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**Ochratoxin A and citrinin loads in stored wheat grains: impact
of grain dust and possible prediction using ergosterol
measurement**

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Running Head : Mycotoxigenicity of grain dust

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Abstract

Crop storage should be carried out under hygienic conditions to ensure safe products, but sometimes grain dust which has settled from previous storage may be left over and incorporated to the following stored grains. This paper describes the results obtained using a lab model developed in order to assess the impact of grain dust incorporation for its direct contribution as a contaminant but also as an inoculum in stored wheat. Settled grain dust (4 samples) released from Belgian grain storages were collected and analyzed by HPLC for ergosterol, ochratoxin A (OTA) and citrinin (CIT) content. For OTA and for ergosterol, there was a high degree of variability in concentrations found in the dust samples (from 17.3 to 318 ng g⁻¹ and from 39 to 823 ug g⁻¹, respectively) whilst for CIT, the range was less significant (from 137 to 344 ng g⁻¹). Incorporation of grain dust into wheat storage contributed to an increase in the concentrations of mycotoxins in the stored grain. Dust acts as a contaminant and as an inoculum. According to these two ways, patterns of mycotoxin generation vary with the nature of the mycotoxin, the mycotoxigenic potential of dust and the water activity of the wheat. OTA and CIT showed a very versatile image when considering the amounts of toxins produced under the selected experimental conditions. The development of a robust tool to forecast the mycotoxigenicity of dust was based on the determination of ergosterol content as a general marker of fungal biomass. Present results suggest that this predictive tool would only be valid for predicting the contamination level of CIT and OTA at reasonable moisture content (14-20 %). The potential risk of having highly contaminated batches from stock to stock may thus occur and this paper discusses possible pathways leading to OTA and CIT contamination either under wet or dry storage

conditions. We therefore, recommend taking precautionary measures not only by controlling and maintaining moisture at a reasonable level during storage of the raw materials but also by paying more attention to the cleaning of the stores before loading in the new harvests

Key words: Cereals, Storage, Grain dusts, Fungal growth, Ergosterol, Mycotoxins

Introduction

Grains entering store carry a wide range of microorganisms, including bacteria, yeasts, and filamentous fungi, the population structure being dependent on field climatic conditions and harvesting processes (Magan *et al.* 2003). If harvesting is performed at the right time, crop storage should be done under hygienic conditions for safe products. Poor post-harvest management can lead to rapid deterioration in grain quality, severely decreasing germinability and nutritional values of stored grains. This is why an efficient preservation system is important for the sustainable use of food resources. However, it was observed that the storage is always performed under some environmental contamination risks (dusts, insects, birds or rodents). Dusts generated during the previous cereal-storage can be colonized by microorganisms like toxigenic fungi, which in conducive environmental conditions, may proliferate and produce mycotoxins. Ochratoxin A (OTA) has been found on a large number of agricultural products after storage (Smith *et al.* 1994, Chandelier *et al.* 2004). OTA, in combination either with sterigmatocystin or citrinin (CIT), has often been found in stored wheat, barley, oat and corn (Abramson *et al.* 1999). In Belgium, analyses of different commodities including raw foodstuffs and processed food performed during 2001-2002 have shown that of the 184 samples investigated, OTA contamination was above the limit of quantification in 73 samples (40%) and that cereal grain samples were contaminated up to $25.4 \mu\text{g kg}^{-1}$ (Chandelier *et al.* 2004). Note that this contamination exceeded the current maximum level of $5 \mu\text{g kg}^{-1}$ (EC 2002). Nowadays in Europe, cereals are frequently stored at the farm for economic reasons (low prices, use as feed). Under such

conditions, the mycotoxin risk increases because grains are not always harvested at a sufficiently low water activity to guarantee the absence of fungal growth during the storage period. In addition, the farmers' storage facilities (barns, lofts, attics, silos) are more difficult to clean up thoroughly in comparison with the more sophisticated commercial storage facilities. There can also be more important moisture sources in the farmers' storages (leaks in the roof, humidity from the walls, etc) and, hence an increased risk of mycotoxin contamination at the farm. So, Pussemier *et al.* (2005) have reviewed data from different countries and concluded that atypical severe contaminations of cereals by OTA (up to 1000 $\mu\text{g kg}^{-1}$ as observed in Poland, for example) could be linked to bad storage conditions in individual farms from either the organic or the conventional side. Based on these findings, mycotoxins can be a serious contamination problem in poorly stored grains intended for human consumption. It can also be a problem for animal health because cereals can be used to prepare animal feed and also because grain dusts collected in large grain storage facilities can be used, as a source of fiber and/or cellulose, for incorporation into feed.

Today, microbiological analyses and, more particularly, the detection of fungal genes involved in mycotoxin biosynthesis, are more and more performed in order to detect mycotoxigenic fungi. It is often proposed to use such screening tools in order to forecast mycotoxin risks in food or feed commodities (Nicholson 2004). Such approach has been successful for some mycotoxins and the links between mycotoxigenic fungi and mycotoxin production are not always obvious (Huwig *et al.* 2001, Nicholson 2004). The lack of relationship between mycotoxin-producing fungi and the measured mycotoxins may be due

to the degradation of the fungi after the production of toxin or to the competitive growth of non-toxin producing species (Halstensen *et al.* 2004) or also to the high genetical diversity (Frisvad *et al.* 2005). Moreover, many fungi are not able to produce mycotoxins or produce them in different amounts, depending on the substrate on which they grow (Huwig *et al.* 2001). Therefore, the present investigation was not only initiated to explore the possibilities offered by the chemical analyses of grain dusts derived from cereal storages but also to combine it with incubation assays with wheat grain in order to develop a new experimental tool for the assessment of the mycotoxigenic potential in wheat storages. This study also tried to ascertain whether measurement of ergosterol concentration in stored wheat grains could be useful in forecasting CIT and OTA loads.

