Effects of Grain Sampling Procedures on *Fusarium* Mycotoxin Assays in Wheat Grains

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Running Head: Grain Sampling for *Fusarium* Mycotoxin Assays

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Editorial annotations: R.J. Molyneux (Assoc. Ed.)
Abstract

*Fusarium* mycotoxins are increasingly studied agronomically, chemically and pathologically in the context of food safety, as a means of preventing new major health crises. Reliable mycotoxin techniques and sampling procedures are required for assessment of the effects of different sources of variation on grain mycotoxin content in agronomic experiments. We carried out analyses with the aim of formulating guidelines for grain sampling to increase the reliability of grain mycotoxin measurement in agronomic experiments. We focused on two toxins in wheat samples: deoxynivalenol and nivalenol. With a nested linear mixed model, we estimated that the uncertainty of nivalenol determination was low (±15 µg/kg), whereas that for deoxynivalenol determination was higher (±38 µg/kg). We also found that grinding of the grain decreased the variability of the results. Moreover, despite the heterogeneity in grain mycotoxin content across a given field, we showed that heads can be harvested manually for agronomic experiments provided that sampling is representative (evenly distributed over the entire plot area). Finally, we found that delaying the assay after harvest affected the results obtained and should therefore be avoided.

**Key words:** deoxynivalenol; nivalenol; sampling; sample preparation; sample conservation effect
Introduction

Since the 1990s, several major health crises have shaken the food industry and interest in food safety has increased. One of the key elements of the potential health risk associated with dietary cereals is the accumulation of mycotoxins in grains (1-3). Vomiting, reproductive disturbances, leukoencephalomalacia, pulmonary oedema, impairment of the humoral and cellular immune responses, nervous disorders, myocardial hypertrophy and several cancers may result from the ingestion of mycotoxins (4). Mycotoxins are fungal secondary metabolism products (2, 5) and result from the adaptation of fungal growth to stressful situations (6). The pathogenic fungal complex of the genus *Fusarium* is the principal producer of mycotoxins, notably of deoxynivalenol and nivalenol, in grains of growing crops (7). *Fusarium* and *Microdochium* also cause a cereal disease, *Fusarium* head blight (8-10). *Fusarium* mycotoxins are increasingly being studied in an attempt to prevent new major health crises. Agronomic experiments are carried out to assess the effects of different sources of variation on grain mycotoxin content (10-15). Reliable mycotoxin measurement techniques and appropriate sampling procedures are essential for such studies. Mycotoxin contamination is highly heterogeneous in cereal fields (16) and grain samples (17-21). Almost 90% of the error associated with aflatoxin testing can be attributed to the method used to obtain the original sample (21). Moreover, aflatoxin may only be present at high concentrations in less than 0.5% of the peanut crop and concentrations may be as high as 1,000,000 µg/kg in of contaminated peanuts (17). We assessed the uncertainty of mycotoxin determination and the effect of mycotoxin sampling procedures on mycotoxin contamination levels for the *Fusarium* mycotoxins, deoxynivalenol and nivalenol. The chemical structures of these toxins are presented in Figure 1. We aimed to characterize the errors occurring during each step of the procedure, from the field to the laboratory (Figure 2): sampling in the field (mechanical versus manual methods); sample preparation (flour or grain); and sample conservation.
Materials and methods

Experimental design and mycotoxin analysis

The samples used in this study came from a long-term experiment, the aim of which was to compare different cropping systems and assess the effects of several cropping systems on mycotoxin levels in winter wheat \((I2)\). Nine agronomic treatments were duplicated in this design and two growing seasons were used (2001/02 and 2002/03). Thirty-six plots were available for this methodological study, of which we used only 16. These plots were chosen according to the variability of head blight attacks. Plots A, B, H and P were cropped under a conventional system, M was cropped under an integrated system, I, N and Q were cropped under an integrated direct drilling system whereas C, D, O, F, J, K, L and R were cropped under an organic system. We extended the range of systems and mycotoxin contents studied by also including two farmers' fields cropped under an organic direct drilling system in 2002/03 (plots E and G): we therefore sampled a total of 18 plots.

