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Influence of barley malting operating parameters on T-2 and HT-2 toxinogenesis of *Fusarium langsethiae*, a worrying contaminant of malting barley in Europe

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The fungus *Fusarium langsethiae*, exclusively described in Europe at present, seems to have taken the place of other *Fusarium* species in barley fields over the last 5 years. It has proved to be a highly toxic type-A trichothecene producer (T-2 and HT-2 toxins). The aim of this work was to study the ecotoxinogenesis of this fungus the better to identify and manage the health risk it may pose during the beer manufacturing process. The influence of temperature and water activity on its growth rate and production of toxins are particularly assessed from a macroscopic point of view. Different cultures were grown on sterilized rehydrated barley with a water activity between 0.630 and 0.997 and a temperature ranging from 5 to 35°C. Biomass specific to *F. langsethiae* and T-2 and HT-2 toxins were quantified by real-time polymerase chain reaction and liquid chromatography-mass spectrometry, respectively. It appears that the optimal temperature and water activity for *F. langsethiae* toxinogenesis are 28°C and 0.997. This fungus was able to produce 2.22 g kg^{-1} of these toxins in 16 days on barley in optimal production conditions. The malting process seems to be a critical step because, in its temperature range, specific production was six times higher than under optimal temperatures for fungus growth. In the short-term, this work will help redefine the process conditions for malting. In the medium-term, the results will contribute to the development of a molecular tool to diagnose the presence of this contaminant and the detection of the toxins in barley, from fields to the end product.

Keywords: liquid chromatography/mass spectrometry (LC-MS); trichothecenes; *Fusarium*; mycotoxins; beer; barley; cereals; malt

Introduction

The presence of natural contaminants, like mycotoxins, in food is a recurrent problem in relation to health risks for humans and animals, as widely reported in the scientific literature (D'Mello and McDonald 1997; Scudamore and Livesey 1998; Pitt 2000; Yiannikouris and Jouany 2002). Microscopic fungi of the *Fusarium* genus are known for their capacity to contaminate cereals and to produce highly toxic molecules such as trichothecenes (Desjardins et al. 1993; Miller and Trenholm 1994; Edwards 2009a, 2009b). They are known to be cytotoxic to mammalian cells and to cause alimentary toxic aleukia (ATA) under acute toxicosis (Wannemacher et al. 2000).

Thus, several species (*F. poae*, *F. tricinctum*, *F. sporotrichioides*, and *F. graminearum*) belonging to this genus contaminate malting barley and produce T-2 toxin and its deacetylated form HT-2 toxin (Chen et al. 2009). The highly toxic type A trichothecenes: T-2 and HT-2 toxins, are of special interest because T-2 toxin has been shown to induce DNA fragmentation and to inhibit protein synthesis (Beasley 1989). Currently, there is no legislation concerning these toxins but, in the near future, maximum levels for T-2 and HT-2 trichothecenes will be regulated by the European Commission as announced in EU NO 1881/2006.

The problem of contamination by toxinogenic fungi becomes serious when disequilibrium suddenly occurs inside the producing population. In fact, it seems that, for 5 years, the well-known species producing T-2 and HT-2 toxins, such as *F. sporotrichioides*, have been supplanted in the barley fields by another species, *F. langsethiae*. This species is newly described in Europe and is proving to be a major T-2 and HT-2 toxin producer (Torp and Nirenberg 2004). It has been isolated from kernels of straw cereals in several European countries. *F. langsethiae* shares common morphological characteristics with *F. poae* and the isolates were originally named 'powdery *F. poae*'.

With the aim of contributing to future legislation in France, and of controlling the health risk due to the presence of this new toxin producer on barley for

malting, the ecotoxicogenesis conditions of *F. langsethiae* were investigated on sterilized rehydrated malting barley. Temperature and water activity (a_W) are the primary environmental factors that influence growth and mycotoxin production by several Fusaria species in cereals (Magan and Lacey 1984; Ramirez et al. 2006). In the present work, their influence on growth and toxin production were particularly assessed from a macroscopic point of view. Different cultures were carried out on sterilized rehydrated barley with a_W ranging from 0.63 to 0.99 and temperature ranging from 5 to 35°C. Biomass and final mycotoxin concentrations were quantified at the end of culture by real-time polymerase chain reaction (PCR) and liquid chromatography/mass spectrometry (LC-MS), respectively.

