreting these results it needs to be taken into account that in the mild climate of Ontario the variations of temperatures, rain and soil water content are much lower than in warmer climates.

Maiorano et al. (2009) presented a preliminary version of FUMAGrain, a dynamic risk assessment model developed with data from the northern regions of Italy. The elements of the pathosystem were simulated by three submodels: maize development, *F. verticillioides* infection and fumonisins synthesis, and European corn borer wounding activity on maize grain. Systemic infections were not taken into account. Inputs to the final model are planting date, hourly meteorological data including temperature, relative humidity, wind speed and rain intensity, information on the phenological development of the variety planted, and information on chemical treatment against European corn borer. FUMAGrain gives an initial risk alert at the end of flowering based on the meteorological conditions during this phase. A second alert follows maturation when an assessment is made of maize grain moisture, European corn borer damage to the ear, and fumonisin synthesis risk. The model was calibrated to Dutch conditions using survey data and found to reasonably represent an effect of weather on the accumulation of DON and zearalenone (Van Asselt et al., 2012).

Torelli et al. (2012) developed a neural network model suitable for predicting fumonisins, DON, and ZON contamination of maize at harvest time. They gathered the following data during two years at 98 farms on: location of the crop, FAO class, sowing and harvest time, presence or absence of irrigation, pesticide treatment against the European corn borer, grain moisture (%) of the crop at harvest, daily minimum, and maximum temperatures and rainfall were taken from three weather stations. The estimation resulted in the neural network model that used irrigation, chemical treatment against the European corn borer and harvest date but not the weather data. Though the model reached quite a good level of fit for a training dataset and reasonable level for a validation dataset, the omission of the weather variables probably tells that the weather data was not very useful for the fields.

Predictive models have been tried relating weather to DON concentrations to assess the effect of expected climate change. For example, Van der Fels-Klerx et al. (2012) used data from 717 wheat fields in the Netherlands and Scandinavia to develop a regression model that would be more widely valid than a model based on data from a small geographical area. Van der Fels-Klerx et al. (2012) concluded that mostly the expected climate change by 2050 would increase DON concentrations in wheat. However, it is very difficult to know the validity of a model because not only weather but also growing systems and field ecosystems will change. Even generating weather data for a future situation is hard because shifting the means of temperature, humidity and rainfall do not suffice. Also the variability – on all scales from daily to decadal – needs to be well-described to provide useful data for predicting the impacts of climate change. In principle, it is known that very often the

extreme or at least uncommon weather events result in high toxin concentrations (Marvin et al., 2013) but current predictive models require detailed weather data.

Some other disease prediction models have been published (DeWolf, 2003; Molineros et al., 2005; Del Ponte et al., 2005; Moschini, 1996), but they are not calibrated to predict toxin levels. Eiblmeier (2006) reported on a model under development in Germany that used weather just before and around heading, together with agronomic variables, to predict DON. A Swiss model by Forrer et al. (2006) correctly predicted 78% of wheat samples with DON concentrations lower than 0.5 ppm, but the authors warned that improvements still need to be made before predictions are publicized. All of these models in development use similar weather variables to DONcast and recognize several of the same key agronomic variables for correction. DONcast will be the only one predictive model for DON in wheat to be commercialized (Schaafsma & Hooker, 2007).

Schmidt-Heydt et al. (2011) studied with microarray technique the effect of temperature and water activity on gene expression, growth and DON production by German strains of *F. culmorum* and *F. graminearum*. They both required high water activity for DON production, especially *F. culmorum* greater than 0.93. *F. culmorum* had a little, narrow temperature optimum at 15°C. Temperature range for *F. graminearum* is wider, from 15 to 25°C, with an optimum at 20°C. Using regression they developed a model that predicted immediate DON production directly and through gene expression based on temperature and water activity.

Based on the results mentioned above of the predictive model studies, development of predictive models needs to include climatic variables as the most important effects, followed by the various effects of agronomic practices (Schaafsma & Hooker, 2007). Schaafsma et al. (2001) showed the influence of year was the primary contributing factor to deoxynivalenol content at harvest. In that study, a year alone accounted for 48% of the variability of DON among the four years. The effect of wheat variety accounted for the most variability in DON amongst agronomic variables (27%), but less than the effect of year or weather. Tillage systems that maintained residue from previous crops on the soil surface were only a minor contributor to the final DON accumulation at harvest, which was associated with maize residues on the soil surface and crop rotation. Similar results were reported in another study from Canada (Guo et al., 2006), one each from the Netherlands (Schepers et al., 2006) and the Czech Republic (Sip et al., 2006), two from the United Kingdom (Edwards and Rumiana, 2006), Germany (Eiblmeier, 2006; Koch et al., 2006), and Switzerland (Forrer et al., 2006).

Two aspects of epidemic development are significant: 1) spore production: sufficient rainfall about 8–10 days before and during anthesis facilitates production of both ascospores and conidia; 2) spore dispersal and infection: adequate rainfall is needed to disperse ascospores and conidia, followed by periods of warm, humid conditions that are conducive for infection of ears. According to Schaafsma & Hooker (2007) from four to seven days before heading and three to 10 days after heading represent the most significant contributors to the variation in DON. The period four to seven days before heading very likely

corresponds to inoculum production. In this time, rainfall amounts of 5 mm per day trigger an increase in DON potential, and daily minimum air temperatures less than 10 °C limit DON potential (Andries et al., 2000; De Wolf et al., 2000; Osborne et al., 2000).

Similarly, studies by Schaafsma & Hooker (2007) focusing on weather variables on the critical periods anthesis and soft dough correspond to infection during flowering and fungal growth. The number of rainy days, and days with relative humidity over 75% at 11:00 h increase DON potential. In contrast, daily maximum temperatures over 32 °C and average temperatures less than 12 °C limit DON potential. During the fourth critical period, near maturity, daily maximum temperatures exceeding 32 °C limit DON, and rainfall events near harvesting favour DON accumulation. All critical times were determined from multiple regression analysis using real field data.

In addition, an interesting detail is reported in the studies of Hooker et al. (2002) regarding the first critical period (four to seven days before heading), that levels of inoculum may be reduced with excessive rain. The quadratic relationship between rain (four and seven days before heading) and DON suggests a maximum concentration of DON with two days of rain, then a decrease with at least three days of rain. Others have suggested that excessive rainfall may inhibit the development of perithecia, impede the release and dissemination of spores, or wash spores off wheat heads (Paulitz, 1996; Hooker et al., 2002). The negative effect of daily temperatures exceeding 32°C was only necessary for predicting DON when rain occurred during the period of infection. High temperatures were not important for predicting DON when conditions were dry during the three- to the six-day period after heading. Temperatures exceeding 30°C have been observed to reduce the growth of *F. graminearum* (Moschini, R. C. & Fortugno, 1996; Pugh et al., 1933; Reid et al., 1999).

In conclusion, the models as mentioned earlier are focused on forecasting field-specific risks of toxins to aid in fungicide application and crop use via toxin analysis. Also, they are descriptive aiming at understanding and demonstrating the effect of environmental factors on toxin concentrations, and assessing conduciveness of regions to toxin accumulation. A well-distributed weather station network can be expected to be available in only a few areas of the world, but satellite data is available for all important production areas. Masuoka et al. (2010) suggested that processed satellite data on weather and soil conditions would be used to classify regions or grid points according to their aflatoxin risk (Vigier et al., 1997).

Table 8 summarizes the effectiveness of various prevention and reduction procedures for mycotoxin contamination in cereal grains.

Table 8. Effectiveness of prevention and reduction of pre-harvest and post-harvest procedures for mycotoxin contamination in cereals.

Control and manage the risk pre-harvest	Reduce the risk	Increase the risk	Neutral risk
High quality seed and resistant cultivars	X (++)		
Crop rotation	X (++)		
Reduced tillage		X (+)	
Deep tillage	X (+)		
Nitrogen fertilization (low to medium by the recommendations)			X
Nitrogen fertilization (high)		X (+)	
Lodging		X (+)	
Organic farming	X (+)		
Physico-chemical factors of the soil:			
• clay	X (+)		
• silty soil	X (+)		
• coarser silty soil		X (+)	
• sandy soil		X (+)	
Long-term transport		X (+)	
Environmental conditions:			
• precipitation and long-term high humidity (>80 %) during cereal flowering		X(+++)	
• mean temperature and humidity (>80 %) a two-week period before harvest		X(++)	
Pesticide treatments:			
• fungicides	X (+)		
• herbicides			X
Biological control agents	X (+)		
Control and manage the risk post-harvest			
Harvesting:			
• high moisture % of the crop		X (+)	
• dry harvest conditions and low moisture % of the crop	X (+)		
Storage:			
• moisture content below 14,6 %	X (+)		
• moisture content over 14,6 %		X (+)	
Interactions of fungi during drying and storage:			
• changes in water content		X (+)	
Impact of sorting and dehulling	X (+++)		
Use of preservatives	X (+)		
Processing – milling, bread making and malting process			
Milling and bread making			X
Malting process		X(+)	
Mycotoxin inactivation (feed, animal husbandry)	X (+)		
Forecasting mycotoxin risk	X (+)		

+: low impact; ++: medium impact; +++: high impact

4 AIMS OF THE STUDY

The aims of the present study were:

1. to produce updated information of *Fusarium* species in Finnish cereal grains
2. to define the changes in *Fusarium* mycotoxins in Finnish cereals in the years 1987–2014
3. to determine, on the basis of the mycotoxin contents and agronomic factors behind the studied samples, how to control and manage the *Fusarium* mycotoxin risk
4. to predict by modeling the magnitude of the mycotoxin risk.

5 MATERIALS AND METHODS

5.1 Collection of samples

5.1.1 Samples at the turn of the 1990s (I)

Representative samples (2-3 kg) of Finnish and imported cereals and feeds from the 1987 and 1988 crops were collected (I). The collected sample material from the Finnish State Granaries, the feed industry and private farmers included oats, wheat, barley, rye, soy granules, rapeseed, turnip rapeseed, maize gluten, fish meal, poultry feed and pig feed were collected. Samples of imported oats, wheat, barley and rye were collected from the State Granaries. Altogether 145 samples of grains (food), 61 samples of grains (feed) and 96 samples of industrial feeds and feedstuffs were collected for mycotoxin determinations.

5.1.2 Samples at the turn of the 2000s (II)

The oat samples were collected after harvest during official and agronomy trials conducted by MTT Agrifood Research Finland in 1997–1999 (II). The official variety trials conducted at 8-10 locations were managed following standard protocol (Figure 13). There were two types of agronomy trials, the first included comparison of oat cultivars grown in conventional and organic farming systems at six locations, and the second used five nitrogen rates (0, 40, 80, 120 and 160 kg N per ha) with four oat cultivars at two locations (Figure 13). More detailed information on the trials has been published in 2000 and 2003 (publication II: Järvi et al., 2000; Euroola et al., 2003). After harvest, the grains were immediately dried with warm air in a flat bed grain drier to a moisture content of below 14%. The oat grains were sorted with a 2.0-mm sieve and hulled with a laboratory hulling machine (BT 459) using air pressure. Oat groats were milled with a falling number hammer mill using a 1.0-mm sieve. The total number of oat samples analysed were 147, 147 and 99 in 1997, 1998 and 1999, respectively.