Mycotoxin analyses were performed by the Qualtech laboratory (Vandoeuvre-les-Nancy, France). Levels of the trichothecenes nivalenol and deoxynivalenol were determined. Each sample (flour or grain) received by the laboratory was homogenised at least three times, in a mixer/divider. A small quantity of each sample was taken (20-25g), and in the case of grain samples was ground. Trichothecenes were determined by gas chromatography-mass spectrometry (GC-MS). This method was validated by the French norm NF EN ISO/CEI 17025. The assay laboratory estimates the measurement error for trichothecenes at 20\% (differences in extraction rate and errors in sample preparation in the laboratory assay are included).

In addition, according to laboratory assay data, the detection limit \((d_l)\) was 30 \(\mu g/kg\) for trichothecenes, and the quantification limit \((q_l)\) was twice the detection limit (60 \(\mu g/kg\)).
the purpose of this study, mycotoxin contents below \( d_i \) or \( q_i \) were assigned values equal to half of these limits: 15 and 30 µg/kg, respectively.

**Effect of sample preparation and mycotoxin measurement uncertainty**

Twenty-four samples from the 18 plots were harvested mechanically (roughly 2 kg in total). They were dried at 80 °C for 48 h. Half of the 24 available samples (nos. 1 to 12) were completely ground and three flour subsamples of roughly 300g each were used for mycotoxin analysis; this procedure for sample preparation before analysis was called “flour-flour” (flour subsamples taken from a sample already ground into flour). For each of the twelve remaining samples, three grain subsamples of roughly 300g each were taken. The subsamples from samples 13 to 15 were completely ground and sent for mycotoxin analysis; we called this procedure “grain-flour” as the original sample was in the form of grain and only converted to flour after subsampling. The subsamples from samples 16 to 24 were not ground and sent directly for analysis (referred to as the “grain-grain” procedure, as both sample and subsample are in grain form). The mycotoxin content data obtained for these 24 samples were also used to evaluate the uncertainty of mycotoxin determination.

Mycotoxin analyses are destructive, making it impossible to carry out several measurements on the same sample. It is therefore difficult to characterise the repeatability and reproducibility of the assays rigorously because these two parameters must be determined for a single sample (22). However, we estimated the uncertainty of the assays by dividing each of the 24 samples into three subsamples . Measurement uncertainty characterises the dispersion of the values that could reasonably be attributed to the measurand (22-24) and may be estimated using a linear mixed model (25). We used the following model to describe our data:

\[
Y_{ijk} = \mu + \alpha_i + (A_i)_{ij} + \varepsilon_{ijk}
\]
where $Y_{ijk}$ is the measured toxin content; $\mu$ the general mean toxin content; $\alpha_i$ a variable for the $i$th sample, which has fixed effect; $(A_i)_j$ a variable for the $j$th preparation, which has random effect because the toxin content of the $j$th preparation depended on the toxin content of the sample $A_i$; and $\epsilon_{ijk}$ the standard error, relating to $\kappa$, the mycotoxin content of the subsample. The classic proc glm with the random option program of SAS software was used to calculate the intrapreparation method variance as the difference between the variance of $(A_i)_j$ and the standard error. Measurement uncertainty was then calculated as half the confidence interval (CI) estimated using the following equation:

$$\text{Measurement uncertainty} = \frac{\text{CI}}{2} = \left[ \frac{1}{((1-\alpha/2)(n-1)) \times \sqrt{\text{variance}(A)_j - \text{variance}\epsilon_{ijk})/n} \right] / 2$$

where $n$ is the number of subsamples.

We used all the mycotoxin content data, regardless of the method of sample preparation, to estimate the mean square (population variance) from the variability of a given set of mycotoxin measures. We have therefore estimated mycotoxin measurement uncertainty according to the mixed model described above but with the $(A_i)_j$ term eliminated. Thus, $Y_{ij}$ was described by the following relationship: $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$. The preparation methods were not distinguished so $j$ stands for the $j$th subsamples of the $i$th sample. In this model, $\epsilon_{ij}$, which characterises the modelling error, was associated with the maximum value of variability for the assay. The maximum measurement uncertainty can therefore be expressed as:

$$\text{Measurement uncertainty} = \frac{\text{CI}}{2} = \left[ \frac{1}{((1-\alpha/2)(n-1)) \times \sqrt{\text{variance}\epsilon_i}/n} \right] / 2$$