This work was in line with a national research programme, the aim of which is to better identify all aspects of the health risk incurred following the appearance of *F. langsethiae* on malting barley.

Materials and methods

Microbial strain

The microbial strain used was the microscopic fungus *Fusarium langsethiae* isolated from the harvest of 2008. It was provided by the Institut Français de la Brasserie et de la Malterie (serial number 35-2008). Conidia were obtained from solid culture on Petri dishes filled with PDA medium. Spores were harvested after a 5-day incubation period. Four 1-cm³ cubes of agar-microbial carpet were cut up and then transferred to a Schott bottle containing sterilized barley kernels. The culture was shaken energetically in order to distribute the microorganisms uniformly throughout the culture.

Culture conditions

Sterilized rehydrated barley (Cellar variety) was used as a culture medium. Culture parameters (temperature and water activity ranges) were chosen according to the ecology of the fungus *F. langsethiae*. Different volumes of distilled water were added to 250 ml Schott bottles containing 50 g of barley kernels to obtain different values of a_W. They were then sterilized at 121°C for 20 min. Cultures were performed under static conditions in thermostatically controlled incubators for 7 and 23 days. To ensure the proper aeration of each culture, the vial stopper was half unscrewed. A Schott bottle filled with sterilized rehydrated barley was incubated at 28°C, and the fungal biomass and the concentrations of toxins were quantified during the culture. This was used as a negative control to ensure the culture material was sterile.

Analytical methods

Water activity was measured using a portable instrument (display instrument HygroPalm Aw1® and water activity probe HygroClip AW-DIO®; Rotronic AG, Basserdorf, Switzerland). After growth of *F. langsethiae* on rehydrated barley, the whole samples were dried overnight at 70°C and ground before biomass and toxin analysis. *F. langsethiae* DNA analysis (DNA quantification) and multiplex trichothecene quantifications were performed by QualtechTM (Vandoeuvre les Nancy, France). DNA quantifications were made by real-time PCR (LightCycler, Roche Diagnostics, Meylan, France) using primers developed by QualtechTM.

Trichothecene determinations were performed simultaneously by LC-MS/MS (TSQ Quantum Ultra, Thermo Fisher Scientific, Cergy Pontoise Cedex, France). The method was validated according to standard NF V03 110 (Analysis of Agrifood – Protocol of Characterization for the Validation of a Quantitative Method of Analysis by Construction of an Accuracy Profile). The T-2 and HT-2 detection limit was $0.05\,\mu g\,l^{-1}$. The limit of quantification of toxins was equal to twice the detection limit. The biomass detection limit was two *F. langsethiae* genomes/2500 barley genomes. The limit of quantification of biomass was equal to twice the detection limit.

Each sampling was carried out in triplicate on three independent cultures, and the arithmetic mean calculated. Experimental errors were the gap-types determined from the arithmetic mean.

Moisture determination

Initial 'dry' kernel moisture (M_{DK}) amounted to 10%. Kernel moisture (M_K) according to the quantity of water added was determined as follows: W_B is the quantity of barley used for the experiments; and W_{H_2O} is the quantity of water added to the kernels.

$$M_K = \frac{(W_B \times M_{DK}) + W_{H_2O}}{W_B + W_{H_2O}} \times 100$$

Results

Moisture and water activity of malting barley

The 'water added – a_W' profile specific to the barley is presented in Figure 1. The barley sorption isotherm was confirmed to be of type II with a sigmoid form. It showed the quantity of water actually available for microorganisms (a_W) in relation to the total amount of water added to the kernel. Thus, the profile specific to cellar barley allowed us to select five values of a_W to test, from 0.629 to 0.997 (Table 1). Knowing the ability
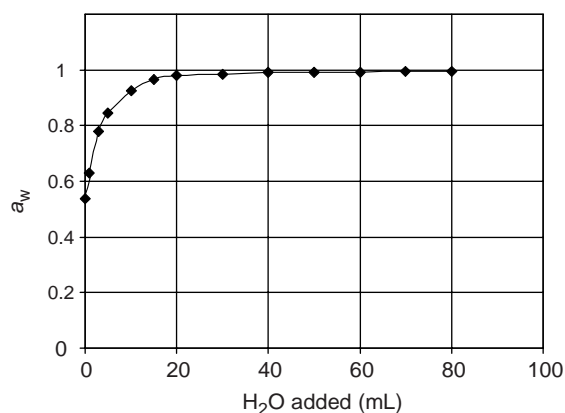
Figure 1. Sorption isotherm of barley: evolution of a_W according to the quantity of water added to 50 g of barley.