The varieties studied were Leila, Kolbu, Salo, Belinda, Veli, Roope, Aarre, Katri, Puhti and Yty. Kolbu and Roope have yellow husks, and the other varieties have white husks. Leila and Kolbu are cultivars developed in Norway, Salo and Belinda in Sweden, and Veli, Roope, Aarre, Katri, Puhti and Yty in Finland. The oat varieties selected for the project included the most popular cultivars as well as new varieties on the National list of cultivars in Finland.



Figure 13. Location of the trial sites in Finland. Reprinted from Hietaniemi et al. (2004) with permission. Copyright 2004 Agricultural and Food Science.

5.1.3 Samples for developing a predictive model (III)

Data were collected as a part of the Finnish safety monitoring programme from 470 oat fields in Finland between 2000 and 2009, from 171 oat fields in Norway between 2004 and 2008, and from 33 oat field trials in Sweden between 2006 and 2009 (III). The Finnish dataset was divided into 257 fields from the southern region (FIab, CAP subsidy areas A and B) and 213 fields from the northern region (FIc, CAP subsidy area C) (Mavi 2009). The Norwegian dataset was divided into 93 fields from Solør (NOs), a district within the province of Hedmark in south-eastern Norway, and 78 fields from other parts of Norway (NOns). The oat field trials in Sweden (SE) were located in the southern and central part of the country, but were not divided into geographical regions due to the limited number of observations. Field data included DON levels in the oats crop, agronomical factors and weather data. All samples were taken from unprocessed oats grain after harvest. DON levels in samples from Finland were analysed by a GC-MS method at MTT Agrifood Research Finland (Hietaniemi et al. 1991, 2004, 2016). Samples from Norway were analysed by a multi-toxin LC-MS/MS analysis at the Finnish Food Safety Authority Evira, Finland, according to the method described by Kokkonen and Jestoi (2009) (publication III). DON levels in samples from Sweden were analysed by GC at the Swedish University of Agricultural Sciences (Pettersson 1998, publication III).

The agronomical factors were flowering date and harvest date (ordinal date), period between flowering and harvest (days), tillage system (ploughed or not), pre-crop (oats as pre-crop or not), and soil type (silty or not). Weather data were collected from the nearest weather station, most often within 20 km from each field. Weekly weather variables were calculated for the time period from four weeks before the week of flowering to four weeks after the week of flowering (totally nine weeks). The weekly weather variables were mean temperature (°C), number of days with a relative humidity exceeding RH 80%, and rainfall

(mm). The selection of weather variables were based on results from work with preliminary DON prediction models in Norway.

Samples that had a DON level below the limit of quantification (LOQ) were assigned the LOQ value, or, in the case of Norway, the limit of detection (LOD) value if below the LOD. The LOQ for data from Finland was 25 ng/g, and for data from Norway and Sweden 100 ng/g. The LOD for data from Norway was 50 ng/g. To stabilize the variance, DON levels were log₁₀ transformed before statistical analysis.

5.1.4 Samples during 2000–2014 from the FinMyco project and from the Finnish Safety Monitoring Programme for up-to-date information (IV)

Representative 2 kg cereal samples (oats, barley, wheat and rye) were collected for FinMyco project in 2005 and 2006 after harvest either from Finnish farms by ProAgria advisors and private farmers, or from crop trials by Agrifood Research Finland MTT (IV). The total number of cereal samples was 390 in 2005 and 166 in 2006. Since 2000, the Finnish Safety Monitoring Programme (FSMP) of cereals has been conducted by the Finnish Cereal Committee (see www.vyr.fi) together with ProAgria, the Finnish Food Safety Authority Evira and MTT Agrifood Research Finland, from which the important mycotoxin issues have been raised as the focus of research group efforts. In the context of the FSMP, the Finnish Food Safety Authority Evira has been responsible for collecting representative samples of oats, barley, malting barley, spring wheat, rye and winter wheat. The samples were collected by farmers. Farms were randomly selected from the National Farm Register. Those with fewer than five hectares of cultivated area were not included in the sampling programme. The total amount of samples received from farmers per year varied between 989 and 2,128. Sample size was 1.5–2 kg. Samples were selected for the FSMP by area and variety. Sample size was on average 300 g. Between 2000 and 2014, the number of samples collected for trichothecene and zearalenone analysis varied between 120 and 199: 120 samples were collected in 2000–2005, 170 samples per year from 2006 to 2012 and in 2014, and 199 samples in 2013. Moniliformin (MON), beauvericin (BEA) and enniatins (ENNs) were not analysed in these FSMP samples (IV).

All the above-mentioned samples were given an individual sample code in the Laboratory Information Management System (LIMS) by Agrifood Research Finland MTT laboratories. The samples were divided into subsamples with a laboratory scale grain divider (Riffelteiler RT 12.5, Retsch) for all research laboratories in this study, either as grains or in milled form. All grains were milled with a falling number hammer mill using a 2.0 mm sieve. The cultivation history of each sample was recorded, but extensive information was not available for all the samples from 2005 and 2006. The samplers were given instructions on sample collection as follows: since FHB and mycotoxins may occur as colonies in threshed crops, the samples from a dried grain batch to be sent to the research

project must consist of several separate subsamples. Whenever possible, an automatic sample collection system in the dryer should be used for sample collection.

Related to the sample collection, accurate weather data in 2002–2014 were collected to understand better the possible risk factors, such as the weather at the beginning of the growing season, prolonged humid weather around anthesis, and a protracted wet period before harvesting.

5.2 Analytical methods

Trichothecenes and ZON in 1987–1988, 1997–1999 and in the FSMP 2005–2014 were analysed immediately after each year's harvest. Determinations of trichothecenes, zearalenone, *Fusarium* species, MON, BEA and ENNs were made during the FinMyco study between 2005 and 2007. After the 1987–1988, 1997–1999 and 2005–2006 FinMyco harvests and harvests during FSMP in 2000–2014, grain samples were stored at RT before analysis.

5.2.1 Trichothecene and zearalenone analysis

Trichothecenes were analysed as described by Publications I, II, and IV. The laboratories of Agrifood Research Finland MTT apply a quality control system in accordance with SFS-EN ISO/IEC 17025:2005. In brief, 20 g of ground grain samples were extracted with 84% acetonitrile. Raw extract was purified with MycoSep #227 SPE column (RomerLabs). The extract volume of oats sample and maize reference material for clean-up was standardised at 6.1 ml for better repeatability in 2006. The cleaned-up extract was transferred to a silylated test tube and evaporated to dryness. DON, DAS, 3-AcDON, 15-AcDON, F-X, NIV, T-2, HT-2 and 19-nortestosterone (internal standard) were identified and quantified as their trimethylsilylether derivatives by GC-MS. The LOQ was 25 µg/kg for all trichothecenes. The method for trichothecenes (DON, 3-AcDON, NIV, T-2 and HT-2) has been accredited since 2003. The reference material for DON was either Maize Flour CRM 378 (BCR) or Maize Flour BRM 003001 (Biopure). Recovery tests were needed for quality control for the other seven trichothecenes.

ZON was analysed as described in Publication IV. ZON from barley, rye and wheat samples were extracted in the same way as for trichothecenes. The raw extract (7.5 ml) was purified using a MycoSep #226 SPE column (Romer Labs Methods: Zearalenone HPLC MycoSep™ 226 Method, zon-lc-01-00.3 2000). The cleaned-up extract (4 ml) was evaporated to dryness in a silylated test tube. In oat samples, 1% of acetic acid was added to the extraction solvent (acetonitrile: water: acetic acid 84:15:1) (Romer Labs Application Brief: Rapid, Accurate Quantitation of OTA in Corn by liquid-chromatograph-fluorescence detector (HPLC-FLD), App. 4-01-031006 2003). Part of the raw extract (4 ml) was evaporated to dryness in a rotary evaporator. The residue was dissolved in dichloromethane (5 ml), dried with Na₂SO₄ and cleaned up with a silica SPE column (Baker) according to the

official method of AOAC with some modifications (AOAC 1984). ZON was identified and quantified using HPLC equipped with a fluorescence detector.

5.2.2 Determination of *Fusarium* species (IV)

The determination of *Fusarium* species was carried out using a traditional microbiological method for grain harvested in 2005 and 2006 (IV). For the isolation of *Fusarium* species, 100 grains of each sample were cultured on a PCNB medium (Nash & Snyder medium; Nelson et al. 1983) at 22°C. The resulting colonies were inoculated for identification on potato dextrose agar (PDA) medium and cultured in the dark. *Fusarium* species were determined from cultures using the microscope and contamination % values for each species were determined for the identified colonies. Contamination % was defined as the percentage of kernels investigated that were contaminated by each *Fusarium* species.

5.2.3 DNA analysis (IV)

DNA was extracted from the surfaces of grain samples (10 g per sample) and from ground grain samples (50–100 mg per sample) with Sigma's GenElute™ Plant Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA), as described by Yli-Mattila et al. (2008). DNA was extracted from pure cultures using the chloroform/octanol method as described by Yli-Mattila et al. (1998). *Fusarium* species-specific TaqMan primers and probes in real-time PCR and the GeneAmp® 5700 cycler were used for the quantification of the DNA levels of these species as described by Yli-Mattila et al. (2006, 2008, 2009).

5.3 Statistical analyses

In the study of Publication II statistical analyses regarding the DON toxin differences in various varieties of oats were accomplished using Data Desk 6.1.1 (Data exploration and visualization; Velleman and Hoaglin, 1981).

In the study of Publication III and partly in Publication IV the relationship between DON levels and geographical, agronomical and weather variables was examined by using the General Linear Model (GLM). In the study of Publication III an initial correlation analysis showed that all pair-wise combinations of weekly relative humidity data were highly correlated (coefficient of determination (R^2) > 0.7). Therefore, a new variable comprising the number of days with a relative humidity exceeding RH 80% during the whole nine-week period before and after flowering was calculated and used in the statistical analysis. Date of harvest and the number of days between flowering and harvest were also highly correlated ($R^2 = 0.8$) and only the latter variable was used in the analysis.