Materials and methods

Reagents

Pure crystalline form of ergosterol (ERG), OTA and CIT were obtained from Sigma Chemical Co. (St Louis, MO, USA). Ergosterol solution was prepared in *n*-hexane, while the stock solutions of the mycotoxin standards were prepared in methanol, ethanol or acetonitrile. The concentrations were measured by spectrophotometry. Further dilutions were made in corresponding solvent and multi-mycotoxin standards were made in 50 % methanol and stored at -20°C until use.

Acetonitrile, methanol, ethanol, ethyl acetate, isopropanol, amyl alcohol, *n*-hexane, hydrochloric acid (37 %), phosphoric acid, were obtained from J.T. Baker (Deventer, The Netherlands), Riedel-de Haën (Seelze, Germany) or Biosolve (Valkenswaard, The

Netherlands). They were either of HPLC grade or of analytical grade. SPE columns Chromabond[®] XTR (70 ml, 14500 mg) were obtained from Macherey-Nagel (Düren, Germany). Microfilters type Millex[®]-HV, SLHV 013NL (0.45µm) and Durapore[®] membrane filters (type 0.22 µm GV, GVWP04700) were purchased at Millipore Corporation (Bedford, MA, USA).

Potassium hydroxide pellets were obtained from VWR International whereas sodium sulphate (exsiccated) was obtained from Riedel-De Haën AG (Seelze Hannover).

Water of HPLC-quality was obtained from a Milli-Q system of Millipore (Molsheim, France).

Grain dusts

Four settled-grain dusts were collected from storage-companies (2) and private farmers (2) established in the main agricultural region of Belgium (loamy region in central Belgium). In each case, composite dusts (with grain debris if any) were collected from cereal (wheat and/or winter barley) storages, which were performed between 2001 and 2003. On delivery, dust samples were sieved to remove particles larger than 2.0 mm. The sifted dusts were stored at +4°C in polypropylene cans. Before use, they were mixed to ensure homogeneity.

Experimental set-up

All the incubation experiments (preliminary assessment and extended incubation trials) were conducted with natural wheat grains (*Triticum aestivum*) Decan cultivar. Wheat grains [60g, 10.4 % moisture content (m.c.)] were dispersed into 300 ml-Erlenmeyer flask.

For the preliminary assessment, sterilized (autoclaved at 121°C for 20 min) and non-sterilized batches were incubated with grain-dust (1 %, w/w) at 25°C in the dark for 28 days with two occasional shakings per week. A control treatment without dust addition to the grains was carried out to determine the normal fungal activity patterns. At the end of the incubation period, the products were autoclaved, ground and stored at -20°C until analysis. All these preparations were performed in triplicate for sterilized and non-sterilized trials.

For the extended incubation trials, a case-control study design was adopted, using non-sterilized grains only. Wheat grains (60 g per Erlenmeyer flask, 10.4 % m.c.) were adjusted to 14, 17 and 20 % m.c. with the addition of the appropriate volume of sterile distilled water dispersed into the Erlenmeyer. Moistened samples were roughly shaken and mixed with grain dust powder (1 % w/w). They were then statically incubated in a thermostatically control room at 25°C for 4 weeks. Samples were daily shaken during the first 5 days and thereafter once a week basis till the end of the trials. Storage environmental conditions were kept at target moisture content (14, 17 and 20 %) and temperature (25°C) conditions. Sample weights were weekly monitored and additional humidification treatment was necessary in order to avoid changing final target water availability. At the end of the incubation period, samples were autoclaved at 121°C for 20 min and were thereafter dried at 103°C for 18 hours, ground and stored at -20°C until analysis.

A control treatment without dust but with water addition to reach the target moisture content of the wheat grains was carried out to determine the normal fungal activity patterns. All these preparations were run in triplicate at each moisture content for the different dust samples.

At the end of the experiments, fungal growth was assessed by ergosterol determination as an indicator of total fungal contamination in wheat .

Ergosterol determination in cereals and grain dusts by Liquid chromatography

Ergosterol analysis in wheat and grain-dusts was carried out as described by Bakan *et al.* (2001) and Pietri *et al.* (2004). Briefly, a sample (5 g) of wheat-flour or dust thoroughly mixed was transferred into a 250-ml centrifuge container. Methanol (150 ml), ethanol (50 ml) and potassium hydroxide (10 g) were added and roughly mixed. The mixture was refluxed for 30 min at 80°C, cooled to 20°C with water (20 ml) and filtered through a folded filter paper in a separator funnel. Additional methanol (25 ml) was added to rinse the flask and cake. The supernatant was isolated and filtered through the filter paper. Saponified mixture was then intensively shaken for 1 min with *n*-hexane (50 ml) and let rest for 10 min to allow complete separation of layers. Upper layer (*n*-hexane) was then isolated and dried over anhydrous sodium sulphate (Na₂SO₄) into 250 ml- round bottom flask. A second extraction was similarly performed and the organic extracts were pooled and concentrated (5-20 ml) at the vacuum rotation evaporator (Büchi) at 40°C.