Figure 2. Biomass production (number of genomes/1000 barley genomes (Go)) after 7 days versus temperature and a_W.

Table 1. Correspondence between the quantity (ml) of water added to the kernel, the moisture (%) of the grain, and the water activity (a_W) measured. Initial 'dry' kernel moisture was 10%.

H_2O added (ml)	a_W measured	Moisture (%)
1	0.629	11.8
5	0.845	18.2
20	0.982	35.7
50	0.992	55.0
70	0.997	62.5

of microscopic fungi to develop at low water activity (Corry 1987) and the water absorption capacity of barley, we were able to determine the values of a_W to test.

Effect of environmental parameters on growth

The results obtained from the quantification of the biomass produced after 7 days of culture according to two parameters: a_W and temperature are plotted on Figure 2. It shows that this fungus is able to develop at temperatures between 22 and 30°C and at an a_W between 0.845 and 0.997. The optimal growth conditions found for the barley were for $a_W = 0.992$ and at 28°C. A residual growth was observed at low a_W. In contrast, no production of biomass was observed at 5 and 35°C.

Effect of environmental parameters on production

The results of the T-2 and HT-2 toxin concentrations reached after 7 days of culture on barley are presented in Figure 3. It appears that there was twelve times more T-2 toxin (Figure 3(a)) than HT-2 toxin (Figure 3(b)) after 7 days regardless of the conditions of a_W and temperature during culture. The optimal conditions for biosynthesis were close to those for growth (i.e.
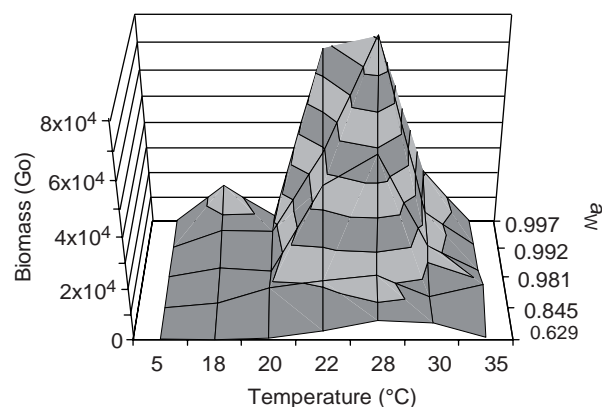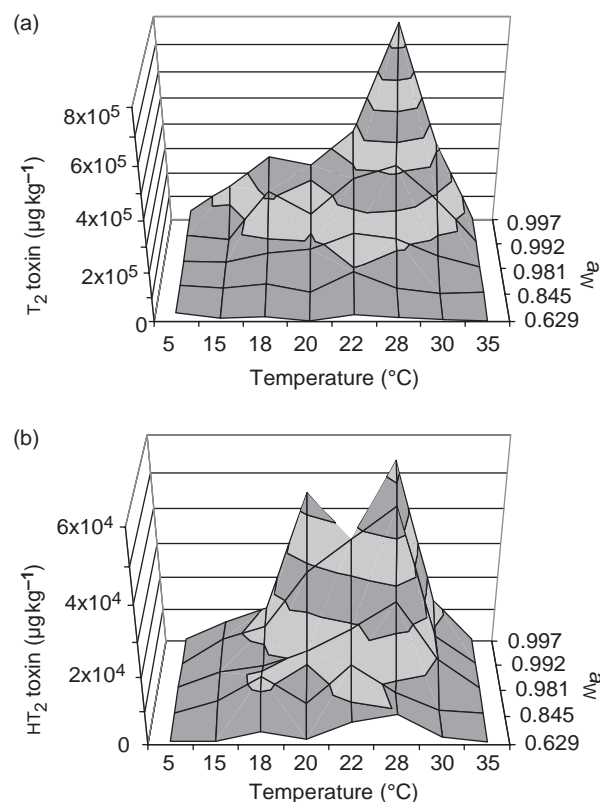

(a)

(b)

Figure 3. T-2 toxin (a) and HT-2 toxin (b) productions ($\mu g \, kg^{-1}$) after 7 days versus temperature and a_W.