First in Publication III, differences in mean log DON levels between regions were analysed. Second, the proportion of variation in log DON levels explained by year-to-year variation within each region was estimated, hypothesizing that large between-year differences indicate that weather is of significant importance for variability in DON levels

(Schaafsma and Hooker, 2007). Third, univariate analyses were performed to estimate the effect of each agronomical variable on DON levels. In these analyses, region was added as a blocking factor to adjust for any regional differences in the distribution of agronomical factors. Fourth, backward stepwise regression was performed including all significant agronomical variables from the univariate analysis and all weather variables as independent factors.

In the study of Publication IV the statistical analyses (analysis of variance – ANOVA; and Tukey's test) were performed with Statistix for Windows 7.0 (Analytical Software, Tallahassee, FL, USA). Those p-values < 0.05 were considered to be statistically significant. R^2 , regression slope and P (significance of the regression slope) were calculated using SigmaPlot 2001 version 7.1 (SPSS Inc.). The original DNA and toxin concentrations were transformed to logarithmic values in order to obtain a more normal distribution for the values of toxin and DNA concentrations.

Year-to-year weather variations in the above-mentioned study were examined using a calculation model where a weekly growth stage of cereals was used as the x-axis. The flowering day is used as the zero point for the x-axis (Figure 4, publication IV). Mycotoxin and weather (temperature, rainfall, relative humidity) data from the FinMyco study and from the Finnish grain safety monitoring programme are used as the source of the calculation model. Both of the above-mentioned research datasets include spatial, agronomic and weather information. The information on the cultivation fields was collected from spatial records. From the agronomic information the sowing day was needed to calculate a flowering day. Weather data from all the available Finnish Meteorological Institute's official weather stations around Finland were used for research purposes. These meteorological data were used to generate the curves for rainfall, temperature and humidity. In addition, effective temperature sums (ETS) from the meteorological data were used as a basis for the determination of the flowering day. All the above-mentioned data processing was performed using the FileMaker Pro database platform; Figure 4 in Publication IV was created using Microsoft Excel.

6 RESULTS AND DISCUSSION

6.1 Results at the turn of the 1990s (I)

Despite the wet and rainy summer of 1987, the concentrations of *Fusarium* toxins were relatively low in Finnish grains (feed) in the 1987 crop. The summer of 1988 was warmer than that of 1987, with less rainfall until the beginning of autumn. Apparently, *Fusarium* fungi were more abundant in grains from the 1988 crop and the levels of *Fusarium* toxins were also higher than those in the 1987 crop. The results showed that over 90% of all Finnish grain and feed samples contained DON from 7 to 300 µg/kg and over 30% of samples contained smaller amounts (13–120 µg/kg) of 3-AcDON. The most toxic trichothecenes, T-2, HT-2 and NIV, and also zearalenone, were found at low concentrations in 1–10 % of the samples analysed. Six lots of oats containing toxic levels of DON (1.3–2.6 mg/kg) and 3-AcDON (0.2–0.6 mg/kg) were found in Finnish grains.

The results showed that, without exception, 3-AcDON levels of the samples with high toxin concentrations were 10–20% of the corresponding DON levels. We also found that the same samples contained DON, 3-AcDON and sometimes NIV and ZON simultaneously, but T-2 and HT-2 were generally found alone or occasionally with DON.

Fungi isolations of selected analytical samples showed that in those cereals with high concentrations of *Fusarium* toxins there also appeared to be much more moldiness and a high occurrence of *Fusarium* fungi. The most frequently encountered species of *Fusarium* were *F. graminearum*, *F. avenaceum*, *F. tricinctum*, *F. moniliforme* and *F. oxysporum*. The most important and the most productive species associated with the production of the *Fusarium* toxins (DON, 3-AcDON, NIV and ZON) was *F. graminearum*. According to the present results, the highest concentrations of DON and 3-AcDON were found in oat samples. Among the cereals investigated, oat samples seemed to be the most susceptible to the development of *Fusarium* toxins.

In contrast to oats, relatively low toxin concentrations were found in samples of wheat, barley and rye. The highest concentrations were 700 µg/kg (DON) and 106 µg/kg (3-AcDON). The samples of pig feed studied contained the highest toxin levels detected among the feeds, but on average they were low, with only a few exceptions. The highest concentrations of *Fusarium* toxins found in pig feeds were DON (1,216 µg/kg), 3-AcDON (87 µg/kg), NIV (67 µg/kg), T-2 (90 µg/kg) and ZON (42 µg/kg). It is remarkable that feedstuffs such as maize gluten, soy granules, rapeseed and turnip rapeseed were almost free of mould toxins. The contents of *Fusarium* toxins in samples collected in Finland from the 1987 and the 1988 crops seemed to be at the same level or somewhat lower than those reported earlier (Ylimäki et al., 1979; Karppanen et al., 1985; Tanaka et al., 1988; Sundheim et al., 1988; Wood and Carter, 1989). The average contents of all *Fusarium* toxins were below the present advisory or official tolerance limits.

6.2 Results at the turn of the 2000s (II)

The results of the official variety and nitrogen fertilization trials and comparison of conventional and organic cultivation, suggest that future research and follow-up on trichothecenes must be emphasized and continued. The results of the trials showed that the mycotoxin DON was found most frequently in Finnish oats during 1997–1999. According to the results on an average 55% of the oat samples respective to the official variety trials in 1997–1999 contained DON within the range of 50–896 µg/kg. Correspondingly, the range and frequencies of other toxin findings were as follows: 3-AcDON 50–310 µg/kg (5%), NIV 50–575 µg/kg (16%), T-2 toxin 50–349 µg/kg (1%) and HT-2 toxin 50–507 µg/kg (4%). In comparison to previous studies, the contents of trichothecenes in grains appeared to be similar or lower to those reported earlier in the Northern or Southern Hemisphere (Karppanen et al., 1985; Tanaka et al., 1988; Sundheim et al., 1988; Wood et al., 1989; Hietaniemi and Kumpulainen, 1991; Hietaniemi and Kumpulainen, 1993; Müller and Schwadorf, 1993; Rizzo, 1993; Pettersson et al., 1995; Groves et al., 1999; Janardhana et al., 1999; Döll et al., 2002; Langseth and Rundberget, 2001; Rizzo et al., 2001; Eskola, 2002; Schollenberger et al., 2002; Salay and Mercadante, 2002). The results also showed that no distinct differences were found in DON contents of various varieties. The differences in DON concentrations between organic and conventional cultivation were small. In addition, the results showed that the use of various nitrogen fertilization levels only slightly affected the trichothecene concentrations.

Nevertheless, the importance of background factors with respect to samples is often forgotten in monitoring the quality of grains, not to mention a deeper familiarity with their impact and, through the same, better control over grain quality. Evidently, the incidence of rain during heading time represents a risk factor (McMullen et al., 1997; Döll et al., 2002; Oldenburg et al., 2000; Langseth et al., 2001). The present results also showed that more precise research into the effects of cultivation methods in relation to fungi and toxins is necessary (Norred, 2000). Good familiarity with, the effects of the preceding crop, rotation, seed purity, various soil preparation methods, direct sowing and pesticides on the formation of mycotoxins is a minimal requirement. A question of its own right also concerns breeding for resistance against *Fusarium* fungi and their associated toxins (de Vries, 2000; Hollins et al., 2003). Knowledge of these factors is a prerequisite for good cultivation-related directives that industry and farmers should follow to ensure high-quality production of oats both in Finland and internationally.

6.3 Developing a predictive model (III)

The relationship between weather data and agronomical factors and deoxynivalenol (DON) levels in oats was examined with the aim of developing a predictive model (Lindblad et al., 2012, publication III). Data were collected from a total of 674 fields during periods of up to 10 years in Finland, Norway and Sweden, and included DON levels in the harvested oats

crop, agronomical factors and weather data. The agronomical factors were flowering date and harvest date (ordinal date), period between flowering and harvest (days), tillage system (ploughed or not), pre-crop (oats as pre-crop or not), and soil type (silty or not). A silty soil is defined as $\geq 80\%$ silt and $< 12\%$ clay. Flowering and harvest was earliest in Sweden and latest in Finland (CAP subsidy area C). Unploughed fields were less common in the Finnish regions and in Sweden than in the Norwegian regions, where about one-third of the fields were unploughed. Oats as a pre-crop was most common in Finland. Silty soils were most common in Norway (Solør area) and least common in Sweden and Norway (other parts of Norway).

The results show there was a large regional variation in DON levels, with much higher levels in one region in Norway compared with other regions in Norway, Finland and Sweden. Agronomical factors or weekly weather data did not, or only to a limited degree, explain the variation in DON levels. Including more variables than region in a multiple regression model only increased the adjusted coefficient of determination from 0.17 to 0.24, indicating that very little of the variation in DON levels could be explained by weather data or agronomical factors. Thus, it does not seem to be possible to predict DON levels in oats based on the variables included in this study (III).

The fact that the annual variation in regional DON levels was limited in oats indicates that between-year variation in weather conditions had little influence on DON levels. This is in contrast with DON levels in wheat, where variation between years has been shown to explain a large part of the total variation (Schaafsma and Hooker, 2007). The results of earlier studies on oats vary. Whereas Langseth and Elen (1997) found a correlation between DON levels and precipitation in July, the study of Publication II could not explain regional differences in DON levels based on weather conditions around flowering. Since variables such as temperature and humidity indeed are important factors in the epidemiology of *Fusarium* species (Osborne and Stein, 2007), it is possible that access to field-specific weather data, instead of data from weather stations within a certain distance from the field, could improve possibilities to predict DON levels in oats based on weather data. One possibility may also be to include data on weather conditions during the period just before harvest time, or to use weather data with a more detailed time scale than weekly.

The present study of Publication III shows that DON in oats is a significant problem, especially in the Solør region of Norway, but also to some extent in other regions in Finland, Norway and Sweden. The data also show that the DON levels in Finland were more stable over years than in Norway. This could indicate that the increase of the incidence of *F. graminearum* that has occurred in Norway in recent years is still a minor toxin producer in Finland. According to Publication III unpublished trials in Sweden show that there are obvious differences in fungal communities between regions, indicating that this may be the reason for regional differences in mycotoxin content in grains. Further more detailed monitoring of regional differences in species distribution of fungal communities and their changes during the oats cultivation period is needed, as well as efforts to understand which

factors favour infection, growth and/or mycotoxin production of DON-producing *Fusarium* in certain regions.

A recent study by Kaukoranta et al. (submitted in 2016) used the data of FSMP 2002–2014 to investigate the infection of oats by *F. langsethiae* and T-2 and HT-2 toxins influenced by weather and climate. The effect of weather on the total concentration of T-2 and HT-2 mycotoxins and incidence of *Fusarium* spp. in harvested spring oats grain was analysed using partial correlation analysis with moving seven-day time window and regression analysis. According to Kaukoranta et al. (submitted paper 2016) moisture was related to the incidence and T-2 + HT-2 concentration 30–40 days before mid-anthesis, and in interaction with temperature near harvest. From mid-anthesis to harvest the toxin accumulation was favoured by high temperatures. However, without other than weather variables, R^2 of regression was only 0.10. When adding to an equation cereal pre-crops, ploughing, and the incidence of *F. langsethiae* R^2 raised to 0.42. Based on the results of Kaukoranta et al. (submitted in 2016) more research is clearly needed to predict T-2+HT-2 more reliably during the growing season, and towards improving practices among farmers and grain industry.

