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

7

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14 Running Head: Grain Sampling for *Fusarium* Mycotoxin Assays

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30

31 **Abstract**

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

46

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

49

50

51 **Introduction**

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

76

## 77 **Materials and methods**

### 78 *Experimental design and mycotoxin analysis*

79 The samples used in this study came from a long-term experiment, the aim of which was to  
80 compare different cropping systems and assess the effects of several cropping systems on  
81 mycotoxin levels in winter wheat (12). Nine agronomic treatments were duplicated in this  
82 design and two growing seasons were used (2001/02 and 2002/03). Thirty-six plots were  
83 available for this methodological study, of which we used only 16. These plots were chosen  
84 according to the variability of head blight attacks. Plots A, B, H and P were cropped under a  
85 conventional system, M was cropped under an integrated system, I, N and Q were cropped  
86 under an integrated direct drilling system whereas C, D, O, F, J, K, L and R were cropped  
87 under an organic system. We extended the range of systems and mycotoxin contents studied  
88 by also including two farmers' fields cropped under an organic direct drilling system in  
89 2002/03 (plots E and G): we therefore sampled a total of 18 plots.

90

91 Mycotoxin analyses were performed by the Qualtech laboratory (Vandoeuvre-les-Nancy,  
92 France). Levels of the trichothecenes nivalenol and deoxynivalenol were determined. Each  
93 sample (flour or grain) received by the laboratory was homogenised at least three times, in a  
94 mixer/divider. A small quantity of each sample was taken (20-25g), and in the case of grain  
95 samples was ground. Trichothecenes were determined by gas chromatography-mass  
96 spectrometry (GC-MS). This method was validated by the French norm NF EN ISO/CEI  
97 17025. The assay laboratory estimates the measurement error for trichothecenes at 20%  
98 (differences in extraction rate and errors in sample preparation in the laboratory assay are  
99 included).

100 In addition, according to laboratory assay data, the detection limit ( $d_l$ ) was 30  $\mu\text{g}/\text{kg}$  for  
101 trichothecenes, and the quantification limit ( $q_l$ ) was twice the detection limit (60  $\mu\text{g}/\text{kg}$ ). For

102 the purpose of this study, mycotoxin contents below  $d_l$  or  $q_l$  were assigned values equal to  
103 half of these limits: 15 and 30  $\mu\text{g}/\text{kg}$ , respectively.

104

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

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

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

125

$$Y_{ijk} = \mu + \alpha_i + (A_i)_j + \varepsilon_{ijk}$$

126 where  $Y_{ijk}$  is the measured toxin content;  $\mu$  the general mean toxin content;  $\alpha_i$  a variable for  
 127 the  $i^{\text{th}}$  sample, which has fixed effect;  $(A_i)_j$  a variable for the  $j^{\text{th}}$  preparation, which has random  
 128 effect because the toxin content of the  $j^{\text{th}}$  preparation depended on the toxin content of the  
 129 sample  $A_i$ ; and  $\epsilon_{ijk}$  the standard error, relating to  $k$ , the mycotoxin content of the subsample.  
 130 The classic *proc glm* with the *random option* program of SAS software was used to calculate  
 131 the intrapreparation method variance as the difference between the variance of  $(A_i)_j$  and the  
 132 standard error. Measurement uncertainty was then calculated as half the confidence interval  
 133 (CI) estimated using the following equation:

$$134 \quad \text{Measurement uncertainty} = \text{CI} / 2 = \left[ t_{((1-\alpha/2)(n-1))} \times \sqrt{(\text{variance}(A_i)_j - \text{variance}\epsilon_{ijk}) / n} \right] / 2$$

135 where  $n$  is the number of subsamples.

136

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

$$145 \quad \text{Measurement uncertainty} = \text{CI} / 2 = \left[ t_{((1-\alpha/2)(n-1))} \times \sqrt{(\text{variance}\epsilon_{ij}) / n} \right] / 2$$

146 This model is based on three assumptions. The first is equality of the variances for each level  
 147 of variables. The other assumptions are normality and independence of the variables with  
 148 random effect: kurtosis and skewness coefficients and the distribution of residues with respect  
 149 to predicted values were also assessed.