$a_W = 0.997$ and 28°C). The production and growth ranges were also similar: from 18 to 30°C and at a_W between 0.845 and 0.997. There was no toxin production under extreme temperatures of 5 and 35°C.

Specific production

The specific production represents the quantity of toxins produced divided by the biomass present after 7 days of culture. Table 2 shows the values of specific

Table 2. Specific production according to culture conditions (a_W and temperature) after 7 days on barley. Specific production represents toxins produced divided by biomass produced ($\mu g\,kg^{-1}$ number of genomes^{-1}).

Temperature (°C)	a_W	Biomass (Go/1000 barley Go)	T-2 ($\mu g\,kg^{-1}$)	HT-2 ($\mu g\,kg^{-1}$)	Specific production
18	0.982	3.8E+03	6.5E+04	7.6E+03	19.1
	0.992	7.3E+03	2.2E+05	1.2E+04	31.6
	0.997	1.5E+04	2.7E+05	1.0E+04	18.6
20	0.982	2.3E+03	8.0E+04	1.2E+04	39.4
	0.992	7.1E+03	1.2E+05	2.8E+04	21.4
	0.997	2.1E+03	2.3E+05	4.4E+04	131.6
22	0.982	3.7E+04	1.5E+05	1.9E+04	4.5
	0.992	3.8E+04	2.7E+05	3.8E+04	8.0
	0.997	6.8E+04	3.7E+05	3.0E+04	5.9
28	0.982	4.8E+04	1.2E+05	2.7E+04	3.1
	0.992	8.1E+04	3.2E+05	4.7E+04	4.6
	0.997	4.6E+04	7.8E+05	5.3E+04	18.0

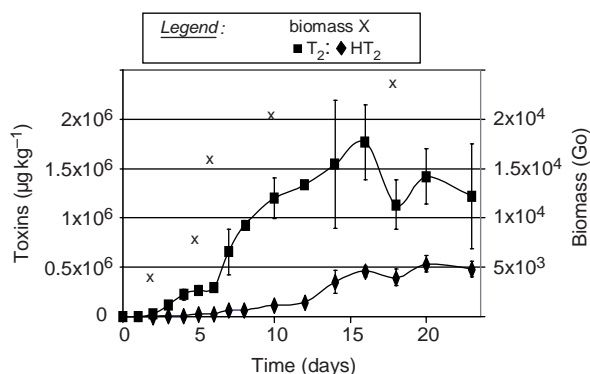Figure 4. Biomass (genomes/1000 barley genomes (Go)) and toxin ($\mu g\,kg^{-1}$) concentration variations with time (days) on barley. $a_W = 0.997$ and temperature $= 28°C$. Biomass, crosses; T-2 toxin, squares; and HT-2 toxin, diamonds.

productions for different culture conditions (temperatures $= 18$, 20, 22 and $28°C$; $a_W = 0.982$, 0.992 and 0.997). This table indicates that, below the optimum growth temperature, the specific production is, on average, six times higher.

Validation by kinetics, acquisition of new information

The a_W was adjusted at the beginning of the culture and checked after 14 days of culture. Its variation was less than 3% compared with the initial value. Culture in optimal conditions for toxinogenesis ($a_W = 0.997$ and $28°C$) was monitored for 23 days; the results are shown in Figure 4. The strain produced the maximum quantity of T-2 toxin: $1.77\,g\,kg^{-1}$ in 16 days and $0.53\,g\,kg^{-1}$ for HT-2 toxin in 20 days. The two toxins seemed to be produced at two different times. The first production of toxin T-2 began after 2 days of culture, i.e. 4 days before HT-2 toxin production. The second phase of T-2 and HT-2 toxin production began after

6 and 12 days of culture, respectively. According to the data, growth started after 2 days. The lag-phase appeared to be short. Growth and production were partially uncoupled.