6.3.1 The effect of climate change in the near future

Management and control of the mycotoxin risk is a complex problem. In the pre-harvest phase the components of a management scheme are as follows: 1) high-quality seed and resistant cultivars; 2) field management; 3) monitoring of environmental conditions; 4) pesticide treatments and 5) application of biological control agents. These components have to adjusted correctly to minimize the risk. In the post-harvest phase 1) harvesting and storage, 2) interactions of fungi during drying and storage, 3) impact of sorting and dehulling, 4) use of preservatives and processing – milling, bread making and malting process should find their own place to manage and control the mycotoxin risk. From the pre-harvest parts environmental conditions, weather impacts, are clearly dominating over all. The effect of weather conditions on the risk is crucial and depends on specific conditions.

According to a United Nations Environment Programme (UNEP) Frontiers 2016 Report ‘Emerging Issues of Environmental Concern’ climate change is already underway, with shifting weather patterns that will present serious challenges to agricultural productivity. Each of the past several decades has been significantly warmer than the previous one. The period 2011–2015 was the hottest on record, and 2015 was the hottest year since modern observations began in the late 1800s (World Meteorological Organization (WMO), 2016). The 2013 global assessment released by the Intergovernmental Panel on Climate Change reports that since 1950 the frequency of heat waves has increased in large parts of Europe, Asia, and Australia; that the frequency and intensity of droughts have increased in the Mediterranean and West Africa; and that the frequency and intensity of heavy precipitation events are likely to increase in North America and Europe (Hartmann et al., 2013).

Aflatoxins are a type of mycotoxin produced by a species of *Aspergillus* fungi. About 4.5 billion people in developing countries are exposed to uncontrolled and unmonitored amounts of aflatoxins (Williams et al., 2004). The risk of aflatoxin contamination, particularly in maize, is expected to increase in higher latitudes due to rising temperature. A recent model study predicts that aflatoxin in maize will become a food safety issue for Europe, especially in the most likely scenario of a 2°C increase in global temperature. Areas at high risk of aflatoxin outbreaks include Eastern Europe, the Balkan Peninsula, and the Mediterranean (Battilani et al., 2016, Material available under Public License, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4828719/>).

Extreme climatic conditions reduce yields, increase fungal contamination and postharvest losses. They also trigger biophysical reactions in plants in response to environmental stresses. These reactions include production of mycotoxins and concentrating chemical compounds that are harmful to animal and human health. In such cases, either the plant itself or invading microbes can produce specific chemical compounds at levels toxic to human health.

6.4 Updated survey of *Fusarium* species and toxins in Finnish cereal grains (IV)

6.4.1 FinMyco project in 2005–2007

It seems clear that *F. langsethiae* is increasing in Finnish cereal grains (Parikka et al., 2008). It is found in cultivated areas throughout of Finland, but mostly in the south, and in oat samples. Based on contamination, *F. culmorum* was a more common and abundant DON producer than *F. graminearum* in Finland in 2005–2006. Based on qPCR and chemical determinations, *F. graminearum* seems to be the main DON producer in Finnish oats, barley and spring wheat, especially in samples with high DON levels (Yli-Mattila et al., 2008). This is in accordance with the results obtained from Norwegian grain samples (Elen et al., 2007, unpublished results), and the qPCR results of Sarlin et al. (2006) with Finnish barley. According to Publication IV the R^2 value in barley was higher when *F. culmorum* DNA was summed with *F. graminearum* DNA, which means that *F. culmorum* was also producing a significant amount of DON in barley. The DON produced by *F. culmorum* in barley might explain the poor correlation between *F. graminearum* DNA and DON and the better correlation between TMTRI DNA and DON levels by Sarlin et al. (2006). In the present work there was a highly significant correlation between *F. graminearum* DNA and DON in all three cereals (Yli-Mattila et al., 2009), as *F. graminearum* is an indicator species for high levels of DON.

F. poae is the main producer of NIV, especially in Northern Europe, while *F. langsethiae* and *F. sporotrichioides* are the main producers of T-2 and HT-2 toxins (Pettersson, 1991; Salas et al., 1999; Jestoi, Paavanen-Huhtala, et al., 2004; Torp & Langseth, 1999; Bottalico & Perrone, 2002; Thrane et al., 2004). In Finland a correlation between *F. poae* DNA and

NIV levels has been found in barley and oats, while the correlation between *F. langsethiae* + *F. sporotrichioides* DNA and HT-2+T-2 levels is also clear (Yli-Mattila et al., 2008, 2009). The R^2 between *F. graminearum* DNA and DON was about 0.50 (0.49–0.61) when DNA was extracted from the grain surfaces. Higher R^2 values (0.78–0.99) between *F. graminearum* DNA and DON in oats, barley and spring wheat were obtained when DNA was extracted from ground grain. The R^2 values between *F. langsethiae*/*F. sporotrichioides* DNA (TMLAN) and HT-2+T-2 levels were also clearly higher when DNA was extracted from ground grains (Yli-Mattila et al., 2009).

The highly significant correlation between DON and *F. graminearum* DNA levels in ground oat grains in FinMyco samples from 2005–2006 (Yli-Mattila et al., 2009) is in accordance with the results obtained by Yli-Mattila et al. (2011, 2013), and they also found a significant correlation between ZON and *F. graminearum* DNA whereas there was no correlation between DON and *F. culmorum* DNA.

DAS, F-X and 15-AcDON were not detected in cereal samples in either 2005 or 2006. In 2005 ZON was detected in six samples, with the highest ZON concentration of 230 $\mu\text{g kg}^{-1}$ found in a malting barley sample. In 2006 ZON was detected in only one sample. The content of 3-AcDON was monitored in oats in 2005 and the results have a strong correlation to high DON concentrations. Few individual DON concentrations over 1250 $\mu\text{g kg}^{-1}$ were discovered in barley and spring wheat samples, or in oats over 1750 $\mu\text{g kg}^{-1}$ in 2005. The highest DON concentration of 9300 $\mu\text{g kg}^{-1}$ was found in 2005 in an oat sample. All DON concentrations detected in the studied cereal samples were under the MRLs in 2006. DON concentrations were also very low throughout the study in winter wheat and rye samples.

The level of NIV concentrations were similar to those reported earlier in oat crops from 1997 to 1999, according to Publication II. However, positive NIV findings were found more in oat and barley samples in 2005 and 2006 than in the 1987 and 1988 crops (I). In other previous studies (Eskola et al., 1998; Pettersson, 1991; Yli-Mattila et al., 2004a) the conclusions are similar to the prevalence found in this study, which means that the occurrence of NIV varies greatly between different years and cereals and exceptionally high values were found only infrequently. In Finland, it has also been noted that warmer and drier weather conditions favour increased contents in grains of NIV and NIV-producing fungi (*F. poae*) (unpublished results). This conclusion is also supported by Placinta et al. (1999), in which remarkably high levels of NIV were reported from Vietnam (Wang et al., 1995a), Japan (Yoshizawa, 1997) and New Zealand (Lauren et al., 1996).

It is obvious that the contents of T-2 and HT-2 in cereals have increased in Finland in the 2000s (I, II, IV). In 1987–1988 and 1997–1999, positive findings (above LOQ) of T-2 and HT-2 toxins were found only in 1–10% of the studied oat and barley samples (I, II). Also, according to Edwards et al. (2009, 2009a, 2009b, 2009c) and Pettersson et al. (2008), results from Nordic countries suggest that the occurrence of these highly toxic compounds has increased dramatically over the last decade. On the basis of the work of Edwards et al. (2009) and Edwards (2009a, 2009b, 2009c), the data on the contents of T-2+HT-2 and DON in oats, barley and wheat show signs of mutual exclusion. This means that when T-2+HT-2

concentration is high, DON concentration is low, and vice versa. The same trend is also observed in Finland (unpublished results). The results of the same paper also raise the question of the impact of agronomy. There are limited data on the impact of agronomy on the trichothecene A-type compared with B-type toxins such as DON.

There are many probable reasons for these changes in *Fusarium* mycobiota and the level of the risk of the mycotoxins: cultivation techniques have changed a lot and climate change is now a reality. The use of reduced tillage and no tillage has increased substantially in Finland since the 1990s. Therefore, more debris from the previous crop is left on the field, which increases the growth of *Fusarium* fungi and leads to the possibility of fungi diversification.

6.4.2 Finnish safety monitoring programme during 2000-2014 – big deal for control and manage the risk of mycotoxins and safety in Finnish cereal grains (IV)

The Finnish Safety Monitoring Programme (FSMP) has been carried out as part of a National Quality Strategy in Finland since 1999 (Hietaniemi et al., 2008, 2010, 2014, IV). Since 2000, the FSMP of cereals has been conducted by the Finnish Cereal Committee (see www.vyr.fi) in cooperation with ProAgria, The Finnish Food Safety Authority Evira and MTT Agrifood Research Finland from which the important mycotoxin issues have been raised for research groups to focus on. The aim of this programme has been the systematic analysis and documentation of grain quality and safety data, including the agronomic variables behind each sample. In the monitoring study mycotoxins and *Fusarium* fungi have been determined from regionally representative Finnish cereal samples. Altogether 170–190 cereal samples per year from the farmer's silos around Finland have been collected for the mycotoxin survey. The Finnish Food Safety Authority Evira has been responsible for collecting representative samples of oats, barley, malting barley, spring wheat, rye and winter wheat. From the samples, trichothecenes such as DON, DAS, 3-AcDON, 15-AcDON, FX, NIV, T-2 and HT-2 and both zearalenone and ochratoxin A have been analysed by validated GC-MS and HPLC methods, respectively.

Based on the results of FSMP 2000–2014 oats is the most sensitive to *Fusarium* infestation in Finland and exceptionally high DON and T-2+HT-2 concentrations have increased during the new Millennium (Figures 14-25). According to the FSMP, DON toxin levels in oats and spring wheat increased markedly in 2011–2014. The rainy and moist summer of 2012 was highly exceptional: median toxin levels especially in oat samples rose but still over 90% of the analysed samples were below the limit values assigned for food grade grain. The year 2013 was worse than the previous year with respect to mycotoxins; 31% of the oats grain batches did not fulfil the norms for food-grade grain. However, it must be noted that in 2013 the collection of samples focused in particular on high-risk areas. In 2014 the situation normalized and 85% of the analysed oat samples were acceptable for food use. Once again, these years brought the tools of risk management into play and under critical

evaluation. It is greatly emphasised that the drying of cereals should be done carefully even in years in which cultivation and weather conditions are good. During years when higher contamination risk has been noticed, optimized rotation of grain during warm air drying and/or sorting after drying is recommended in order to reject the light kernels and waste which includes the highest mycotoxin contents. Sorting and dehulling of cereals (oats, barley) is always done in the food industry before milling.