150

151 The effects of preparation procedures on measurement variability were investigated for plots  
152 A, B, C and P, Q, R for which two types of sample preparation were carried out. The  $Y_{ijk} =$   
153  $\mu + \alpha_i + (A_i)_j + \varepsilon_{ijk}$  model was used to estimate (i) interpreparation variance, i.e. the variance  
154 of  $(A_i)_j$  and (ii) intrapreparation variance, i.e. the ratio between the difference between  
155 interpreparation variances (those of  $(A_i)_j$ ) and the standard error (those of  $\varepsilon_{ijk}$ ) and the number  
156 of subsample mycotoxin content values ( $k$ ). We investigated whether there was an  
157 interpreparation effect or an intrapreparation effect by means of a  $\text{Chi}^2$  test comparing these  
158 variances and the population variance. These effects were also estimated by calculating the  
159 variation coefficient for mycotoxin content (as the ratio of mean square and mean), and the  
160 standard deviation for each preparation.

161

### 162 ***Effect of the harvest procedure***

163 Three in-field sampling procedures were investigated (one mechanical and two manual  
164 harvest methods) and compared on three plots (P, Q and R). Grain mycotoxin content was  
165 measured just after harvest. For mechanical harvesting, grain sampling was based on the  
166 98/53/CE directive (26), which was subsequently modified by the 2002/27/CE directive (27),  
167 a document that lays down the sampling procedure for official controls of aflatoxin level. This  
168 directive was used because there is no equivalent text dealing with *Fusarium* toxins.  
169 According to this directive, for plots with yields below 1 tonne, 10 samples of 100 g each  
170 must be collected and pooled to give a total sample of 1 kg. The samples (in our case roughly  
171 2 kg) were then dried at 80 °C for 48 h. For the first method of manual harvest (the “hundred  
172 method”), we collected ten randomly selected samples of 100 heads each from each  
173 experimental plot (at least 1 kg). For the second method (the “quadrat method”) we collected  
174 the heads from nine quadrats made up of 1m x 2 adjacent rows from each experimental plot  
175 (900g-1 kg). The harvested heads were dried at 80 °C for 48h and the glumes and rachis were

176 separated from the grains. Grains from all the hand-harvested heads were pooled to give a  
177 total sample per plot for each method. The three samples from each plot were completely  
178 ground and subsamples of flour (each weighing approximately 300g) were sent for mycotoxin  
179 analysis.

180

181

### 182 *Effect of the grain storage procedure*

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

189

190 We evaluated the effect of the different harvest and storage methods used by comparing the  
191 variances associated with these methods with the estimated population variance for each  
192 toxin, by means of a Chi<sup>2</sup> test. If a significant effect was observed, Bonferroni correction was  
193 applied.

194

## 195 **Results**

### 196 *Mycotoxin measurement uncertainty*

197 For each sample, the various mycotoxin measurements obtained are presented in **Table 1**. We  
198 checked that  $\epsilon_{ij}$  for deoxynivalenol and nivalenol analyses were randomly distributed (results  
199 not shown) and followed a Gaussian distribution: the coefficients of kurtosis and skewness for  
200 deoxynivalenol were 0.65 and 0.25 respectively, and those for nivalenol were 2.328 and –

201 0.23, respectively. This variable with a random effect was therefore normally distributed and  
202 independent. No significant differences were observed in the variance of  $\epsilon_{ij}$  (according to  
203 Bartlett's test with  $\alpha \leq 35\%$ ) for nivalenol. For deoxynivalenol content, eight samples  
204 (numbers 1, 9, 14, 16, 17, 18, 20, 23) presented  $\epsilon_{ij}$  variances significantly higher than those  
205 for the other 16 samples. To take into account the three assumptions on which the model was  
206 based, the uncertainty of nivalenol determinations was calculated using all the samples  
207 whereas that for deoxynivalenol was estimated using the 16 samples for which no significant  
208 inequality was observed in the variances of  $\epsilon_{ij}$  (according to Bartlett's test with  $\alpha = 10\%$ ).

209

210 For nivalenol, measurement uncertainty was 15  $\mu\text{g}/\text{kg}$  (mean square: 956.4), whereas for  
211 deoxynivalenol, measurement uncertainty was at least 38  $\mu\text{g}/\text{kg}$  (mean square: 153.6). It  
212 should be noted that (i) if the samples excluded due to heterogeneity in variance were  
213 included, then measurement uncertainty was even higher for deoxynivalenol (81  $\mu\text{g}/\text{kg}$ ) and  
214 (ii) six of the eight samples excluded from the calculation of deoxynivalenol measurement  
215 uncertainty corresponded to grain-grain preparations rather than grain-flour or flour-flour  
216 preparations.