After 7 days, there were $0.73\,g\,kg^{-1}$ of toxins and nearly 12 000 genomes of *F. langsethiae*/1000 barley genomes. This indicates that during this period, on barley the microorganism was in its full development phase. The results demonstrate that the stationary phase had not yet been reached in the experimental conditions. However, these results suggest the interest of performing a kinetic study on the influence of various parameters, whether environmental or not, on toxinogenesis among *F. langsethiae*.

Discussion

Optimal growth and production conditions were similar. In both cases, the temperature was $28°C$ and a_W values were 0.992 and 0.997 for growth and production, respectively. However, as secondary metabolites, T-2 and HT-2 toxins should be produced in conditions not propitious for growth. Conversely, growth should intervene in culture conditions unfavourable to the biosynthesis of these mycotoxins (Hodgson 2000). The residual growth observed at low a_W confirms the fact that the microscopic fungi are able to grow in poor available water conditions (Corry 1987; Nielsen et al. 2004; Samapundo et al. 2005). In this study, $2.22\,g\,kg^{-1}$ of toxins (T-2 and HT-2) had been produced after 16 days of culture ($a_W = 0.997$ and $28°C$). This quantity of toxins produced by *F. langsethiae* on barley was compared with the data given by different authors concerning type A trichothecenes, which are quite scattered. Most authors even found lower levels (Langseth and Rundberget 1999). Lincy et al. (2008) found a strain of *F. sporotrichioides* able to produce $0.13\,mg\,kg^{-1}$ of T-2 toxin on sorghum. Another strain has been reported to produce $0.941\,mg\,kg^{-1}$ of

T-2 toxin on wheat (Mateo et al. 2002). One study has been performed on well-identified *F. langsethiae* on German oats where $0.623 \, \text{mg} \, \text{kg}^{-1}$ of T-2 and HT-2 toxins were quantified (Schwake-Anduschus et al. 2009). Only one study (Logrieco et al. 1990) found similar levels. These authors reported that $1.6 \, \text{g} \, \text{kg}^{-1}$ of T-2 toxin and $0.5 \, \text{g} \, \text{kg}^{-1}$ of HT-2 toxin were produced by *F. sporotrichioides* on corn kernels.

The present study has shown that more T-2 toxins are found in barley than HT-2 toxins, with ratios between 3 and 20. These results are in agreement with observations made by other authors (Logrieco et al. 2002; Mateo et al. 2002). However, they are in total opposition to those found concerning B-type trichothecenes, where the deacetylated form, DON, is predominant (Langseth and Elen 1996). In some cases, a larger amount of HT-2 toxins has been quantified in cereals, as reported by Langseth and Rundberget (1999), Torp and Langseth (1999), Rafai et al. (2000), Hazel and Patel (2004), and Visconti et al. (2005). Moreover, Lancova et al. (2008) report that only HT-2 toxin was encountered in barley.

F. langsethiae, which was first described on the malting barley from northern Europe (Torp and Nirenberg 2004), seems to have migrated southward. Beer industry professionals have developed a hypothesis to explain this migration. It could be one direct consequence of the climate change that has lasted over the past 5 years (Krstanović et al. 2005). In fact, the temperature and the water activity ranges enabling the growth of *F. langsethiae* are close to those of other *Fusarium* trichothecenes producers (Torp and Nirenberg 2004). *F. langsethiae* might have supplanted other species thanks to its growth rate. However, this does not seem to be the case because, as described in the literature, a factor that differentiates *F. langsethiae* from *F. poae* is its slower growth (Torp and Nirenberg 2004).

The production of biomass and toxins reach optimal values at a_W of 0.992 and 0.997, respectively (see the sorption isotherm results shown in Figure 1). Despite the relatively similar a_W values, moisture contents are significantly different (55% and 62%, respectively, at optimal biomass and toxin production conditions). As shown in Figure 1, the sorption isotherm reaches an asymptote at moisture contents above 50% (i.e. 40 ml of water added to 50 g of barley kernels). Hence, addition of water to the medium does not increase a_W, i.e. the water available for microbial activity.