Quantitation was performed by HPLC. The chromatographic system consisted of a Waters 510 pump (Milford, MA, USA) equipped with an automatic WISP model 712 injector (Waters, Milford, MA, USA), a Chromspher Si (100 x 3.0 mm i.d., 3 µm particles) column, a Diode array detector (Thermo Finnigan Spectra System UV 6000LP) set at 282 nm.

OTA and CIT determination in wheat and grain dusts by Liquid chromatography

The method is derived from a multi-mycotoxin analysis method based on the method described by Pierard *et al.* (2004) for 6 mycotoxins and adapted to the larger set of 15 mycotoxins including OTA and CIT. This method has been described in detail elsewhere (Tangni and Pussemier 2005). Briefly, a 10 g-portion of flour was transferred into a centrifuge container and mixed with 50 ml of acetonitrile-water (90:10) acidified with phosphoric acid (pH = 1.5). The mixture was triturated for 3 min with the Ultra-Turrax device CAT x 620 (Staufen, Germany) fitted with a T17-V shaft at 13500 rpm to produce slurry. The supernatant was then isolated by centrifugation at 1660g during 10 min. A second extraction was identically performed with the remaining cake with 50 ml of acetonitrile-water (90:10, pH = 1.5). The combined supernatants were then concentrated and further processed for clean up on a SPE column Chromabond® XTR (Macherey-Nagel).

The chromatographic system consisted of a system of two Waters 510 pumps (Milford, MA, USA) equipped with an automatic WISP model 712 injector (Waters, Milford, MA, USA), an Alltima (25.0 x 4.6 mm i.d., 3 µm particles) C18 column and a Waters 474 scanning fluorescence detector (Milford, MA, USA).

Recovery experiments performed with wheat flour samples spiked at 40 ng CIT g⁻¹ and 20 ng OTA g⁻¹ gave averages of 74.0 ± 8.9 % and 92.0 ± 11.0 %, respectively. The analytical method as implemented had detection limits (LOD) of 0.4 and 0.1 ng g⁻¹, respectively, whereas the respective quantification limits o(LOQ) were 1.4 and 0.3 ng g⁻¹.

Data handling and statistical analyses

The calibration curves were calculated using the least-squared method. Experimental results are reported as the mean \pm standard deviation (SD). The increase of ergosterol and mycotoxins (Net production) derived from the incubation experiment was computed by subtracting the amount provided by wheat (control) and dusts from the total amount recovered from the analysis of wheat incubated with dust as follows:

$$\text{Net production} = \text{Total Amount}_{(\text{dust inoculated wheat})} - \text{Amount}_{(\text{wheat})} - \text{Amount}_{(\text{dust})} \quad (1)$$

The statistical significance of data was tested applying non-parametric procedures in order to eliminate difficulties caused by heterogeneous data distribution. Mann-Whitney U-test was used to compare the mean ergosterol level between sterilized and non-sterilized batches and to compare the mycotoxin levels between the control and the treated batches according to humidity level and selected dusts. Spearman correlation analysis was used to study the consistency of the association between the net mycotoxin production and the levels of ergosterol or mycotoxins in dust as well as between the ergosterol level, CIT and the sum of the OTA and CIT concentrations in the incubated samples. As data had skewed distributions, they were plotted on a log-scale. Effects with $p < 0.05$ were deemed as statistically significant. Statistical analyses were performed by the statistical program SPSS (Release 10.0.5. 1999).

Results

Ergosterol and mycotoxins in grain-dusts: screening of collected dusts

The concentrations of ergosterol and mycotoxins in four grain-dusts are presented in table I.

“ [Insert table I about here] ”

The ergosterol content of the four dust samples selected for this study ranged from 39 to 823 $\mu\text{g g}^{-1}$ of dust. High CIT and OTA contaminations were recorded for all dust samples (ranges: 137 – 343 ng CIT g^{-1} and 17.3 – 318.0 ng OTA g^{-1}). One must note that for these two toxins, there was a high degree of variability in the concentrations found in the 4 dust samples and that the highest concentrations of 344 ng CIT g^{-1} and 318 ng OTA g^{-1} were found in dust B which was associated with mouldy grains and grain debris. Based on this result, the dust sample with the low ergosterol content was used for a preliminary trial aimed at assessing the influence of the intrinsic fungal population in wheat whereas, the 3 others were chosen for the extended incubation trials with grains.

Preliminary trial on sterilized and non-sterilized wheat samples

Table II presents a comparison of the mean ergosterol contents under intrinsic moisture content in sterilized and non-sterilized wheat grains after incubation at 25°C for 28 days.

“ [Insert table II about here] ”

Comparing the ergosterol production in sterilized and non-sterilized batches, the Mann-Whitney test did not reveal any statistically significant difference ($p = 0.667$) between ergosterol production in these two batches, showing thus that similar fungal activity occurred whether the grains are sterilized or not. So, under 10.4 % m.c. (drier conditions) the addition of grain dust increased the net production of ergosterol as a result of fungal biomass increase. From this preliminary experiment it may be concluded that : i) the increase of fungal biomass as measured by the ergosterol concentrations is a consequence of the loading with dust and is not due to the development of the intrinsic fungal microflora naturally present in wheat (no difference between sterilized and non-

sterilized batches) ; ii) even at low moisture content there is evidence that some fungal biomass growth is observable on grains when they are contaminated with grain dust.

3. *Changes in ergosterol contents in grains inoculated with dusts upon incubation under various moisture contents*

The concentrations of ergosterol provided by wheat, dusts and incubation process are depicted in figure 1, for three different dust samples and three moisture contents.