217

218 For plots A, B and C, the standard deviations of deoxynivalenol and nivalenol measurements  
219 were lower after the flour-flour procedure than after the grain-grain procedure, with values of  
220 62 and 5 versus 98 and 9, respectively. With the exception of the deoxynivalenol  
221 measurements for plot A, the variability of measurements (estimated by the coefficient of  
222 variation on each plot) was lower for analyses on flour samples than for those on grain  
223 samples (**Figure 3a**). For grain samples taken for plots P, Q and R, variability was also lower  
224 if subsamples were ground (grain-flour procedure) than if they were not ("grain-grain"  
225 procedure), except for the deoxynivalenol measurements for plot P (**Figure 3b**). The standard

226 deviation was 54 for deoxynivalenol and 3 for nivalenol for samples sent for analysis in the  
227 form of flour, versus 76 and 16, respectively, for samples sent for analysis in the form of  
228 grain. The results from plots A, B, C, P, Q and R therefore suggest that the variability of the  
229 mycotoxin measurements may be reduced by early grinding of the samples. The results  
230 obtained with the  $Y_{ijk} = \mu + \alpha_i + (A_i)_j + \epsilon_{ijk}$  model provided no evidence of an intrapreparation  
231 effect: according to the population variance analysis, the variability of intrapreparation  
232 mycotoxin levels (grain or flour) was similar. However, this model revealed an  
233 interpreparation effect on deoxynivalenol contamination ( $\alpha = 0.05$ ) for plots A, B, C and P,  
234 Q, R. There was also an interpreparation effect on nivalenol contamination ( $\alpha = 0.01$ ) for  
235 plots P, Q and R: mycotoxin contamination levels were higher for grain samples than for flour  
236 samples, except for nivalenol contamination in plots P, Q and R, for which the opposite result  
237 was obtained.

238

### 239 *Effect of harvest and sample storage methods*

240 All the results obtained were very similar (**Table 2**), but deoxynivalenol and nivalenol levels  
241 tended to be higher in cases of manual harvest by the quadrat method than in cases of  
242 “hundred harvest” or mechanical harvest. This trend was confirmed by the Chi<sup>2</sup> test ( $\alpha =$   
243 0.10). Bonferroni’s test graded ( $\alpha = 0.05$ ) the deoxynivalenol levels obtained by the quadrat  
244 method were higher than those obtained by the other methods, and nivalenol levels obtained  
245 by the quadrat method were higher than those obtained by the mechanical method.

246

247 Deoxynivalenol levels seemed to be lower when measured two and eight months after harvest  
248 than when they were measured at harvest (**Table 3**). This result was confirmed by the results  
249 of a Chi<sup>2</sup> test with  $\alpha = 0.10$  and a Bonferroni’s test ( $\alpha = 0.05$ ) performed on whole plots. A  
250 similar trend was observed for nivalenol contamination but was found to be non-significant

251 (Chi<sup>2</sup> test with  $\alpha = 0.10$ ) for plots with the four types of storage tested. On plots on which only  
252 two types of storage were tested, the type of storage was found to have a significant effect  
253 (Chi<sup>2</sup> test with  $\alpha = 0.05$ ) on nivalenol contamination. The results of the Bonferroni's test ( $\alpha =$   
254 0.05) showed that nivalenol contamination after eight months of storage was lower than that  
255 with no storage, but no difference was observed between two months of storage and no  
256 storage.

257

## 258 **Discussion**

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

264

265 The laboratory that performed the analysis in this study estimated the variability of its assays  
266 at 20%. We found that the measurement uncertainty for a sample, estimated by means of  
267 mycotoxin analysis on subsamples, was low: **nivalenol determinations were accurate to**  
268 **within 20 µg/kg** up to a minimum nivalenol concentration of 60 µg/kg (the quantification  
269 limit) and the measurement uncertainty was less than 26% of the concentration of nivalenol  
270 measured. Thus, **the subsampling procedure adopted did not increase the variability of**  
271 **mycotoxin concentrations measured.** However, it should be pointed out that these  
272 encouraging results were obtained with only a small number of plots. It would also be useful  
273 to analyse more highly contaminated samples.

274

275 **The accuracy of deoxynivalenol measurement was lower, with a measurement**  
276 **uncertainty of up to 40 µg/kg.** However, this result corresponds to 22% of the measurement  
277 mean, similar to the variability of other analyses. Our results also show that **grinding grain**  
278 **as soon as possible may minimise errors.** Similar results have been obtained for aflatoxin in  
279 shelled peanuts (28) and for deoxynivalenol in wheat (25, 30). Indeed, the trend towards  
280 lower variability when samples or subsamples were ground probably reflected the grinding of  
281 a larger number of grains than would be the case for a grain sample ground in the laboratory  
282 just before testing. This may increase the uniformity of the sample, resulting in lower  
283 variability. These findings require confirmation and should be taken into account in future  
284 agronomic studies.