In this study, the barley kernels were used as a microbial culture medium when studying the influence of temperature and a_W on *F. langsethiae* growth and biosynthetic capacity. Cultures were carried out on grains sterilized by autoclave. This process resulted in the cooking of the grains: starch hydrolysis, kernel swelling and hull break-up. The enhanced synthesis of toxins recorded here must have had an additional cause beyond the influence of an increase in a_W. Possible explanations could be a facilitated access of the microorganisms to certain nutrients resulting from the modification of physical, mechanical and rheological properties of barley grains, such as the physical–mechanical disruption of the kernel hulls during autoclaving, with the addition of water. This would explain the beneficial influence of a large increase of barley moisture content observed on the production of toxins by *F. langsethiae*.

This 'cooking' effect does not mirror what happens in the field. Other studies have used means of sterilization that are non-destructive for the grain. The irradiation of kernels eliminates contaminants without altering kernel integrity (Marin et al. 1998).

First data on the specific production of these toxins by an identified strain of *F. langsethiae* have been presented in the present study (Table 2). Their analysis shows that at temperatures lower than that optimal for growth (28°C), the specific production of these trichothecenes is multiplied by six. These temperatures (18–20°C) are similar to those used during the malting process. Thus, the beer manufacturing process could be critical for the production of toxins. Despite the presence of a small amount of *F. langsethiae*, operating conditions could trigger the production of toxins even to a high level. Furthermore, the specific production data are new and important for the next experimental step, which is to develop a molecular diagnostic tool.

Conclusions

The present work provides for the first time data concerning the influence of temperature and water activity on the production of two worrying type A trichothecenes by *F. langsethiae*: the toxins T-2 and HT-2. Through these two environmental parameters we have found that culture conditions clearly influence the development of *F. langsethiae*. The ecotoxinogenic features of this strain are not surprising, being similar to those of other trichothecene producers. It produces large amounts of toxins (up to $2 \, \text{g} \, \text{kg}^{-1}$ of barley). These data provide interesting and new insights for the implementation of legislation for these toxins. The malting operation seems to be critical in terms of health risk management in the beer manufacturing process. The determination of specific production values highlights conditions for different levels of expression of genes responsible for trichothecene biosynthesis in *F. langsethiae*. Promising outcomes of this study are the establishment and validation of a reactive diagnostic tool for detecting the production of these molecules on barley.

References

Beasley VR. 1989. Trichothecene mycotoxicosis, pathophysiologic effects. Vol. 2. Boca Raton (FL): CRC Press.

Chen X, Steed A, Travella S, Keller B, Nicholson P. 2009. *Fusarium graminearum* exploits ethylene signalling to colonize dicotyledonous and monocotyledonous plants. New Phytol. 182(4):975–983.

Corry J. 1987. Food and beverage mycology. New York (NY): Van Nostrand Reinhold. Chapter: Relationships of water activity to fungal growth. p. 51–99.

D'Mello JPF, Macdonald AMC. 1997. Mycotoxins. Anim Feed Sci Tech. 69:155–166.

Desjardins AE, Hohn TM, McCormick SP. 1993. Trichothecene biosynthesis in Fusarium species: chemistry, genetics, and significance. Microbiol Rev. 57(3):595–604.

Edwards SG. 2009a. Fusarium mycotoxin content of UK organic and conventional oats. Food Addit Contam. 26(7):1063–1069.

Edwards SG. 2009b. Fusarium mycotoxin content of UK organic and conventional wheat. Food Addit Contam. 26(4):496–506.

Hazel CM, Patel S. 2004. Influence of processing on trichothecene levels. Toxicol Lett. 153:51–59.

Hodgson DA. 2000. Primary metabolism and its control in Streptomycetes: a most unusual group of bacteria. Adv Microb Phys. 42:47–238.

Krstanović V, Klapec T, Velić N, Milaković Z. 2005. Contamination of malt barley and wheat by *Fusarium graminearum* and *Fusarium culmorum* from the crop years 2001–2003 in eastern Croatia. Microbiol Res. 160:353–359.

Lancova K, Hajslova J, Poutska J, Krplova A, Zachariasova M, Dostalek P, Sachambula L. 2008. Transfer of Fusarium mycotoxins and 'masked' deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer. Food Addit Contam. 25(6):732–744.

Langseth W, Elen O. 1996. Differences between barley, oats and wheat in the occurrence of deoxynivalenol and other trichothecenes in Norwegian grain. J Phytopathol. 144:113–118.