“ [Insert figure 1 here] ”

In general, for all control and dust inoculated samples the synthesis of ergosterol increases in wheat at high water activity. There is already a net production of ergosterol even at the lower moisture levels and moistening clearly increases this fungal activity (1.9, 2.2, 3.0 $\mu\text{g ergosterol g}^{-1}$ at 14, 17 and 20 % m.c., respectively).

The Kruskal-Wallis test performed for different types of dusts indicated a significant moisture content effect ($p = 0.027$) in ergosterol accumulation in stored grains inoculated with dust. This significant effect was observed for all the tested dusts with various patterns. This means that water availability and the nature of the grain dust materials are two major inductive factors with various patterns for fungal proliferation in wheat. So, the fungal growth potential of the inoculum was also grain dust specific. Moreover, upon incubation at 20 % m.c., fungal activity, as measured by the ergosterol assay, was clearly higher after the inoculation with the dust that contained the highest initial

ergosterol concentration. This also holds for the incubation at lower m.c., but in a lesser extent than observed at 20 % m.c.

Citrinin and ochratoxin A contaminations by grain dusts

The accumulations of CIT and OTA in stored wheat resulting from the experimental trials performed with the incorporation of three different grain dusts loaded at the rate of 1 % (w/w) in wheat grain batches are depicted in figure 2.

“ [Insert Figure 2 about here] ”

Incorporation of grain dust contributes to the increases of CIT and OTA levels in stored grain. First, contaminated dusts B, C and D provided 1.5, 0.9, and 0.6 ng CIT g⁻¹ of grain. Moreover, storage at various water availabilities generated an additional contribution up to 10 ng CIT g⁻¹ of grain with the dust B under 20 % m.c.. Indeed, control batch content of 0.6 ng CIT g⁻¹ moved up to 12.1 ng CIT g⁻¹ by adding 1 % of grain dust B. The same CIT production pattern also occurred in the case of inoculation with dusts C and D. Generation of OTA was also observed in wheat with tremendously increases from 2.1 ng OTA g⁻¹ up to 39.0 and 52.2 ng OTA g⁻¹ of grain in the cases of dusts D and C under 20 % m.c.. One must note that OTA synthesis did not occur at a low level of water availability. In contrast, the highest OTA contaminated dust B could not significantly generate such higher additional OTA accumulation within the tested moisture contents (Figure 2). In the present study, no significant relationship was observed between these two toxins. So, CIT and OTA accumulation in wheat occurred at various concentration ratios according to the moisture in wheat and the dust material samples.

From all the available data, it appeared that when considering CIT and the cumulative levels of CIT and OTA, positive relationships exist with the ergosterol content in the samples incubated with different dusts under 14, 17 and 20 % m.c. (Figures 3A and 3B). These relations are modelled as outlined by the “equations (2)–(3)”, showing thus that ergosterol is a good quality parameter in assessing CIT and OTA in stored wheat.

“ [Insert Figure 3 about here] ”

$$\log(\text{OTA} + \text{CIT}, \text{ ng g}^{-1}) = (1.298 \pm 0.207) \cdot \log(\text{ergosterol}, \mu\text{g g}^{-1}) + (-0.254 \pm 0.179) \quad (2)$$
$$(r = +0.748, p < 0.0001, n = 33)$$

$$\log(\text{CIT}, \text{ ng g}^{-1}) = (1.102 \pm 0.182) \cdot \log(\text{ergosterol}, \mu\text{g g}^{-1}) + (-0.376 \pm 0.159) \quad (3)$$
$$(r = +0.742, p < 0.0001, n = 33)$$

Note that these equations may be used to roughly forecast CIT + OTA as well as CIT concentrations within one order of magnitude.

Discussion

This study describes the presence of the CIT and OTA mycotoxins in dusts collected in grain storages (either from farmers or from commercial companies). It also reports the potential mycotoxigenicity of the grain dusts when they are incorporated in stored wheat.

Assessment of fungal invasion potential

First, an evaluation of fungal development was performed in all the collected dusts. This evaluation can be carried out mainly either with mycological methods that lead to the estimation of viable fungal population or with the chemical quantification of a specific stable fungal component, evaluating both dead and alive fungal biomass (Gourama and

Bullerman 1995). In the present study, an assessment of mould growth was done by using ergosterol determination, which gives a measure of effective control of total fungal growth and activity in grains. Ergosterol is considered as the principal sterol of fungi and may play an essential role as a component of the cell membrane and other cell constituents. Another reason that ergosterol is a useful indicator of fungal growth is that harsh physical treatments do not affect existing ergosterol concentrations (Bailly *et al.* 1999). Total ergosterol was measured as a more valid estimation than free ergosterol of fungal biomass and mycotoxin production in cereal grains, since both esterified ergosterol and free ergosterol are reported (Abramson *et al.* 1999). The ergosterol content of the four dust samples selected for this study ranged from 39 to 823 $\mu\text{g g}^{-1}$ of dust. As reported by Pietri (2004), cereal of ordinary quality should contain less than 3 $\mu\text{g ergosterol g}^{-1}$ while it is considered to be of a scarce quality over 8 $\mu\text{g ergosterol g}^{-1}$. Moreover, it is well known that in general, the levels of ergosterol in mouldy grain are in the range of 8-15 $\mu\text{g g}^{-1}$ of dry matter (Srzednicki *et al.* 2004).