285

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

294

295 Part of the reason for the choice of this model lies in the fact that a model lacking an  
296 interaction term between the mycotoxin levels of sample and subsample,  $Y_{ijk} = \mu + \alpha_i + \beta_j +$   
297  $\epsilon_{ijk}$ , may be biased by this interaction, should such an interaction exist. Moreover, a classic  
298 model including an interaction between subsample and sample,  $Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \epsilon$   
299  $_{ijk}$ , may be biased by the independence of subsample assays: in our case, the subsamples are

300 taken from the same sample plot and are therefore not true repetitions, which must be taken  
301 from different plots cropped in a similar fashion.

302

303 Despite the heterogeneity of mycotoxin content within a field (16), **mycotoxin**  
304 **contamination may be analysed following harvesting by manual means if the**  
305 **heterogeneity of contamination is taken into account by representative sampling, evenly**  
306 **distributed over the entire area of the plot.** Taking ten samples of one hundred heads  
307 seemed to give better results than analyses of the heads in nine quadrats made up of 2 rows x  
308 1 m, probably simply because the size of the sample considered was greater.

309

310 *Fusarium* mycotoxins are known to be stable to heat and chemical treatments (31, 32), so the  
311 lower levels of mycotoxin contamination recorded when toxin levels were not assessed  
312 immediately after harvest probably does not correspond to a real decrease, resulting instead  
313 from high measurement uncertainty or from changes in the sample during storage. Our  
314 calculations suggest that high measurement uncertainty is not responsible for the observed  
315 decrease. The second possibility, that changes occur in the sample during storage, therefore  
316 appears more likely. Without more data on the question, it is possible for example that mould  
317 could have either modified the grain samples and thus the toxin extraction rate, or have  
318 degraded the toxin with an enzyme such as acetyltransferase Ayt1p (33). This enzyme was  
319 found to be responsible for a decrease in the amount of deoxynivalenol six weeks after  
320 inoculation in a previous study (34). A third explanation is a modification of the ratio of  
321 acetonitrile / water during grain storage: this ratio strongly influences the extraction rate of  
322 deoxynivalenol and nivalenol, and may also explain our results. **It would therefore seem**  
323 **advisable to sort and grind samples immediately after harvest and, if this is not possible,**  
324 **to minimise the time interval between harvest and analysis.**

325

326 These results, which are of potential value for agronomic research, are also likely to be useful  
327 for the harmonisation of mycotoxin-sampling plans (28). They may also contribute to the  
328 standardisation of maximum limits, which currently differ between countries (35), and  
329 thereby facilitate international trade (28, 36).

330

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336

337 **References**

- 338 1. Le Boulc'h, V.; Bedouret, S.; Poyet, V.; Barchietto, T.; Hazouard, D.; De Paepe, I.; Seng,  
339 J.M. Un test innovant pour contrôler la qualité sanitaire des céréales : détection des  
340 champignons potentiellement producteurs d'une famille de mycotoxines, les  
341 trichothécènes. *AFPP - 6<sup>ème</sup> conférence internationale sur les maladies des plantes,*  
342 *Tours, France; 2000, Vol. 1, pp. 451-458.*
- 343 2. Galtier, P.; Comera, C.; Oswald, I.; Puel, O. Mycotoxines : origines et toxicités. *AFPP -*  
344 *6<sup>ème</sup> conférence internationale sur les maladies des plantes, Tours, France; 2000, Vol.*  
345 *1, pp. 77-86.*
- 346 3. Gilbert, J.; Tekauz, A. Review: Recent developments in research on *Fusarium* head blight  
347 of wheat in Canada. *Can. J. of Plant Pathol.* **2000**, *22*, 1-8.
- 348 4. Placinta, C.M.; D'Mello, J.P.F.; Macdonald, A.M.C. A review of worldwide contamination  
349 of cereal grains and animal feed with *Fusarium* mycotoxins. *Anim. Feed Sci. Technol.*  
350 **1999**, *78*, 21-37.
- 351 5. Chelkowski, J. Fungal pathogens influencing cereal seed quality at harvest. In *Cereal*  
352 *grain: mycotoxins, fungi and quality in drying and storage.* (J. Chelkowski, ed.);  
353 Developments in Food Science, 26, Elsevier; Amsterdam, 1991, pp 53-56.
- 354 6. Thouvenot, D. Moisissure et mycotoxine. In *Mycotoxines : un prochain enjeu de sécurité*  
355 *alimentaire ?*; (CERAAF, ed.); Les cahiers de recherche, N°2, 2002, pp 14-17.
- 356 7. Maurin, N.; Chenet, I. Espèces de *Fusarium* présentes dans les cultures de céréales en  
357 France. *Phytoma.* **1993**, *453*, 20-22.
- 358 8. Cassini, R. Facteurs favorables ou défavorables au développement des fusarioses et  
359 septorioses du blé. *Proc. Meeting of sections cereals and physiology, Dijon; 1970, pp.*  
360 *271-279.*