This model is based on three assumptions. The first is equality of the variances for each level of variables. The other assumptions are normality and independence of the variables with random effect: kurtosis and skewness coefficients and the distribution of residues with respect to predicted values were also assessed.
The effects of preparation procedures on measurement variability were investigated for plots A, B, C and P, Q, R for which two types of sample preparation were carried out. The $Y_{ijk} = \mu + \alpha_i + (A_i)_j + \epsilon_{ijk}$ model was used to estimate (i) inter-preparation variance, i.e. the variance of $(A_i)_j$ and (ii) intra-preparation variance, i.e. the ratio between the difference between inter-preparation variances (those of $(A_i)_j$) and the standard error (those of $\epsilon_{ijk}$) and the number of subsample mycotoxin content values ($k$). We investigated whether there was an inter-preparation effect or an intra-preparation effect by means of a $\chi^2$ test comparing these variances and the population variance. These effects were also estimated by calculating the variation coefficient for mycotoxin content (as the ratio of mean square and mean), and the standard deviation for each preparation.

**Effect of the harvest procedure**

Three in-field sampling procedures were investigated (one mechanical and two manual harvest methods) and compared on three plots (P, Q and R). Grain mycotoxin content was measured just after harvest. For mechanical harvesting, grain sampling was based on the 98/53/CE directive (26), which was subsequently modified by the 2002/27/CE directive (27), a document that lays down the sampling procedure for official controls of aflatoxin level. This directive was used because there is no equivalent text dealing with *Fusarium* toxins. According to this directive, for plots with yields below 1 tonne, 10 samples of 100 g each must be collected and pooled to give a total sample of 1 kg. The samples (in our case roughly 2 kg) were then dried at 80 °C for 48 h. For the first method of manual harvest (the “hundred method”), we collected ten randomly selected samples of 100 heads each from each experimental plot (at least 1 kg). For the second method (the “quadrat method”) we collected the heads from nine quadrats made up of 1m x 2 adjacent rows from each experimental plot (900g-1 kg). The harvested heads were dried at 80 °C for 48h and the glumes and rachis were
separated from the grains. Grains from all the hand-harvested heads were pooled to give a total sample per plot for each method. The three samples from each plot were completely ground and subsamples of flour (each weighing approximately 300g) were sent for mycotoxin analysis.

**Effect of the grain storage procedure**

The effect of the grain storage procedure was investigated on several plots. In each case, subsamples of approximately 300g were collected for mycotoxin analysis. Five kinds of storage process were tested: storage at room temperature for eight months (plots A, B and C); at 4 °C for two months (plots H, I and J); at –20°C for two months (plots H, I and J); at room temperature for two months (plots H, I and J and M, N and O); and no storage at all (assay performed immediately after harvest) for all nine plots.

We evaluated the effect of the different harvest and storage methods used by comparing the variances associated with these methods with the estimated population variance for each toxin, by means of a Chi² test. If a significant effect was observed, Bonferroni correction was applied.

**Results**

**Mycotoxin measurement uncertainty**

For each sample, the various mycotoxin measurements obtained are presented in Table 1. We checked that $e_{ij}$ for deoxynivalenol and nivalenol analyses were randomly distributed (results not shown) and followed a Gaussian distribution: the coefficients of kurtosis and skewness for deoxynivalenol were 0.65 and 0.25 respectively, and those for nivalenol were 2.328 and –
0.23, respectively. This variable with a random effect was therefore normally distributed and independent. No significant differences were observed in the variance of $\varepsilon_{ij}$ (according to Bartlett’s test with $\alpha \leq 35\%$) for nivalenol. For deoxynivalenol content, eight samples (numbers 1, 9, 14, 16, 17, 18, 20, 23) presented $\varepsilon_{ij}$ variances significantly higher than those for the other 16 samples. To take into account the three assumptions on which the model was based, the uncertainty of nivalenol determinations was calculated using all the samples whereas that for deoxynivalenol was estimated using the 16 samples for which no significant inequality was observed in the variances of $\varepsilon_{ij}$ (according to Bartlett’s test with $\alpha = 10\%$).

For nivalenol, measurement uncertainty was 15 µg/kg (mean square: 956.4), whereas for deoxynivalenol, measurement uncertainty was at least 38 µg/kg (mean square: 153.6). It should be noted that (i) if the samples excluded due to heterogeneity in variance were included, then measurement uncertainty was even higher for deoxynivalenol (81 µg/kg) and (ii) six of the eight samples excluded from the calculation of deoxynivalenol measurement uncertainty corresponded to grain-grain preparations rather than grain-flour or flour-flour preparations.