Therefore, it can be postulated that grain dusts merit special attention, as they can be inoculums for fungal growth in new grain deliveries in existing storage facilities. The addition of dust to wheat grain may increase the mycotoxin contents in grain as a contaminant and/or as an inoculum allowing the fungal proliferation with mycotoxin production during storage. In the present study, these two patterns were explored in mycotoxin accumulation in grain during storage.

Natural occurrence of mycotoxins in grain dusts: chemical assessment of their contaminant potential

Chemical analysis of the dusts revealed the presence of quite high concentrations (up to 344 ng CIT g⁻¹ and 318 ng OTA g⁻¹). This means that grain dusts can not only act as an inoculum but also as a contaminant source. Considering the authorized maximum level of OTA in cereals of 5 ng g⁻¹, the presence of 1.6 % (w/w) of dust B in grains can indeed be considered as a threat to exceed the legal limits. For many mycotoxins, the grain dusts may thus represent an additional route of contamination, with the subsequent result that stored grains can be contaminated with toxic substances. In addition, the use of low-grade of grain dusts in animal feeding may increase animal exposure to mycotoxins, which may affect breeding performance as well as milk and meat quality values. Moreover, as these grains are processed during milling, dust particles may be dispersed in the environment and thus increase the exposure to mycotoxins by inhalation among grain workers since aerial contamination by these particles was observed in Italian grain mill (Dacarro *et al.* 2005). One can note that storage work generated, on average, more than 5 times the dust obtained through threshing, so expectedly, storage work was found to be an important exposure determinant (Halstensen *et al.* 2004).

Effect of grain dusts on fungal development and mycotoxin production: inoculum effect

Intrinsic cereal microflora has limited effect on ergosterol production (sterilized versus non-sterilized wheat) and fungal growth, as measured with ergosterol index, shows that fungal development caused by the dust inoculum is possible even at low water activity levels. Extended trials at different moisture contents have shown that fungal growth in grains is not only linked to the water availability but also to the nature of the dust material and particularly to its initial ergosterol content. In addition, it appears that, for the main

relevant storage mycotoxins (OTA and CIT), an increase of the concentrations is observed as a direct consequence of the inoculum effect (net production). For OTA, this is especially true when the moisture level is very high (20 %) whilst for CIT net production is observed whatever the moisture status of the grains. Results suggest that within the tested moisture levels, a positive relationship could be established between OTA + CIT concentrations and ergosterol content in incubated or stored grains. This could be explained by the fact that many fungal species and chemotypes are present at various levels; some of them favoring preferentially OTA production while other fungi produce CIT according to the moisture conditions. The measurement of both mycotoxins and the summing up of measured concentrations could help to provide some leveling of these effects. Our results agree with a recent study performed by Abramson *et al.* (2005) upon the farm storage of durum wheat during 20 weeks at 20 % of m.c.. Indeed, OTA and CIT were found to be the main contaminants in stored grains at high concentrations (6.5 and 11.6 $\mu\text{g g}^{-1}$, respectively). Nevertheless, these mycotoxins were absent at 16 % m.c.. Here, the incorporation of dust allows the contamination by CIT at low water availability. The concentrations of CIT and OTA found in this study may also increase according to the storage time.

The usefulness of chemical analysis as a tool to predict the mycotoxigenic potential of grain dust.

OTA and CIT production show a very versatile image when considering the amounts of toxins produced according to the nature of grain dust and the water activity in grain maintained during incubation. For CIT, there is a steady increase in mycotoxin with moisture and this, for the three different dusts selected in this study whilst for OTA, there is

clearly a drastic increase in toxin production and this, only at the highest level of moisture. This seems to corroborate the idea that many species and chemotypes act together and that their expressions depend on local conditions (nature of dust, humidity level). It seems thus obvious that a predictive tool based on identification of the microbial species/chemotype will be of limited use under such conditions.

An alternative solution could be the development of a rather robust tool based on the chemical analysis of grain dust and the quite easy determination of its ergosterol content as a general marker of fungal biomass. Present results suggest that this predictive tool could be valid for predicting the contamination level of OTA and CIT taken together within the tested moisture content (14 - 20 %). It clearly appears that, at higher water activity, rather unpredictable situations will dominate with hot spots of OTA. Considering that OTA and CIT can act synergistically and that high moisture levels such as 20 % will not be representative of the normal storage conditions of wheat in bulk, it is worth recommending to further study the relevance of such a tool in order to assess the mycotoxigenic potential of grain dust and the associated risks for contamination of wheat storages.

Implication for safe management of cereal storage

The results gathered in this study as well as in other studies show that mycotoxin production can occur either at high moisture level and/or at high (ergosterol rich) dust loads. In the latter case, a high m.c. is not necessary for mycotoxin production as depicted in figure 4. This could have important consequences for the management of cereal storages, especially in traditional farms that are not specifically equipped to keep grains under ideal storage conditions.

“ [Insert Figure 4 about here] ”

In fact, two steps could be identified as critical for the contamination of stored cereals. In a first step, a primary mycotoxigenic inoculum could be produced in spots with high humidity (e.g., along the walls made of concrete or bricks in silo's or lofts) by allowing the grains to turn mouldy (ergosterol content higher than $8 \mu\text{g g}^{-1}$) and to produce ergosterol rich dust (as high as $800 \mu\text{g g}^{-1}$ as measured in this study).

In a second step, this mycotoxigenic dust could act as a secondary inoculum for the bulk of stored cereals (especially when new deliveries are loaded in the storage facility). It is important to note that this second step in the general process of mycotoxin production in storages does not necessarily require a high moisture level. Therefore, it seems worth recommending to not only control the moisture level of the bulk of the stored cereals (which is generally already performed) but also to pay attention to any other possible way to contaminate the grains with ambient dust. It seems particularly important to very carefully discard mouldy grains, grain debris and dust from the storage facility before a new arrival of harvested grains. So, clearing the remains of previous harvests and cleaning the stores before loading in the new harvests might be some basic sanitary measures against storage deterioration.