- 361 9. Parry, D.W.; Jenkinson, P.; McLeod, L. *Fusarium* ear blight (scab) in small grain cereals -  
362 a review. *Plant Pathol.* **1995**, *44*, 207-238.
- 363 10. Champeil, A.; Doré, T.; Fourbet, J.F. Review - *Fusarium* head blight: Epidemiological  
364 origin of the effects of cultural practices on head blight attacks and the production of  
365 mycotoxins by *Fusarium* in wheat grains. *Plant Sci.* **2004**, *166*, 1389-1415.
- 366 11. Dill-Macky, R.; Jones, R.K. The effect of previous crop residues and tillage on *Fusarium*  
367 head blight of wheat. *Plant Dis.* **2000**, *84*, 71-76.
- 368 12. Champeil, A.; Fourbet, J.F.; Doré, T. Influence of cropping system on *Fusarium* head  
369 blight and mycotoxin levels in winter wheat. *Crop Protect.* **2004**, *23*, 531-537.
- 370 13. Teich, A.H. Epidemiology of wheat (*Triticum aestivum* L.) scab caused by *Fusarium* spp.  
371 In *Fusarium: mycotoxins, taxonomy and pathogenicity*; (J. Chelkowski, ed.); Elsevier;  
372 Amsterdam, 1989, pp 269-282.
- 373 14. Lemmens, M.; Haim, K.; Lew, H.; Ruckenbauer, P. The effect of nitrogen fertilization on  
374 *Fusarium* head blight development and deoxynivalenol contamination in wheat. *J.*  
375 *Phytopathol.* **2004**, *152*, 1-8.
- 376 15. Cromey, M.G.; Shorter, S.C.; Lauren, D.R.; Sinclair, K.I. Cultivar and crop management  
377 influences on fusarium head blight and mycotoxins in spring wheat (*Triticum*  
378 *aestivum*) in New Zealand. *New Zealand J. Crop Hortic. Sci.* **2002**, *30*, 235-247.
- 379 16. Raimbault, J.M.; Orlando, D.; Grosjean, F.; Leuillet, M. Méthodes d'échantillonnage et de  
380 quantification des mycotoxines - Réussir à trouver des aiguilles dans une meule de  
381 foin. *Perspect. Agri.* **2002**, *278*, 32-35.
- 382 17. Whitaker, T.B.; Wiser, E.H. Theoretical investigations into the accuracy of sampling  
383 shelled peanuts for aflatoxin. *J. Am. Oil Chem. Soc.* **1969**, *46*, 377-379.
- 384 18. Armitage, D. Grain sampling methods to achieve consumer confidence and food safety. In  
385 *HGCA Research Review*; Home Grown Cereals Authority; London UK, 2003.

- 386 19. Hart, P.; Schabenberger, O. How to sample wheat to accurately determine vomitoxin  
387 levels. <http://www.msue.msu.edu/imp/modab/26309701.htm>, 1999, date on last  
388 accessed 18<sup>th</sup> February, 2004.
- 389 20. Codex Alimentarius Commission. Report of the 33rd session of the codex alimentarius  
390 committee on food additives and contaminants. ALINORM 01/12A. FAO/WHO;  
391 2001, pp. 300.
- 392 21. GIPSA. Grain fungal diseases and mycotoxin reference. GIPSA, Technical Services  
393 Division, Kansas City, 1999, pp. 54.
- 394 22. Feinberg, M. *La validation des méthodes d'analyse - Une approche chimiométrique de*  
395 *l'assurance qualité au laboratoire*; Masson: Paris, 1996, pp. 397.
- 396 23. ISO. Guide to the Expression of Uncertainty in Measurement. *2001 ALINORM 01/23*.  
397 1993.
- 398 24. Codex Alimentarius Commission. Report of the 23rd session of the codex committee on  
399 methods of analysis and sampling - ALINORM 01/23. FAO/WHO; 2001, pp. 51.
- 400 25. Hart, L.P. Variability of vomitoxin in truckloads of wheat in a wheat scab epidemic year.  
401 *Plant Dis.* **1998**, 82, 625-630.
- 402 26. Commission Directive. Commission directive 98/53/EC of the 16 July 1998 on laying  
403 down the sampling methods and the methods of analysis for the official control of the  
404 levels for certain contaminants in foodstuffs. *Official Journal* **1998**, 201, 93-101.
- 405 27. Commission Directive. Commission directive 2002/27/EC of the 13 March 2002  
406 amending Directive 98/83/EC on laying down the sampling methods and the methods  
407 of analysis for the official control of the levels for certain contaminants in foodstuffs.  
408 *Official Journal* **2002**, 75, 44-45.
- 409 28. Whitaker, T.B. Standardisation of mycotoxin sampling procedures: An urgent necessity.  
410 *Food Control* **2003**, 14, 233-237.