For plots A, B and C, the standard deviations of deoxynivalenol and nivalenol measurements were lower after the flour-flour procedure than after the grain-grain procedure, with values of 62 and 5 versus 98 and 9, respectively. With the exception of the deoxynivalenol measurements for plot A, the variability of measurements (estimated by the coefficient of variation on each plot) was lower for analyses on flour samples than for those on grain samples (Figure 3a). For grain samples taken for plots P, Q and R, variability was also lower if subsamples were ground (grain-flour procedure) than if they were not (“grain-grain” procedure), except for the deoxynivalenol measurements for plot P (Figure 3b). The standard
deviation was 54 for deoxynivalenol and 3 for nivalenol for samples sent for analysis in the form of flour, versus 76 and 16, respectively, for samples sent for analysis in the form of grain. The results from plots A, B, C, P, Q and R therefore suggest that the variability of the mycotoxin measurements may be reduced by early grinding of the samples. The results obtained with the \( Y_{ijk} = \mu + \alpha_i + (A_i)_j + \epsilon_{ijk} \) model provided no evidence of an intrapreparation effect: according to the population variance analysis, the variability of intrapreparation mycotoxin levels (grain or flour) was similar. However, this model revealed an interpreparation effect on deoxynivalenol contamination (\( \alpha = 0.05 \)) for plots A, B, C and P, Q, R. There was also an interpreparation effect on nivalenol contamination (\( \alpha = 0.01 \)) for plots P, Q and R: mycotoxin contamination levels were higher for grain samples than for flour samples, except for nivalenol contamination in plots P, Q and R, for which the opposite result was obtained.

\[
\text{Effect of harvest and sample storage methods}
\]

All the results obtained were very similar (Table 2), but deoxynivalenol and nivalenol levels tended to be higher in cases of manual harvest by the quadrat method than in cases of “hundred harvest” or mechanical harvest. This trend was confirmed by the Chi\(^2\) test (\( \alpha = 0.10 \)). Bonferroni’s test graded (\( \alpha = 0.05 \)) the deoxynivalenol levels obtained by the quadrat method were higher than those obtained by the other methods, and nivalenol levels obtained by the quadrat method were higher than those obtained by the mechanical method.

Deoxynivalenol levels seemed to be lower when measured two and eight months after harvest than when they were measured at harvest (Table 3). This result was confirmed by the results of a Chi\(^2\) test with \( \alpha = 0.10 \) and a Bonferroni’s test (\( \alpha = 0.05 \)) performed on whole plots. A similar trend was observed for nivalenol contamination but was found to be non-significant.
(Chi$^2$ test with $\alpha = 0.10$) for plots with the four types of storage tested. On plots on which only two types of storage were tested, the type of storage was found to have a significant effect (Chi$^2$ test with $\alpha = 0.05$) on nivalenol contamination. The results of the Bonferroni’s test ($\alpha = 0.05$) showed that nivalenol contamination after eight months of storage was lower than that with no storage, but no difference was observed between two months of storage and no storage.

Discussion

The results of any assay are biased by measurement uncertainty resulting from the variability of the sample (dependent on the method used to select samples, sample size, sample quality) and variability of the measurements (dependent on the measurement method, operator, kind of analytical method and number of analytical measurements) (25, 28). Sampling constitutes the greatest source of error, followed by subsampling and analysis (29).

The laboratory that performed the analysis in this study estimated the variability of its assays at 20%. We found that the measurement uncertainty for a sample, estimated by means of mycotoxin analysis on subsamples, was low: nivalenol determinations were accurate to within 20 $\mu$g/kg up to a minimum nivalenol concentration of 60 $\mu$g/kg (the quantification limit) and the measurement uncertainty was less than 26% of the concentration of nivalenol measured. Thus, the subsampling procedure adopted did not increase the variability of mycotoxin concentrations measured. However, it should be pointed out that these encouraging results were obtained with only a small number of plots. It would also be useful to analyse more highly contaminated samples.
The accuracy of deoxynivalenol measurement was lower, with a measurement uncertainty of up to 40 µg/kg. However, this result corresponds to 22% of the measurement mean, similar to the variability of other analyses. Our results also show that grinding grain as soon as possible may minimise errors. Similar results have been obtained for aflatoxin in shelled peanuts (28) and for deoxynivalenol in wheat (25, 30). Indeed, the trend towards lower variability when samples or subsamples were ground probably reflected the grinding of a larger number of grains than would be the case for a grain sample ground in the laboratory just before testing. This may increase the uniformity of the sample, resulting in lower variability. These findings require confirmation and should be taken into account in future agronomic studies.