In recent years the number of positive findings and higher contents of DON in spring wheat and barley have reported (Figures 15, 16). On the other hand, winter cereals with low toxin concentrations from year to year have caused a lot of discussion about that why the toxin contents are consistently so low (Figures 18, 19, 24, 25). One explanation is that these winter-crops have different growth rhythms which favour vigorous and healthier growth of plant and time to better growth climatic conditions at critical infestation points. In most cases, the harvest conditions for winter cereals at the beginning of August are favorable. Increased positive findings from 2000-2014 concerning T-2+HT-2 levels in barley and malting barley should be noted (Figures 22, 23). However, it is gratifying that a significant part of Finnish cereal grains meet also the EU limits and recommendation values set for DON and T-2 and HT-2 toxins in baby food, respectively.

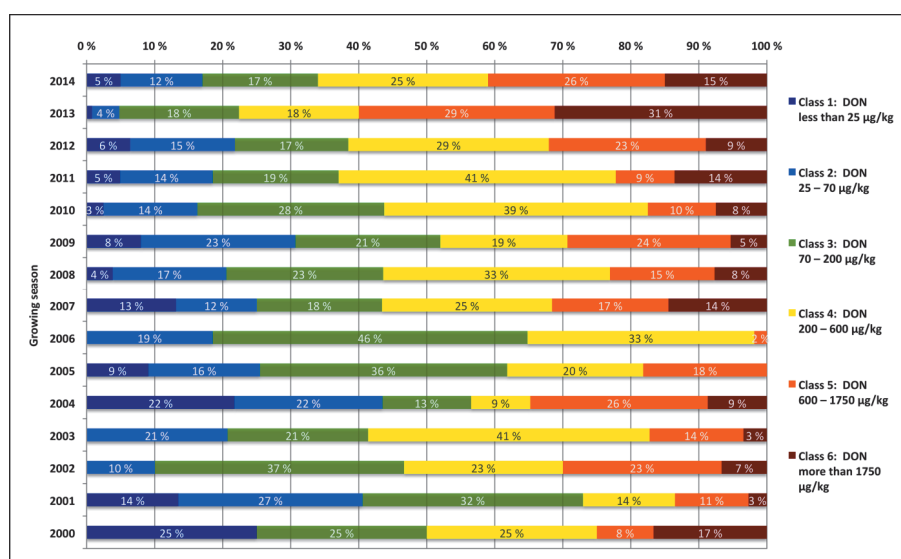


Figure 14. DON levels in oat samples which were classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014.

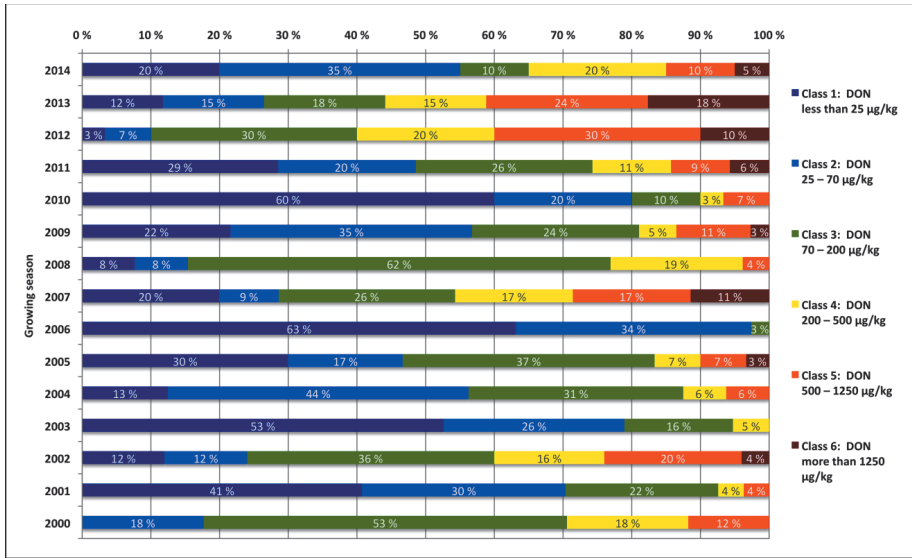


Figure 15. DON levels in spring wheat samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014.

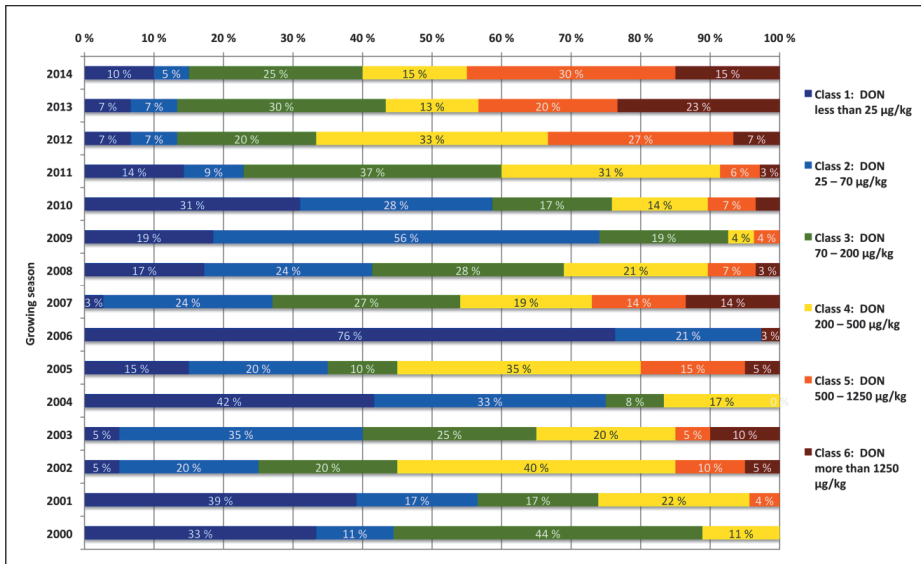


Figure 16. DON levels in barley samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014.

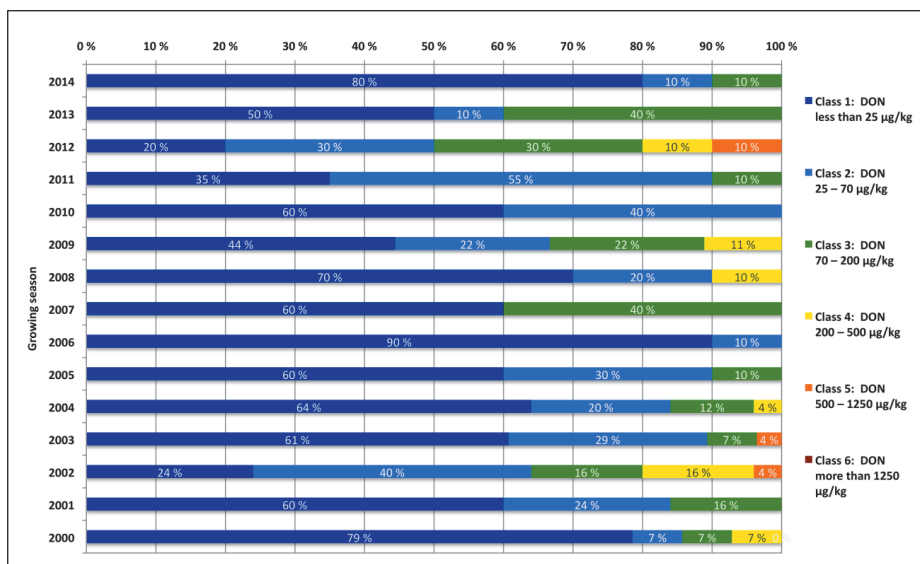


Figure 17. DON levels in malting barley samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014.

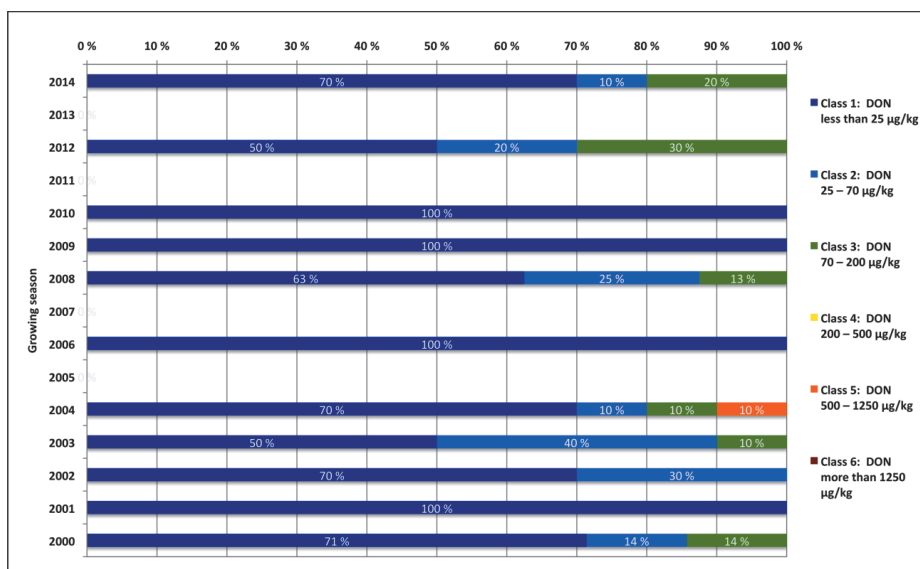


Figure 18. DON levels in winter wheat samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014. Winter wheat samples were not collected in 2005, 2007, 2011 and 2013.



Figure 19. DON levels in winter rye samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014. Winter rye samples were not collected in 2005, 2007, 2009, 2011 and 2013.