- 411 29. Whitaker, T.B.; Dickens, J.W. Variability of aflatoxin test results. *J. Am. Oil Chem. Soc.*  
412 **1974**, *51*, 214-218.
- 413 30. Whitaker, T.B.; Hagler, W.M., Jr.; Giesbrecht, F.G.; Johansson, A.S. Sampling, sample  
414 preparation, and analytical variability associated with testing wheat for  
415 deoxynivalenol. *J. AOAC Int.* **2000**, *83*, 1285-1292.
- 416 31. Vidal, D.R. Propriétés immunosuppressives des mycotoxines du groupe des  
417 trichothécènes. *Bulletin de l'institut Pasteur.* **1990**, *88*, 159-192.
- 418 32. Quillien, J.F. Les mycotoxines. Institut National de la Recherche Agronomique (INRA) -  
419 Centre de Réseaux pour l'Innovation en Agriculture et Agroalimentaire (CRIAA);  
420 2002, pp. 24.
- 421 33. Mitterbauer, R.; Adam, G. *Saccharomyces cerevisiae* and *Arabidopsis thaliana*: Useful  
422 model systems for the identification of molecular mechanisms involved in resistance  
423 of plants to toxins. *Eur. J. of Pl. Pathol.* **2002**, *108*, 699-703.
- 424 34. Miller, J.D.; Young, J.C. Deoxynivalenol in an experimental *Fusarium graminearum*  
425 infection of wheat. *Can. J. of Pl. Pathol.* **1985**, *7*, 132-134.
- 426 35. Food and Agriculture Organization. Worldwide regulations for mycotoxins 1995- A  
427 compendium. FAO Food and Nutrition; 1997, pp. 64.
- 428 36. FAO/WHO/UNEP. Report of the Third Joint FAO/WHO/UNEP International Conference  
429 on Mycotoxins - MYC-CONF/99/REPe. FAO/WHO/UNEP International; 1999, pp.  
430 22.
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433 **Table 1:** Mycotoxin levels were determined for three subsamples per sample, with each  
 434 sample corresponding to a plot. No result indicates that no analysis was carried out; <d<sub>l</sub>  
 435 mycotoxin not detected (< 30 µg.kg<sup>-1</sup>); <q<sub>l</sub> mycotoxin level lower than the quantification limit  
 436 (< 60 µg.kg<sup>-1</sup>).

437

Preparation when sampling	sample sent for analyse	Sample	Plot	Deoxynivalenol, µg/kg			Nivalenol, µg/kg		
				sub sample 1	sub sample 2	sub sample 3	sub sample 1	sub sample 2	sub sample 3
Flour	Flour	1	A	150	160	320	<d <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>
		2	B	240	290	240	<d <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>
		3	C	330	310	390	<q <sub>l</sub>	<q <sub>l</sub>	<d <sub>l</sub>
		4	D	60	100	120	<q <sub>l</sub>	<q <sub>l</sub>	<d <sub>l</sub>
		5	E	370	380		<q <sub>l</sub>	<q <sub>l</sub>	
		6	F	110	90	<d <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>
		7	G	200	190		<q <sub>l</sub>	<q <sub>l</sub>	
		8	H	240	250	250	<q <sub>l</sub>	<q <sub>l</sub>	<q <sub>l</sub>
		9	I	800	650	600	120	150	150
		10	J	110	100	130	60	60	80
		11	K	110	100		60	<d <sub>l</sub>	
		12	L	100	<d <sub>l</sub>		70	<d <sub>l</sub>	
Grain	Flour	13	P	190	160	100	<q <sub>l</sub>	<q <sub>l</sub>	<q <sub>l</sub>
		14	Q	550	400	430	80	80	70
		15	R	110	90	120	<q <sub>l</sub>	<q <sub>l</sub>	<q <sub>l</sub>
Grain	Grain	16	A	210	330	170	<d <sub>l</sub>	<d <sub>l</sub>	<q <sub>l</sub>
		17	B	360	120	210	<d <sub>l</sub>	<q <sub>l</sub>	<d <sub>l</sub>
		18	C	350	500	360	<q <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>
		19	M	<q <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>	<d <sub>l</sub>
		20	N	500	700	600	<q <sub>l</sub>	<q <sub>l</sub>	<q <sub>l</sub>
		21	O	350	340	340	<d <sub>l</sub>	60	<q <sub>l</sub>
		22	P	200	240	200	<q <sub>l</sub>	<q <sub>l</sub>	<q <sub>l</sub>
		23	Q	550	340	550	60	<d <sub>l</sub>	60
		24	R	<d <sub>l</sub>	60	110	<d <sub>l</sub>	<d <sub>l</sub>	<q <sub>l</sub>