Conclusions

Grain dusts released from cereal storages were found to be mycotoxigenic. Incorporated in wheat grain storage, they act as a contaminant and/or as an inoculum. The subsequent mycotoxins production varied widely among dust materials and water activity in grain.

Major storage mycotoxins are CIT and OTA that may be forecasted by the levels of ergosterol in stored cereals. On the basis of the present study, it may be stressed that care should be taken during storage preparation in order to reduce possible production of such mycotoxins by grain dusts. So far, these results have been obtained at the lab scale and owing that similar situation can be obtained at a large storage scale, field trials (farm or industrial storage conditions) would be essential to confirm whether similar results can be obtained. So, the cereal stocks would benefit from the safe management with drastic cleaning and drying procedures as a challenge for storing and processing chains.

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List of tables

Table I. Ergosterol, OTA and CIT occurrence in the grain dusts

Table II. Ergosterol contents in batches of sterilized and non-sterilized wheat inoculated with dust
A* (1 %) after storage for 28 days at 25°C and 10.4 % m.c.

Table I. Ergosterol, OTA and CIT occurrence in the grain dusts.

Dusts (origin)	Ergosterol $\mu\text{g g}^{-1}$	CIT ng g^{-1}	OTA ng g^{-1}
A (farm)	38.9	301.3	19.2
B (farm*)	823.3	343.9	318.0
C (storage-company)	110.5	191.8	59.0
D (storage-company)	152.3	137.0	17.3

* Spots with mouldy grains were observed in this storage

Table II. Ergosterol contents in batches of sterilized and non-sterilized wheat inoculated with dust A* (1 %) after storage for 28 days at 25°C and 10.4 % m.c..

	Ergosterol ($\mu\text{g g}^{-1}$ of wheat) derived from			
	inoculated wheat	wheat [†]	dust A	net production
Non-sterilized wheat	3.259	1.534	0.389	1.336 \pm 0.15
Sterilized wheat	3.278	1.383	0.389	1.506 \pm 0.13

* : Ergosterol and mycotoxins contents of this graindust are presented in table 1

† : the results were obtained with the control batches

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List of figures

- Figure 1. Levels of ergosterol in wheat grains inoculated with three dusts (1 %, w/w) after incubation for 28 days at 25°C under 14, 17 and 20 % m.c..
- Figure 2 : Levels of CIT and OTA provided by wheat and dust together with the resulted mycotoxin production on wheat grains moistened at 14, 17 and 20 % m.c. after incubation for 28 days at 25°C.
- Figure 3 : Correlation between the total ergosterol content, CIT + OTA (A) and CIT (B) concentrations in stored wheat after incubation for 28 days at 25°C under 14, 17 and 20 % m.c..
- Figure 4 : Hypothetical pathway for mycotoxin contamination in bulk stored grains under dry conditions.