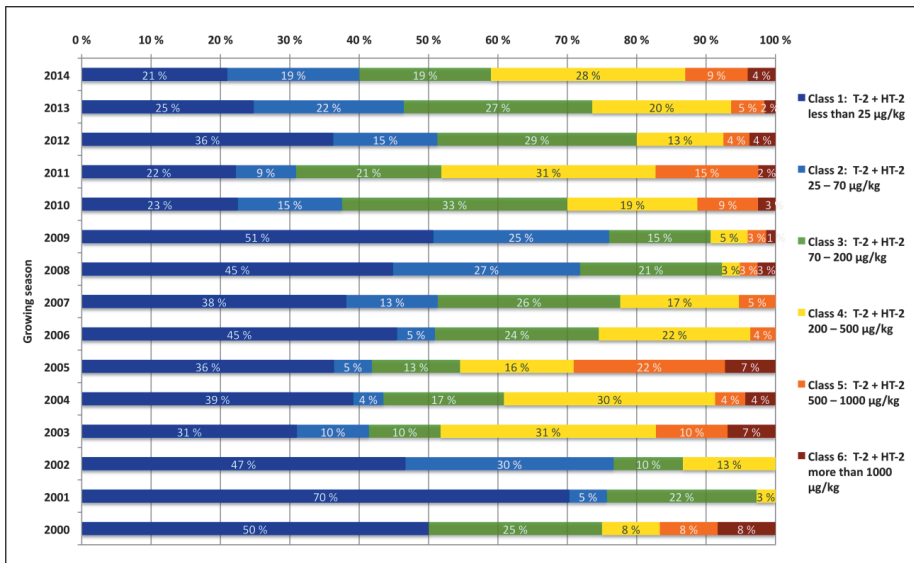


Figure 20. T-2+HT-2 levels in oat samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014.

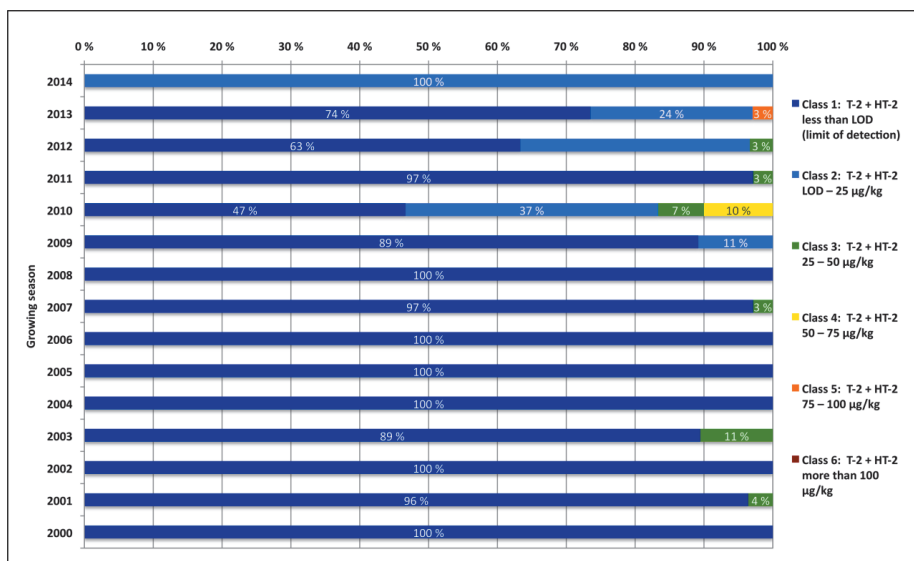


Figure 21. T-2+HT-2 levels in spring wheat samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014.

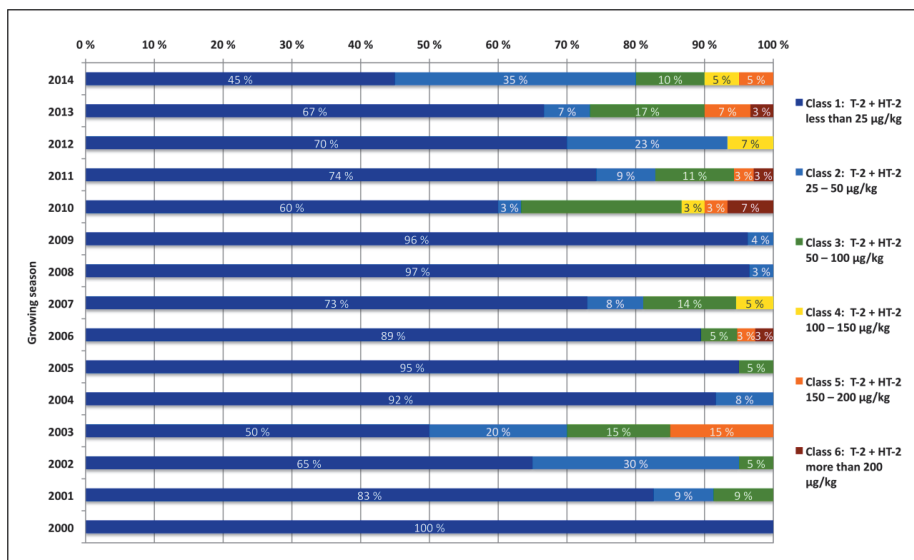


Figure 22. T-2+HT-2 levels in barley samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014.

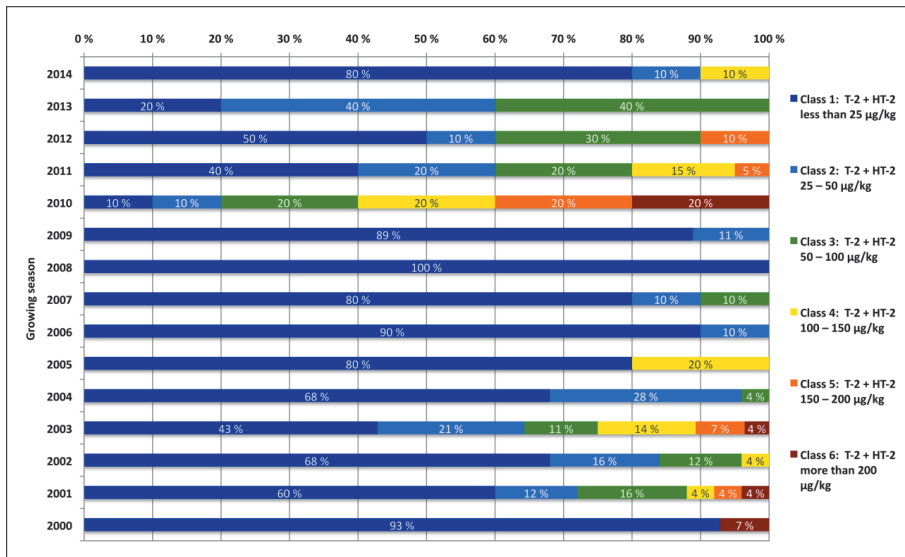


Figure 23. T-2+HT-2 levels in malting barley samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014.

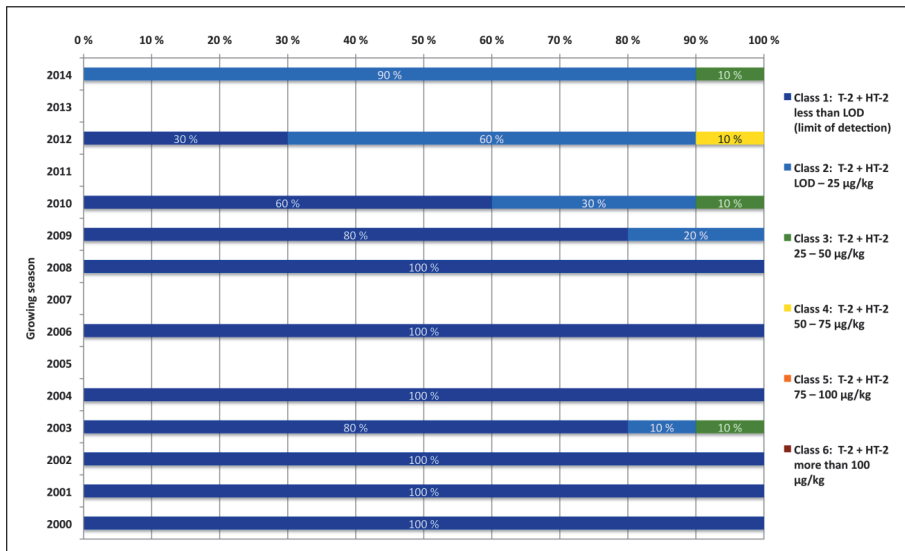


Figure 24. T-2+HT-2 levels in winter wheat samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014. Winter wheat samples were not collected in 2005, 2007, 2011 and 2013.

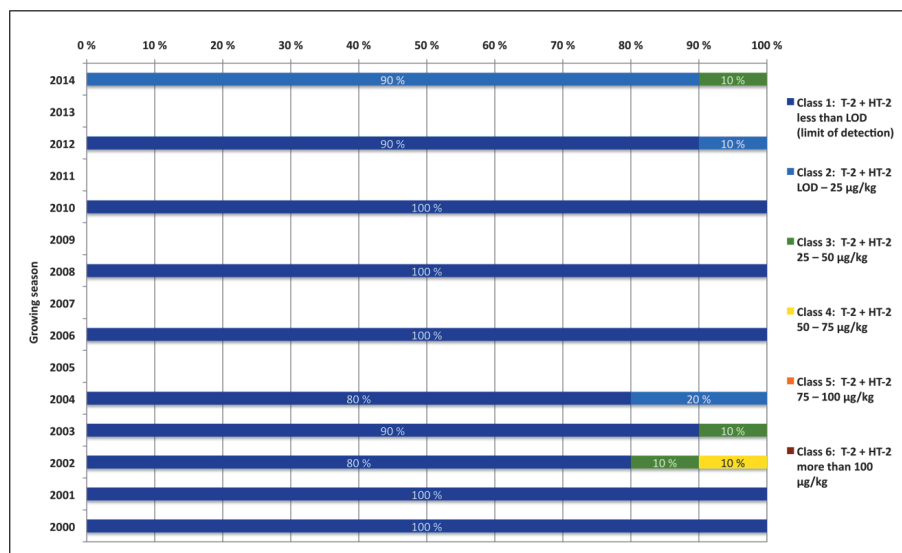


Figure 25. T-2+HT-2 levels in winter rye samples which are classified into six categories over the 2000–2014 period. The results of the figure are based on the Finnish Safety Monitoring Programme 2000–2014. Winter rye samples were not collected in 2005, 2007, 2009, 2011 and 2013.

6.4.2.1 Exposure of *Fusarium* toxins from Finnish cereal grains

The exposure of Finnish population to *Fusarium* toxins was evaluated in a project implemented by the Finnish Food Safety Authority Evira in collaboration with Agrifood Research Finland and the National Public Health Institute (Rautala et al., 2008). Adult intake from cereals and cereal-based products was studied from the data of FSMP during 1999–2007 and the monitoring results of Evira in 2007. According to the results of the study the average DON intake from cereal grains in women (25–74 years old) and men (25–74 years old) was 5.4 µg and 6.3 µg per day, respectively. Corresponding DON P95 values of cereal intake in women and men were 12.4 µg (18 % from acceptable TDI) and 15.3 µg (18 % from acceptable TDI) per day, respectively. Based on the same study, the following intake values for T-2+HT-2 were found: women 1.8 (3.9 P95; 92 % from acceptable TDI) µg, and men 2.2 (5.1 P95; 100 % from acceptable TDI) µg.