438

439 **Table 2:** Mycotoxin contamination determination according to harvest method. <math>\lt; q\_1</math> mycotoxin  
 440 level lower than quantification limit (<math>\lt; 60 \mu\text{g}\cdot\text{kg}^{-1}</math>).  
 441

		Plot P	Plot Q	Plot R	Bonferroni
Deoxynivalenol, $\mu\text{g}/\text{kg}$	Quadrat harvest	350	600	240	a
	Hundred harvest	180	550	160	b
	Mechanical harvest	190	550	110	b
Nivalenol, $\mu\text{g}/\text{kg}$	Quadrat harvest	70	60	90	a
	Hundred harvest	<math>\lt; q_1</math>	70	60	ab
	Mechanical harvest	<math>\lt; q_1</math>	80	<math>\lt; q_1</math>	b

442  
 443

444 **Table 3:** Mycotoxin contamination according to type of storage. No result indicates that no  
 445 analysis was carried out; <d<sub>1</sub> mycotoxin not detected (< 30 µg.kg<sup>-1</sup>); <q<sub>1</sub> mycotoxin level  
 446 lower than the quantification limit (< 60 µg.kg<sup>-1</sup>).  
 447

	Plots	0 months		2 months		8 months	
		no conservation	ambient temperature	4 °C	-20 °C	ambient temperature	
Deoxynivalenol, µg/kg	H	230	240	230	230		
	I	800	500	630	600		
	J	140	140	120	110		
	A	150				220	
	B	310				240	
	C	600				370	
	M	<d <sub>1</sub>	<q <sub>1</sub>				
	N	1100	600				
	O	600	340				
	Nivalenol, µg/kg	H	<q <sub>1</sub>	<q <sub>1</sub>	<q <sub>1</sub>	<q <sub>1</sub>	
I		110	70	90	90		
J		70	50	<q <sub>1</sub>	50		
A		60				<q <sub>1</sub>	
B		440				<q <sub>1</sub>	
C		<d <sub>1</sub>				<q <sub>1</sub>	
M		<d <sub>1</sub>	<d <sub>1</sub>				
N		200	<q <sub>1</sub>				
O		<q <sub>1</sub>	60				

448

449 FIGURES LEGENDS

450

451 **Figure 1:** Chemical structures of type B trichotecenes.