Langseth W, Rundberget T. 1999. The occurrence of HT-2 toxin and other trichothecenes in Norwegian cereals. Mycopathologia. 147:157–165.

Lincy SV, Latha R, Chandrashekar A, Manonmani HK. 2008. Detection of toxigenic fungi and quantification of type A trichothecene levels in some food and feed materials from India. Food Contr. 19(10):962–966.

Logrieco A, Chelkowski J, Bottalico A, Visconti A. 1990. Further data on specific trichothecene production by Fusarium sect. Sporotrichiella strains. Mycol Res. 94(5):587–589.

Logrieco A, Mulè G, Morettiand A, Bottalico A. 2002. Toxigenic Fusarium species and mycotoxins associated with maize ear rot in Europe. Eur J Plant Pathol. 182(4):597–609.

Magan N, Lacey J. 1984. Water relations of some Fusarium species from infected wheat ears and grain. Trans Br Mycological Soc. 83:281–285.

Marin S, Sanchis V, Arnau F, Ramos AJ, Magan N. 1998. Colonisation and competitiveness of Aspergillus and Penicillium species on maize grain in the presence of *Fusarium moniliforme* and *Fusarium proliferatum*. Int J Food Microbiol. 45(2):107–117.

Mateo JJ, Mateo R, Jiménez M. 2002. Accumulation of type A trichothecenes in maize, wheat and rice by *Fusarium sporotrichioides* isolates under diverse culture conditions. Int J Food Microbiol. 72:115–123.

Miller JD, Trenholm HL. 1994. Mycotoxins. St. Paul (MN): Eagan. Chapter: Toxicology of mycotoxins: mycotoxins in grain: compounds other than aflatoxin; p. 359–404.

Nielsen KF, Holm G, Uttrup LP, Nielsen PA. 2004. Mould growth on building materials under low water activities. Influence of humidity and temperature on fungal growth and secondary metabolism. Int Biodeter Biodegr. 54(4):325–336.

Pitt JI. 2000. Toxigenic fungi: which are important? Med Mycology. 38:17–22.

Rafai P, Bata Á, Jacab L, Ványi A. 2000. Evaluation of mycotoxin-contaminated cereals for their use in animal feeds in Hungary. Food Addit Contam. 17:799–808.

Ramirez ML, Chulze S, Magan N. 2006. Temperature and water activity effects on growth and temporal deoxynivalenol production by two Argentinean strains of *Fusarium graminearum* on irradiated wheat grain. Int J Food Microbiol. 106(3):291–296.

Samapundo S, Devlieghere F, de Meulenaer B, Geeraerd AH, van Impe JF, Debevere JM. 2005. Predictive modeling of the individual and combined effect of water activity and temperature on the radial growth of *Fusarium verticilliodes* and *F. proliferatum* on corn. Int J Food Microbiol. 105(1):35–52.

Schwake-Anduschus C, Langenkämper G, Unbehend G, Münzing K. 2009. Occurrence of the Fusarium mycotoxins T-2 and HT-2 in oats from Germany and mycotoxin degradation during cleaning treatment and food processing. Paper presented at: ISM Conference. 1st International Society for Mycotoxicology Conference; Tulln, Austria.

Scudamore KA, Livesey CT. 1998. Occurrence and significance of mycotoxins in forage crops and silage, a review. J Sci Food Agr. 77:1–7.

Torp M, Langseth W. 1999. Production of T-2 toxin by a Fusarium resembling *Fusarium poae*. Mycopathologia. 147:89–96.

Torp M, Nirenberg HI. 2004. *Fusarium langsethiae* sp. nov. on cereals in Europe. Int J Food Microbiol. 95(3):247–256.

Visconti A, Lattanzio VMT, Pascale M, Haidukowski M. 2005. Analysis of T-2 and HT-2 toxins in cereal grains by immunoaffinity clean-up and liquid chromatography with fluorescence detection. J Chromatogr A. 1075:151–158.

Wannemacher RJ, Stanley Z, Wiener MD. 2000. Trichothecenes mycotoxins. In: Zajtchuk R, editor. Medical aspects of chemical and biological warfare. Washington (DC): Department of the Army. p. 656–670.

Yiannikouris A, Jouany JP. 2002. Mycotoxins in feeds and their fate in animals: a review. Anim Res. 51:81–99.