6.4.2.2 How to control and manage the risk

Based on the analytical results of the FSMP knowledge about the agronomic variables, such as growing area, sowing date, the type of soil, variety of the grain, quality of seed and seed dressing, plant rotation, nitrogen fertilization, plant protection procedures during the growing season, growth period, harvesting-related moisture, harvest quantities, harvest date and the method of harvest has been increased and used for cultivation-related directives that farmers and industry should follow to ensure high-quality production of cereal grains in Finland. Every year the *Fusarium* toxin data has been analysed against the agronomical

variables to better understand the causal relationships between toxin production and the cultivation methods used in the field. According to the results a risk assessment for *Fusarium* fungi contamination and the formation of toxins in Finland have been identified (IV). The following cultivation-related guidelines have been made for the grain chain actors to better control *Fusarium* contamination (Table 9, Hietaniemi et al., 2008, 2010, 2014):

- rotation, repeated cultivation of cereals is not recommended
- careful selection of the type of grain and the variety compared to a growth environment
- spring grains are more sensitive to *Fusarium* contamination than winter grains
- pay attention to the quality of seed – sort and dress the seed
- accurate and fast harvest drying; moisture content < 14 %
- sorting will reduce the mycotoxin risk, de-hulling of oats has a significant effect, as much as a 90% drop in toxin contents
- last, but not least, minimize the risks by professional cultivation.

Table 9. Risk table based on agronomic factors from pre-harvest to post-harvest. Data used for the risk evaluation is from the Finnish Safety Monitoring Programme 2000-2014.

Cultivation field			Risk factors for <i>Fusarium</i> fungi				
			no risk	low risk	high risk	critical risk	farmer's observations of risk factors for <i>Fusarium</i> fungi
Location	cropping zone 1	irrespective of grain					
	cropping zone 2	irrespective of grain					
	cropping zone 3	irrespective of grain					
	cropping zone 4	irrespective of grain					
Soil	soil type	clay					
		sand					
		mould					
		mud or muddy clay					
		peat					
	pH of soil is under 6,0	irrespective of grain					
Preceding crop in rotation	2 years growth of same plant in the same area	oats					
		wheat and barley					
	3 years growth of same plant in the same area	oats					
		wheat and barley					
	4 years growth of same plant in the same area	oats					
		wheat and barley					
Sowing and tilling methods	sowing method of growing period	zero-till					
		sowing after tilling					
	tilling method of previous autumn	autumn ploughing					
		low tilling					
		zero-till					
Cultivation process							
Cultivation technique	traditional cultivation	oats					
		wheat and barley					
	organically-grown	irrespective of grain					
Cultivated plant	correlate with grain	oats					
		wheat and barley					
		malting barley					
	correlate with variety	rye and winter wheat					
		oats					
		wheat and barley					
Quality of seed	no quality guarantee	irrespective of grain					
	quality guarantee	irrespective of grain					
	no seed dressing	irrespective of grain					
Rate of fertilization nitrogen	in accordance with cultivation guide of grain						
	out of line with cultivation guide of grain						
Plant protection	herbicides	oats					
		wheat and barley					
	herbicides and plant disease	oats					
		wheat and barley					
	herbicides and plant regulators	oats					
		wheat and barley					
Weather conditions during growing season							
Weather conditions at start of growing season	start of growing season	rainy					
		dry					
Weather conditions during flowering season	flowering season	rainy & RH > 80 %					
		dry					
Weather conditions during harvesting	harvesting season	late					
		rainy & RH > 80 %					
	temperature variation	large					
Harvesting and drying							
Flattening %	under 5 %	irrespective of grain					
	5 - 25 %	oats					
		wheat and barley					
	over 25 %	irrespective of grain					
Drying	warm air drying, humidity %	under 25 %					
		over 25 %					
	non-immediate drying, humidity %	under 14 %					
		over 15 %					
Sorting of grain	unsorted	irrespective of grain					
Storage of grain	storage space	inadequate					

RH = relative humidity

These agronomic guidelines correlate very well with prevention and reduction of contamination by trichothecenes in cereal grains; recommended practices based on good agricultural practices and good manufacturing practice published by Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (Proposed draft revision of the code of practice for the prevention and reduction of mycotoxin contamination in cereals (CAC/RCP 51-2003), 2014; 39th Session Rome, Italy, 27 June–1 July 2016). Besides the above-mentioned cultivation-related guidelines the results from the Finnish Safety Monitoring Programme have been utilised in many other ways, such as in developing a risk table in 2007 based on agronomic factors (Table 9), and a room flat for farmers, industry, various organizations of primary production and authorities. In 2007 the Finnish Cereal Committee published a guide on Fusarium head blight: ‘Fusarium head blight in grain. Cultivation technology measures to reduce the risk of mycotoxins’. The risk Table 9 was updated in 2013 and included in this guide. The guide has provided help for farmers to make choices reducing risks in cultivation plans and estimate the quality of cereal crops in advance.

In addition, many research projects during these years have used or have been using the monitoring data for their purposes. The effects of climate change on incidence of mycotoxins have been studied in the SAFEFOODERA -project in 2009-2011. Also one of the very challenging projects has been the Safe Food - Safe Dairy project concerning the mycotoxins in the feed-dairy chain in Kenya. In the last mentioned project food safety and risk assessment have been the most important targets.

6.5 Sophisticated tools of risk control at farm level

Maintaining and improving the level of competence of rural entrepreneurs and other operators is the main objective of rural development. For an agricultural enterprise, the know-how and competence of the entrepreneur constitute the most important success factors. Production of high-quality raw material for the needs of the food and feed industry, livestock production and other end users is the main objective of the primary production of cereals. Total quality management is a significant competitive factor for domestic grain raw material. It is a central task for quality management to ascertain and document the quality of grain raw material suitable for the intended use for the grain supply chain and to increase cost-effectiveness, productivity and consumer safety.

Quality management of the grain supply chains ‘primary production – food and cosmetic industry – consumer’ or ‘primary production – livestock production – food industry – consumer’ can be significantly improved at the farm by introducing more analytical measurement technology, guidance on use, and logistics management to the process of harvesting, drying and storing of grain. Along with the management of the chain, specialized sections of the chain will also provide possibilities to increase the export of cereals and processed grains, e.g. organic oats, pure oats, baby food and batches of varieties.

At this stage, versatile grain quality analysis, documentation and storage of application-

specific quality adopt a significant role and require the development of existing systems. At present, a major proportion of harvested grain is already being stored in farm silos, and single plot-specific accounting reveals the cultivation history.

6.5.1 Practical operations at farm level

According to the results of the FSMP and the VILJANITTI -project (www.viljanitti.fi) in 2012–2014 more analytical facilities were introduced at the farm level. The introduction of NIT in the processes of reception, drying, pre-cleaning and storage of farm-level grain enabled the measurement of the technical quality of the grain as well as the utilization of results in real time. In 2014 at the field laboratory rapid tests for mycotoxins DON and T-2+HT-2 were also introduced. Analysis certificates and traceability of grain batches increased the confidence of grain buyers and the industry in the purchase and use of cereal raw material. Real-time management of grain quality using NIT increased also the collaboration among the members of farmer communities, grain control between farms and exchange of cultivation knowledge. The work of the Finnish Cereal Committee and the information available through it were brought forth at several farmers' meetings during the development project. The domestic and world market prices of cereals, global cereal balances, cultivation guides and wall hangings, grain passport and the guide for grain trade and contract cultivation were introduced to farmers via the VYR pages.

All assay results and background information on the samples were stored in the grain control data system. It was thus possible to ascertain the traceability of grain batches even to the farmer and field level. Different varieties of variety-specialized cultivation were stored in separate silos, whereby no mixing of grain batches takes place. The grain control data system makes it easy to send electronically signed analysis certificates of technical quality and safety to the grain buyer/customer. Assurance of quality, analysis certificates, documentation of cultivation history and assurance of validity of analysis results are becoming an increasingly significant competitive factor and mainstay for the grain raw material buyers, downstream processors and trade. Very strict requirements are applied in export and supply of grain for the needs of the baby food industry, necessitating comprehensive documentation of grain raw material. Approximately 50 farmers/producers participated in the farmer groups. They gained attention for their results through their co-operation partners in the food industry and these companies utilized their output in their downstream processing.

It was also possible to strengthen the co-operation among farmers by increasing the logistics of grain harvesting and control and storage of grain material streams. This brought cost savings and eco-efficiency by decreasing unnecessary grain transports, energy consumption and emissions, and also eliminated the risk of rejection of grain batches at grain reception.

6.6 Future outlook

In the future, seed conditioning, dressing and the use of certified seed should be prioritized in the management of toxin risk. Often there is no information available on the *Fusarium* infestation and mycotoxin levels of a seed batch. Batch-specific assays for *Fusarium* fungi and mycotoxins should be taken into account in seed management. Of Finnish grains, oats is the most susceptible to *Fusarium* infestation, but there are few studies about its varietal resistance (Figure 26). A great deal of further research will be required in plant breeding, especially in oats. The market is eagerly looking for new varieties capable of preventing *Fusarium* infestation and having low levels of mycotoxins.



Photo: Veli Hietaniemi

Figure 26. High-quality Finnish oats have been closely controlled at the farm level to ensure their safety with regard to mycotoxin content – risk control and quality management procedures have been documented.

The introduction of quick methods on the farm level is a solution to the guidance of grain use and assurance of safety quality (Figure 27). During high-risk years, sampling can be directed to the ripening crop, whereby advance information about the possible mycotoxin risk will be obtained. Besides rapid methods, chromatographic techniques and good analytical know-how will be needed in the Finnish research laboratories in order to confirm the correctness and reliability of the results.



Photo: Veli Hietaniemi

Figure 27. Field laboratory at the Kaski farm in Sastamala, Finland. In the laboratory it is possible to analyze technical (moisture, protein, starch and hectoliter using NIT -technique) and safety (DON, T-2+HT-2 using rapid lateral flow tests) quality.

More research is needed to develop sophisticated optical sorters equipped with high-speed cameras suitable for mycotoxin management. A new sorter which uses near-infrared technology to look at chemical structure and composition of kernels, and to pick out the *Fusarium* -infected kernels appears promising.

More research is also needed concerning risk models. The risk model should be based on a wide and regionally comprehensive weather data set and sampling, accurate data of the cultivation history associated with the samples, and reliable mycotoxin analysis. The short-term goal shall be a user-friendly, electronic predictive model, which allows real-time monitoring of the magnitude of risk. The use of sophisticated farm-specific and farmers' network -specific weather stations should be increased, thus supporting the development of the model as well as farm-specific monitoring of the magnitude of the mycotoxin risk. A more accurate monitoring of weather conditions can also be utilized in the timing of plant protection procedures.