452

453 **Figure 2:** Steps of the grains sampling procedures

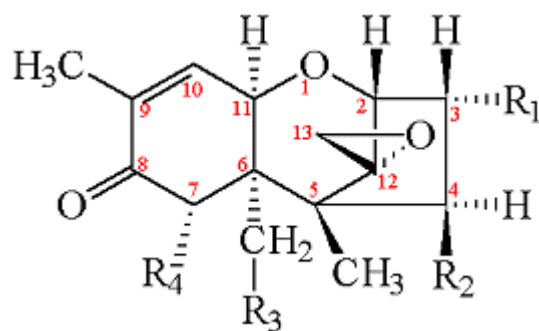
454

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

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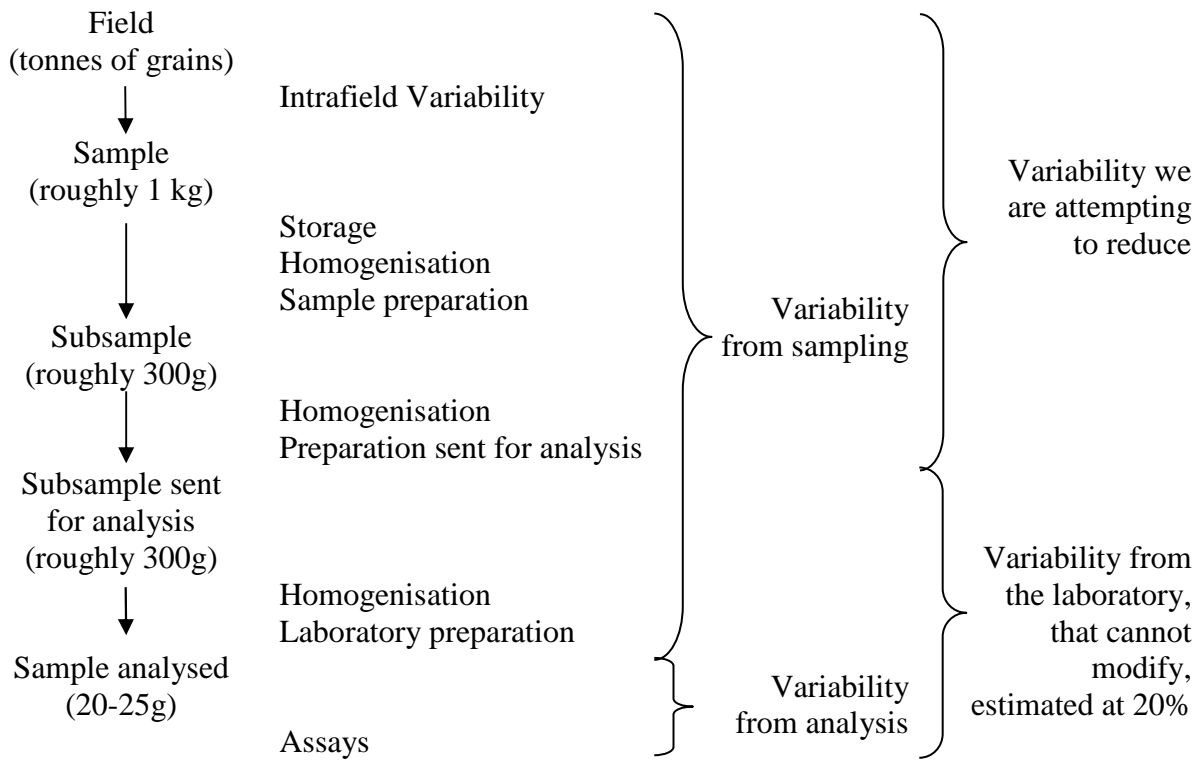
459 Figure 1:  
460



Type B trichothecenes: Deoxynivalenol (R<sub>1</sub> = OH, R<sub>2</sub> = H, R<sub>3</sub> = OH, R<sub>4</sub> = OH)  
Nivalenol (R<sub>1</sub> = OH, R<sub>2</sub> = OH, R<sub>3</sub> = OH, R<sub>4</sub> = OH)

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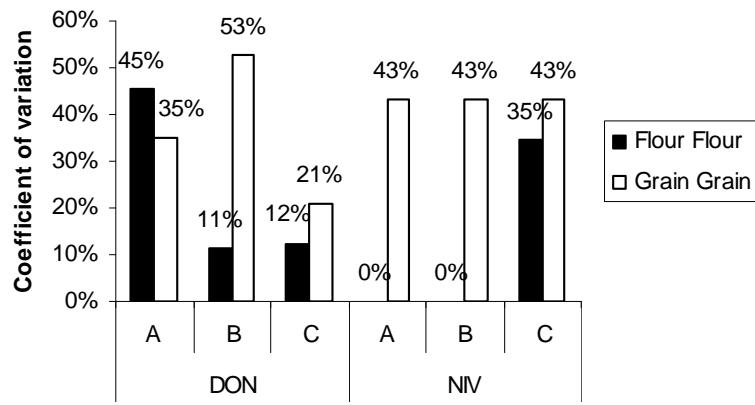
463 Figure 2:  
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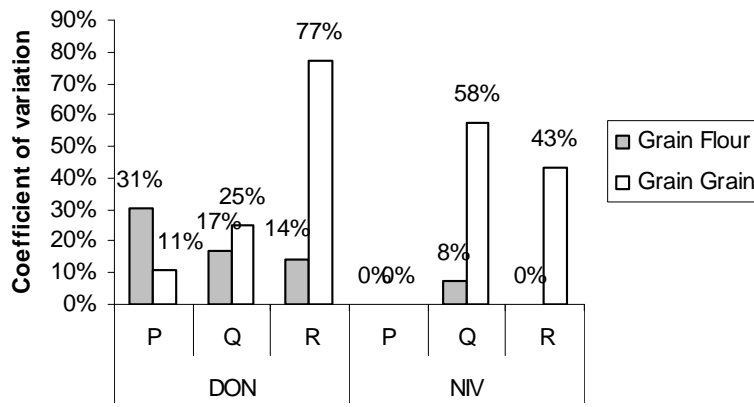
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467 Figure 3a:

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482 Figure 3b:



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