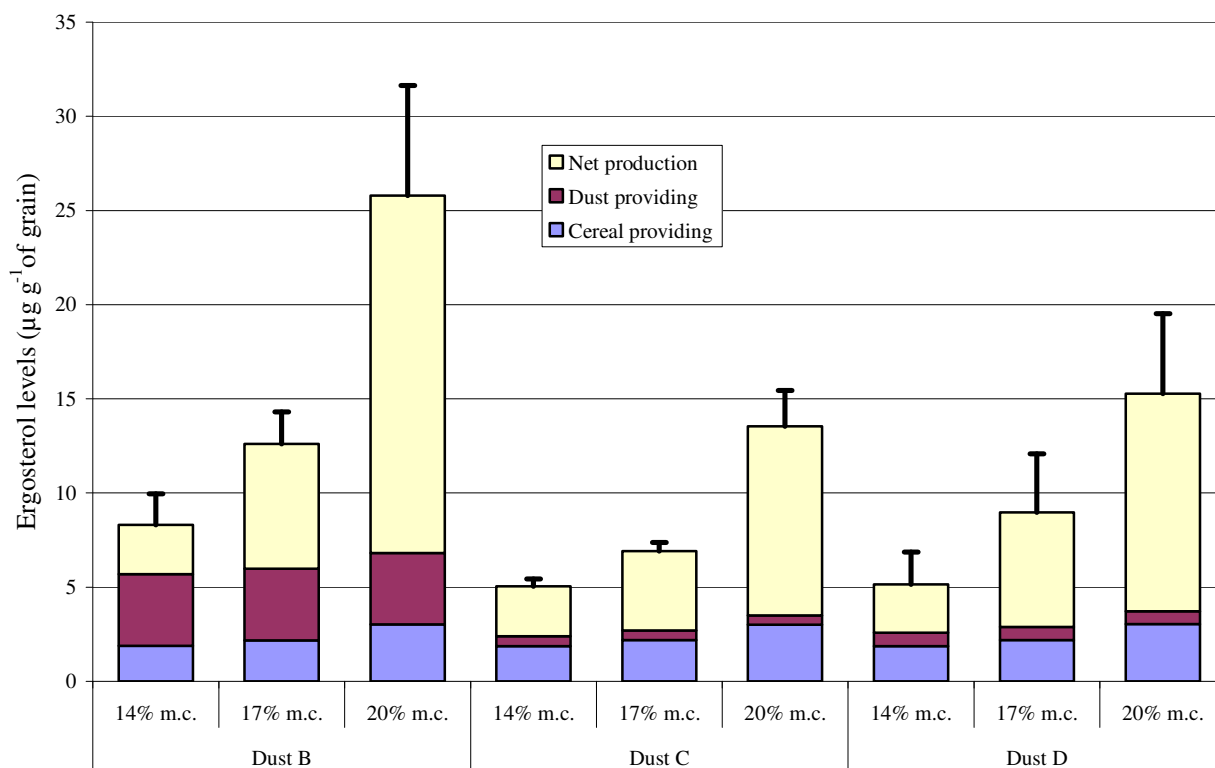


Figure 1. Levels of ergosterol in wheat grains inoculated with three dusts (1 %, w/w) after incubation for 28 days at 25°C under 14, 17 and 20 % m.c..

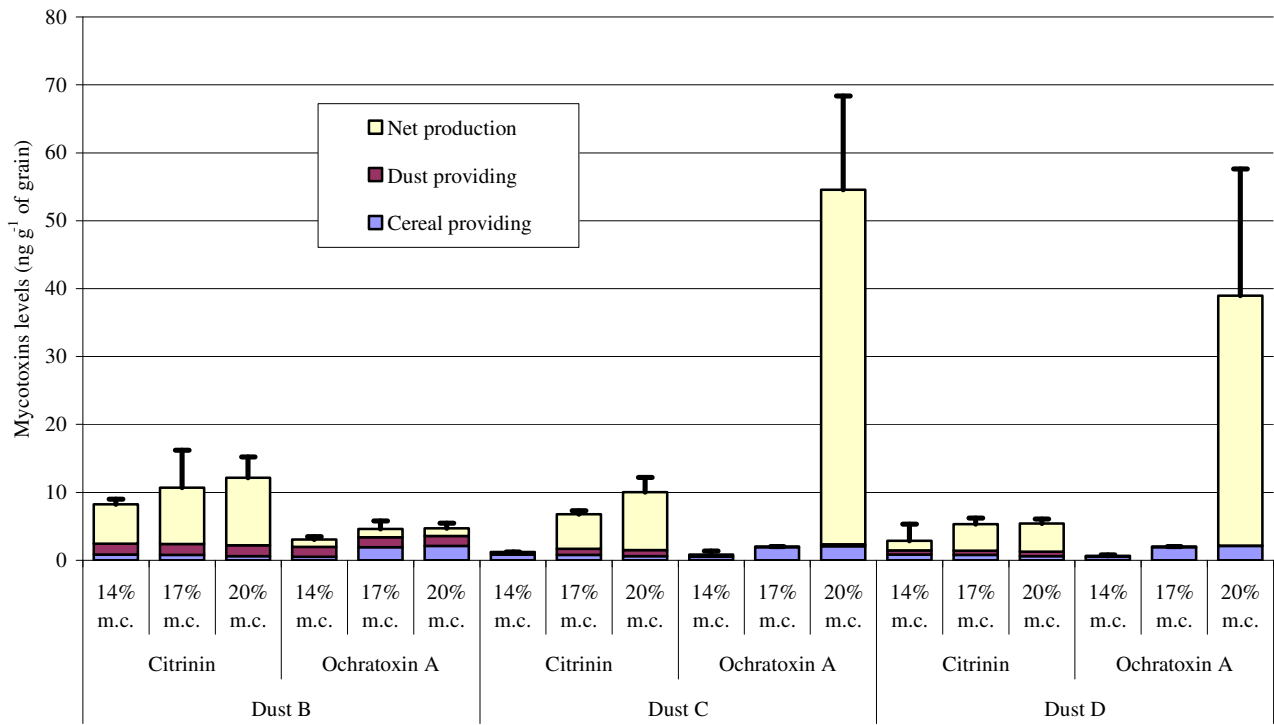


Figure 2 : Levels of CIT and OTA provided by wheat and dust together with the resulted mycotoxin production on wheat grains moistened at 14, 17 and 20 % m.c. after incubation for 28 days at 25°C.