It should also be taken into account, that continuous monitoring of the safety and quality of grains together with analysis of causal connections will confirm that our confidence in safe Finnish food is maintained and transmitted to future generations as well. It is problematic, that intake calculations of mycotoxin levels in cereals are not currently being conducted at regular intervals in Finland. The extensive use of grains, especially the increasing use of oats in animal feed should also be considered so that the well-being and health of animals is secured. Furthermore, the use of mycotoxin binders used as feed additives is one means to reduce the adverse effects of mycotoxins in animals, but future studies on the detoxification of mycotoxins, e.g. microorganisms with mycotoxin degradation activity, will also be required in order to reduce any adverse effects.

Wisdom is learned from history. We need to get back to the basics a little bit more, raise the value of soil health, good water management, high-quality seed and variety, crop rotations, careful monitoring of crops, correctly timed pesticide applications, and to invest in careful harvest, drying and sorting of crops. However, humankind cannot continue to look back at history but continue to improve mycotoxin risk assessment and toxin control – proud to farm in Finland and provide consumers with safe and high-quality cereals and cereal products.

There is a significant need to increase the competitiveness and cost-effectiveness of grain farming in the specialization of national and international markets, and make actors in the cereal chain committed to the production of quality grains, the novel utilization of side streams and the recycling of nutrients. Owing to new product applications and technologies, the use of and demand for high food-quality Finnish oats has increased. Oats are being processed into valuable products such as rolled oats and snack products, health products, pulled oats, flour treatment agents, margarine substitute, protein source, weight control products and cosmetics. The demand for high-quality oats and other cereal grains in the grain-processing industry are increasingly centred on application-specific varieties as well as high technical and hygienic quality. Products of higher nutritional and safety quality have a direct impact on human and animal health.

6.7 Conclusions

According to the results of present study, *Fusarium* fungi and toxins produced by them are an increasing risk factor for Finnish cereal production.

The most common *Fusarium* species found in Finland in this study were *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. sporotrichioides* and *F. langsethiae*. *F. avenaceum* was the most dominant species in barley, spring wheat and oat samples (IV). The occurrence of *F. culmorum* and *F. graminearum* was high in oats and barley. Infection by *Fusarium* fungi was the lowest in winter cereal grains. *F. langsethiae* has become much more common in Finland since 2001. *F. graminearum* has also risen in the order of importance. A highly significant correlation was found between *F. graminearum* DNA and deoxynivalenol (DON) levels in Finnish oats, barley and wheat. Climate change is leading to warmer weather, and this may indicate more changes in Finnish *Fusarium* mycobiota and toxin contents and profiles in the near future.

Changes in *Fusarium* mycotoxins in Finnish cereals were clearly observed in the years 1987–2014 (I, II, IV). At the end of 1980s the results showed that over 90% of all Finnish grain and feed samples contained DON from 7 to 300 µg/kg and over 30% of samples contained smaller amounts (13–120 µg/kg) 3-AcDON. The most toxic trichothecenes, T-2, HT-2 and NIV and also zearalenone were found at low concentrations in 1–10% of the samples analysed. The results of official variety and nitrogen trials at the turn of the 2000s showed that the mycotoxin DON was found most frequently in Finnish oats during 1997–1999. According to the results on an average 55% of the oat samples respective to the

official variety trials in 1997–1999 contained DON within the range of 50–896 µg/kg. Correspondingly, the range and frequencies of other toxin findings were as follows: 3-AcDON 50–310 µg/kg (5%), NIV 50–575 µg/kg (16%), T-2 toxin 50–349 µg/kg (1%) and HT-2 toxin 50–507 µg/kg (4%). The results also showed that no distinct differences were found in DON contents of various varieties. The differences in DON concentrations between organic and conventional cultivation were small. In addition, the results indicated that the use of various nitrogen fertilization levels only slightly affected the trichothecene concentrations. Based on the results of the Finnish Safety Monitoring Programme 2000–2014 oats was the cereal crop most sensitive to *Fusarium* infestation in Finland and DON and T-2+HT-2 concentrations have increased during the new Millennium. According to the FSMP, DON toxin levels in oats and spring wheat increased especially in 2011–2014.

The present results regarding the cause-effect relationships between mycotoxin contents and agronomic factors (II, IV) also showed that more precise research into the effects of cultivation methods in relation to fungi and toxins is necessary. The following important control and management factors were emphasized according to the results of the FSMP: rotation - repeated cultivation of cereals is not recommended; pay attention to the quality of seed - seed dressing is recommended also for oats; careful harvest drying - moisture content < 14%; introduction of rapid test methods and sorting technology at farm level to detect and remove toxins, and last, but not least, minimize the risks by professional cultivation. The results obtained in this study are in very good agreement with the agronomical means suggested by the EU and FAO/WHO for lowering the mycotoxin risk.

In this study, the relationship between weather data and agronomical factors and DON levels in oats was examined with the aim of developing a Nordic prediction model (III). The agronomical factors examined were flowering date and harvest date (ordinal date), period between flowering and harvest (days), tillage system (ploughed or not), pre-crop (oats as pre-crop or not), and soil type (silty or not). A silty soil is defined as $\geq 80\%$ silt and $< 12\%$ clay. The results showed that there was a large regional variation in DON levels, with much higher levels in one region in Norway compared with other regions in Norway, Finland and Sweden. Agronomical factors or weekly weather data did not explain, or explained only to a limited degree, the variation in DON levels. Including more variables in a multiple regression model only increased the adjusted coefficient of determination from 0.17 to 0.24, indicating that little of the variation in DON levels could be explained by either weather data or agronomical factors. Thus, it does not seem to be possible to predict DON levels in oats at the Nordic level based on the variables examined in this study.

Changes in cultivation techniques, profitability issues and climate change bring new risk factors to the safety quality of grains. Weather conditions are the central factor affecting the formation of mycotoxins. It is possible that new fungal species and new mycotoxins will spread to Europe and Scandinavia along with the changing climate (temperature, rainfall/extreme phenomena, CO₂). A warming of climate by two degrees is predicted to bring aflatoxins produced by *Aspergillus* fungi to Europe in addition to the already present *Fusarium* toxins. Aflatoxins are carcinogenic compounds that have caused significant

problems in Africa and Asia. Risk management requires close collaboration with growers, researchers, and grain processing industry on the understanding of mycotoxin risk, critical points and the effects of agronomical and climatic factors on *Fusarium* mold infestation and generation of mycotoxins during the growing season.

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DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

1. **REINO R. LINKO (1967)** Fatty acids and other components of Baltic herring flesh lipids. (Organic chemistry).
2. **HEIKKI KALLIO (1975)** Identification of volatile aroma compounds in arctic bramble, *Rubus arcticus* L. and their development during ripening of the berry, with special reference to *Rubus stellatus* SM.
3. **JUKKA KAITARANTA (1981)** Fish roe lipids and lipid hydrolysis in processed roe of certain *Salmonidae* fish as studied by novel chromatographic techniques.
4. **TIMO HIRVI (1983)** Aromas of some strawberry and blueberry species and varieties studied by gas liquid chromatographic and selected ion monitoring techniques.
5. **RAINER HUOPALAHTI (1985)** Composition and content of aroma compounds in the dill herb, *Anethum graveolens* L., affected by different factors.
6. **MARKKU HONKAVAARA (1989)** Effect of porcine stress on the development of PSE meat, its characteristics and influence on the economics of meat products manufacture.
7. **PÄIVI LAAKSO (1992)** Triacylglycerols – approaching the molecular composition of natural mixtures.
8. **MERJA LEINO (1993)** Application of the headspace gas chromatography complemented with sensory evaluation to analysis of various foods.
9. **KAISLI KERROLA (1994)** Essential oils from herbs and spices: isolation by carbon dioxide extraction and characterization by gas chromatography and sensory evaluation.
10. **ANJA LAPVETELÄINEN (1994)** Barley and oat protein products from wet processes: food use potential.
11. **RAIJA TAHVONEN (1995)** Contents of lead and cadmium in foods in Finland.
12. **MAIJA SAXELIN (1995)** Development of dietary probiotics: estimation of optimal *Lactobacillus* GG concentrations.
13. **PIRJO-LIISA PENTTILÄ (1995)** Estimation of food additive and pesticide intakes by means of a stepwise method.
14. **SIRKKA PLAAMI (1996)** Contents of dietary fiber and inositol phosphates in some foods consumed in Finland.
15. **SUSANNA EEROLA (1997)** Biologically active amines: analytics, occurrence and formation in dry sausages.
16. **PEKKA MANNINEN (1997)** Utilization of supercritical carbon dioxide in the analysis of triacylglycerols and isolation of berry oils.
17. **TUULA VESA (1997)** Symptoms of lactose intolerance: influence of milk composition, gastric emptying, and irritable bowel syndrome.
18. **EILA JÄRVENPÄÄ (1998)** Strategies for supercritical fluid extraction of analytes in trace amounts from food matrices.
19. **ELINA TUOMOLA (1999)** *In vitro* adhesion of probiotic lactic acid bacteria.
20. **ANU JOHANSSON (1999)** Availability of seed oils from Finnish berries with special reference to compositional, geographical and nutritional aspects.
21. **ANNE PIHLANTO-LEPPÄLÄ (1999)** Isolation and characteristics of milk-derived bioactive peptides.
22. **MIKA TUOMOLA (2000)** New methods for the measurement of androstenedione and skatole – compounds associated with boar taint problem. (Biotechnology).
23. **LEEA PELTO (2000)** Milk hypersensitivity in adults: studies on diagnosis, prevalence and nutritional management.
24. **ANNE NYKÄNEN (2001)** Use of nisin and lactic acid/lactate to improve the microbial and sensory quality of rainbow trout products.
25. **BAORU YANG (2001)** Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries and physiological effects of sea buckthorn oils.
26. **MINNA KAHALA (2001)** Lactobacillar S-layers: Use of *Lactobacillus brevis* S-layer signals for heterologous protein production.
27. **OLLI SJÖVALL (2002)** Chromatographic and mass spectrometric analysis of non-volatile oxidation products of triacylglycerols with emphasis on core aldehydes.
28. **JUHA-PEKKA KURVINEN (2002)** Automatic data processing as an aid to mass spectrometry of dietary triacylglycerols and tissue glycerophospholipids.
29. **MARI HAKALA (2002)** Factors affecting the internal quality of strawberry (*Fragaria x ananassa* Duch.) fruit.
30. **PIRKKA KIRJAVAINEN (2003)** The intestinal microbiota – a target for treatment in infant atopic eczema?
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33. **KAISA YLI-JOKIPII (2004)** Effect of triacylglycerol fatty acid positional distribution on postprandial lipid metabolism.
34. **MARIKA JESTOI (2005)** Emerging *Fusarium*-mycotoxins in Finland.
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