The mixed model used made it possible to estimate the mean and the mean square of deoxynivalenol and nivalenol contamination levels of a “field population”. We considered the population to be variable, with a random effect. This made it possible to take into account correlations between several measurements carried out on several subsamples originating from a given sample, although we assumed that assays were independent. In fact, subsample content determinations are independent, but measurement results are not themselves independent because analyses were carried out on subsamples taken from the same given initial sample.

Part of the reason for the choice of this model lies in the fact that a model lacking an interaction term between the mycotoxin levels of sample and subsample, $Y_{ijk} = \mu + \alpha_i + \beta_j + \epsilon_{ijk}$, may be biased by this interaction, should such an interaction exist. Moreover, a classic model including an interaction between subsample and sample, $Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \epsilon_{ijk}$, may be biased by the independence of subsample assays: in our case, the subsamples are
taken from the same sample plot and are therefore not true repetitions, which must be taken
from different plots cropped in a similar fashion.

Despite the heterogeneity of mycotoxin content within a field (16), mycotoxin
contamination may be analysed following harvesting by manual means if the
heterogeneity of contamination is taken into account by representative sampling, evenly
distributed over the entire area of the plot. Taking ten samples of one hundred heads
seemed to give better results than analyses of the heads in nine quadrats made up of 2 rows x
1 m, probably simply because the size of the sample considered was greater.

Fusarium mycotoxins are known to be stable to heat and chemical treatments (31, 32), so the
lower levels of mycotoxin contamination recorded when toxin levels were not assessed
immediately after harvest probably does not correspond to a real decrease, resulting instead
from high measurement uncertainty or from changes in the sample during storage. Our
calculations suggest that high measurement uncertainty is not responsible for the observed
decrease. The second possibility, that changes occur in the sample during storage, therefore
appears more likely. Without more data on the question, it is possible for example that mould
could have either modified the grain samples and thus the toxin extraction rate, or have
degraded the toxin with an enzyme such as acetyltransferase Ayt1p (33). This enzyme was
found to be responsible for a decrease in the amount of deoxynivalenol six weeks after
inoculation in a previous study (34). A third explanation is a modification of the ratio of
acetonitrile / water during grain storage: this ratio strongly influences the extraction rate of
deoxynivalenol and nivalenol, and may also explain our results. It would therefore seem
advisable to sort and grind samples immediately after harvest and, if this is not possible,
to minimise the time interval between harvest and analysis.
These results, which are of potential value for agronomic research, are also likely to be useful for the harmonisation of mycotoxin-sampling plans (28). They may also contribute to the standardisation of maximum limits, which currently differ between countries (35), and thereby facilitate international trade (28, 36).

Acknowledgements

This research was funded by the DGAL (French Ministry of Agriculture) and by INRA. We would also like to thank Alain Bone for tracking down the references, Alex Edelman & Associates, Stéphanie Pineau and Guillaume Laffont for editorial advice and Béatrice Le Fouillen, Christine Souin and students for their contributions to the experimental work.
References


Table 1: Mycotoxin levels were determined for three subsamples per sample, with each sample corresponding to a plot. No result indicates that no analysis was carried out; <<d sub sample 1 sample 3 >> mycotoxin not detected (< 30 µg.kg<sup>-1</sup>); <q sub sample 2 sample 3 >> mycotoxin level lower than the quantification limit (< 60 µg.kg<sup>-1</sup>).