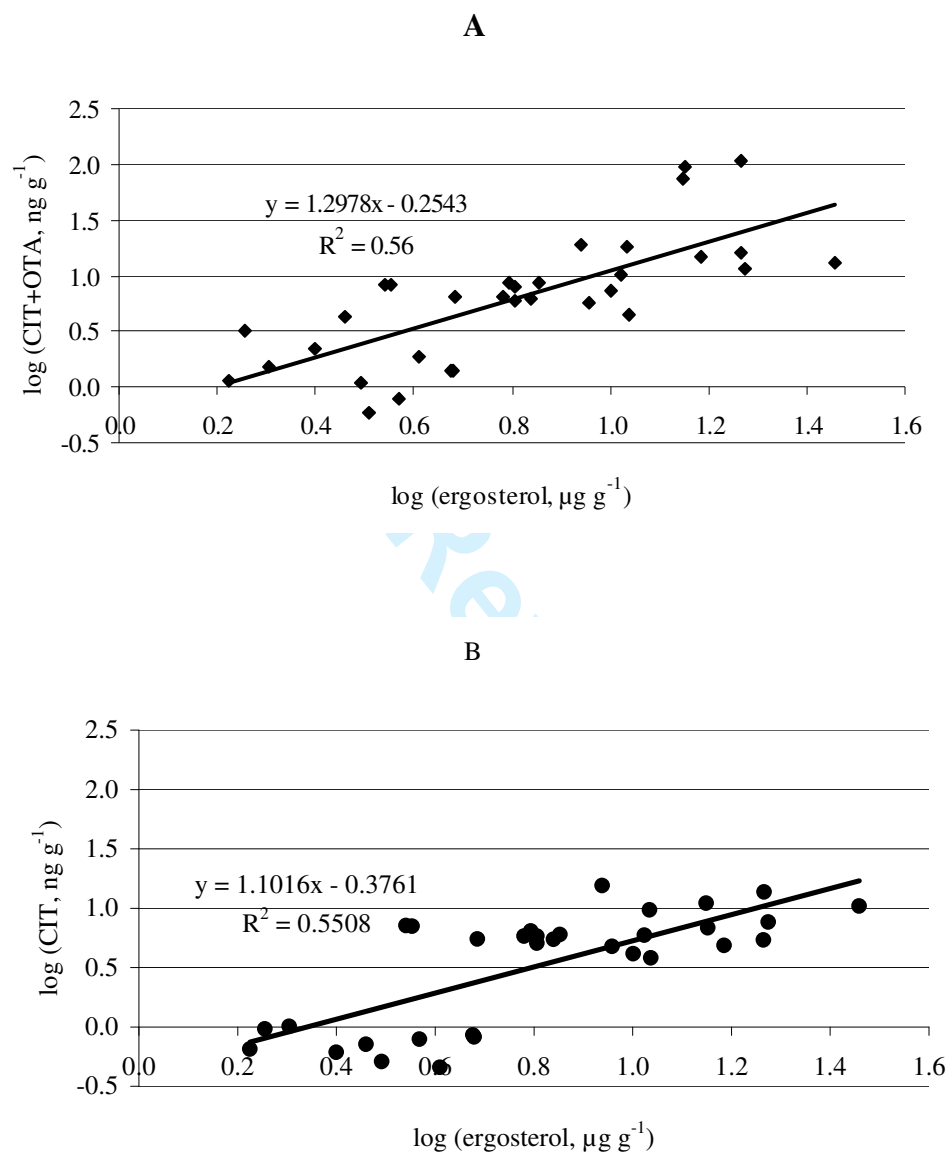


Figure 3 : Correlation between the total ergosterol content, CIT + OTA (A) and CIT (B) concentrations in stored wheat after incubation for 28 days at 25°C under 14, 17 and 20 % m.c..

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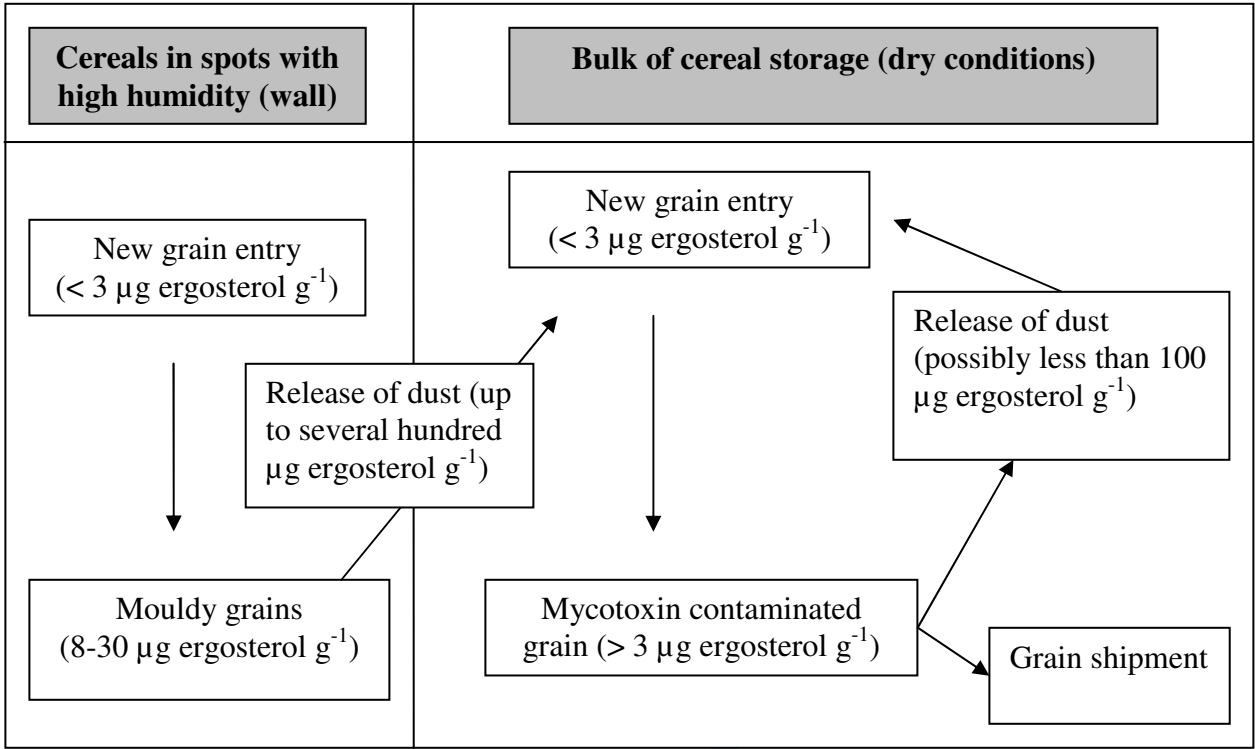


Figure 4 : Hypothetical pathway for mycotoxin contamination in bulk stored grains under dry conditions.