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Sample sent for analyse</th>
<th>Deoxynivalenol, µg/kg</th>
<th>Nivalenol, µg/kg</th>
</tr>
</thead>
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<tr>
<td>Grain</td>
<td>Flour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 A</td>
<td>150</td>
<td>160</td>
<td>320</td>
</tr>
<tr>
<td>2 B</td>
<td>240</td>
<td>290</td>
<td>240</td>
</tr>
<tr>
<td>3 C</td>
<td>330</td>
<td>310</td>
<td>390</td>
</tr>
<tr>
<td>4 D</td>
<td>60</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>5 E</td>
<td>370</td>
<td>380</td>
<td></td>
</tr>
<tr>
<td>6 F</td>
<td>110</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>7 G</td>
<td>200</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>8 H</td>
<td>240</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>9 I</td>
<td>800</td>
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<td>10 J</td>
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<td>100</td>
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</tr>
<tr>
<td>11 K</td>
<td>110</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>12 L</td>
<td>100</td>
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</tr>
<tr>
<td>Grain</td>
<td>Flour</td>
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<tr>
<td>13 P</td>
<td>190</td>
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<td>100</td>
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<td>17 B</td>
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</tr>
<tr>
<td>18 C</td>
<td>350</td>
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<td>360</td>
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<tr>
<td>19 M</td>
<td>&lt;q</td>
<td>&lt;d</td>
<td>&lt;d</td>
</tr>
<tr>
<td>20 N</td>
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<td>23 Q</td>
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<td>24 R</td>
<td>&lt;d</td>
<td>60</td>
<td>110</td>
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Table 2: Mycotoxin contamination determination according to harvest method. *<q* mycotoxin level lower than quantification limit (*< 60 µg.kg$^{-1}$).

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Quadrat harvest</th>
<th>Plot P</th>
<th>Plot Q</th>
<th>Plot R</th>
<th>Bonferroni</th>
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<tr>
<td>Deoxynivalenol, µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hundred harvest</td>
<td>180</td>
<td>550</td>
<td>160</td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>Mechanical harvest</td>
<td>190</td>
<td>550</td>
<td>110</td>
<td></td>
<td>b</td>
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<tr>
<td>Nivalenol, µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hundred harvest</td>
<td>&lt;q</td>
<td>70</td>
<td>60</td>
<td>90</td>
<td>a</td>
</tr>
<tr>
<td>Mechanical harvest</td>
<td>&lt;q</td>
<td>80</td>
<td>&lt;q</td>
<td>&lt;q</td>
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Table 3: Mycotoxin contamination according to type of storage. No result indicates that no analysis was carried out; $<d_m$ mycotoxin not detected (< 30 µg.kg$^{-1}$); $<q_l$ mycotoxin level lower than the quantification limit (< 60 µg.kg$^{-1}$).

<table>
<thead>
<tr>
<th>Plots</th>
<th>0 months</th>
<th>2 months</th>
<th>4 °C</th>
<th>-20 °C</th>
<th>ambient temperature</th>
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<td>no conservation</td>
<td>ambient temperature</td>
<td>4 °C</td>
<td>-20 °C</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>230</td>
<td>240</td>
<td>230</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>800</td>
<td>a</td>
<td>500</td>
<td>b</td>
<td>630</td>
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<tr>
<td>J</td>
<td>140</td>
<td>140</td>
<td>120</td>
<td>b</td>
<td>110</td>
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<tr>
<td>Deoxynivalenol, µg/kg</td>
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<td></td>
<td></td>
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<td>A</td>
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<td>370</td>
</tr>
<tr>
<td>M</td>
<td>$&lt;d_m$</td>
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FIGURES LEGENDS

Figure 1: Chemical structures of type B trichotecenes.

Figure 2: Steps of the grains sampling procedures

Figure 3: Effect of sample preparation on the variability of mycotoxin contamination measurement for plots A, B and C (a) and for plots P, Q and R (b).
Figure 1:

Type B trichothecces: Deoxynivalenol (R1 = OH, R2 = H, R3 = OH, R4 = OH)
Nivalenol (R1 = OH, R2 = OH, R3 = OH, R4 = OH)
Field (tonnes of grains)
  ↓
Sample (roughly 1 kg)
  ↓
Subsample (roughly 300g)
  ↓
Subsample sent for analysis (roughly 300g)
  ↓
Sample analysed (20-25g)

Intrafield Variability

Variability from sampling

Storage
Homogenisation
Sample preparation

Variability from analysis

Homogenisation
Preparation sent for analysis

Variability from analysis

Homogenisation
Laboratory preparation

Variability from the laboratory, that cannot modify, estimated at 20%

Assays

Variability we are attempting to reduce
Figure 3a:

[Bar chart showing coefficient of variation for DON and NIV in flour and grain samples.]

- DON:
  - Sample A: 45%, 35%
  - Sample B: 53%
  - Sample C: 21%

- NIV:
  - Sample A: 43%, 43%, 43%
  - Sample B: 0%, 0%
  - Sample C: 35%

Legend:
- Flour
- Grain
Figure 3b:

![Coefficient of variation for DON and NIV](image)