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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; μ the general mean toxin content; α_i a variable for
 127 the i^{th} sample, which has fixed effect; $(A_i)_j$ a variable for the j^{th} preparation, which has random
 128 effect because the toxin content of the j^{th} preparation depended on the toxin content of the
 129 sample A_i ; and ϵ_{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^{th} subsamples of the i^{th} sample. In this model, ϵ_{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 ε_{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 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 Chi² 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 ϵ_{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 ϵ_{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 ϵ_{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 ϵ_{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 Chi² 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 Chi² 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 (Chi² 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 (Chi² 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 ϵ_{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

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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_l
 435 mycotoxin not detected (< 30 µg.kg⁻¹); <q_l mycotoxin level lower than the quantification limit
 436 (< 60 µg.kg⁻¹).

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 _l	<d _l	<d _l
		2	B	240	290	240	<d _l	<d _l	<d _l
		3	C	330	310	390	<q _l	<q _l	<d _l
		4	D	60	100	120	<q _l	<q _l	<d _l
		5	E	370	380		<q _l	<q _l	
		6	F	110	90	<d _l	<d _l	<d _l	<d _l
		7	G	200	190		<q _l	<q _l	
		8	H	240	250	250	<q _l	<q _l	<q _l
		9	I	800	650	600	120	150	150
		10	J	110	100	130	60	60	80
		11	K	110	100		60	<d _l	
		12	L	100	<d _l		70	<d _l	
Grain	Flour	13	P	190	160	100	<q _l	<q _l	<q _l
		14	Q	550	400	430	80	80	70
		15	R	110	90	120	<q _l	<q _l	<q _l
Grain	Grain	16	A	210	330	170	<d _l	<d _l	<q _l
		17	B	360	120	210	<d _l	<q _l	<d _l
		18	C	350	500	360	<q _l	<d _l	<d _l
		19	M	<q _l	<d _l	<d _l	<d _l	<d _l	<d _l
		20	N	500	700	600	<q _l	<q _l	<q _l
		21	O	350	340	340	<d _l	60	<q _l
		22	P	200	240	200	<q _l	<q _l	<q _l
		23	Q	550	340	550	60	<d _l	60
		24	R	<d _l	60	110	<d _l	<d _l	<q _l

438

439 **Table 2:** Mycotoxin contamination determination according to harvest method. $\lt; q_1$ mycotoxin
 440 level lower than quantification limit ($\lt; 60 \mu\text{g}\cdot\text{kg}^{-1}$).
 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	$\lt; q_1$	70	60	ab
	Mechanical harvest	$\lt; q_1$	80	$\lt; q_1$	b

442
 443

444 **Table 3:** Mycotoxin contamination according to type of storage. No result indicates that no
 445 analysis was carried out; <d₁ mycotoxin not detected (< 30 µg.kg⁻¹); <q₁ mycotoxin level
 446 lower than the quantification limit (< 60 µg.kg⁻¹).
 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 ₁	<q ₁				
	N	1100	600				
	O	600	340				
	Nivalenol, µg/kg	H	<q ₁	<q ₁	<q ₁	<q ₁	
I		110	70	90	90		
J		70	50	<q ₁	50		
A		60				<q ₁	
B		440				<q ₁	
C		<d ₁				<q ₁	
M		<d ₁	<d ₁				
N		200	<q ₁				
O		<q ₁	60				

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449 FIGURES LEGENDS

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

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

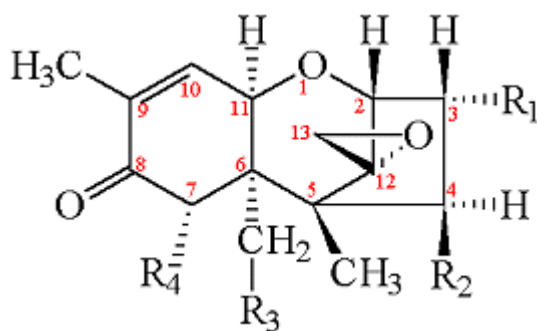
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455 **Figure 3:** Effect of sample preparation on the variability of mycotoxin contamination
456 measurement for plots A, B and C (a) and for plots P, Q and R (b).

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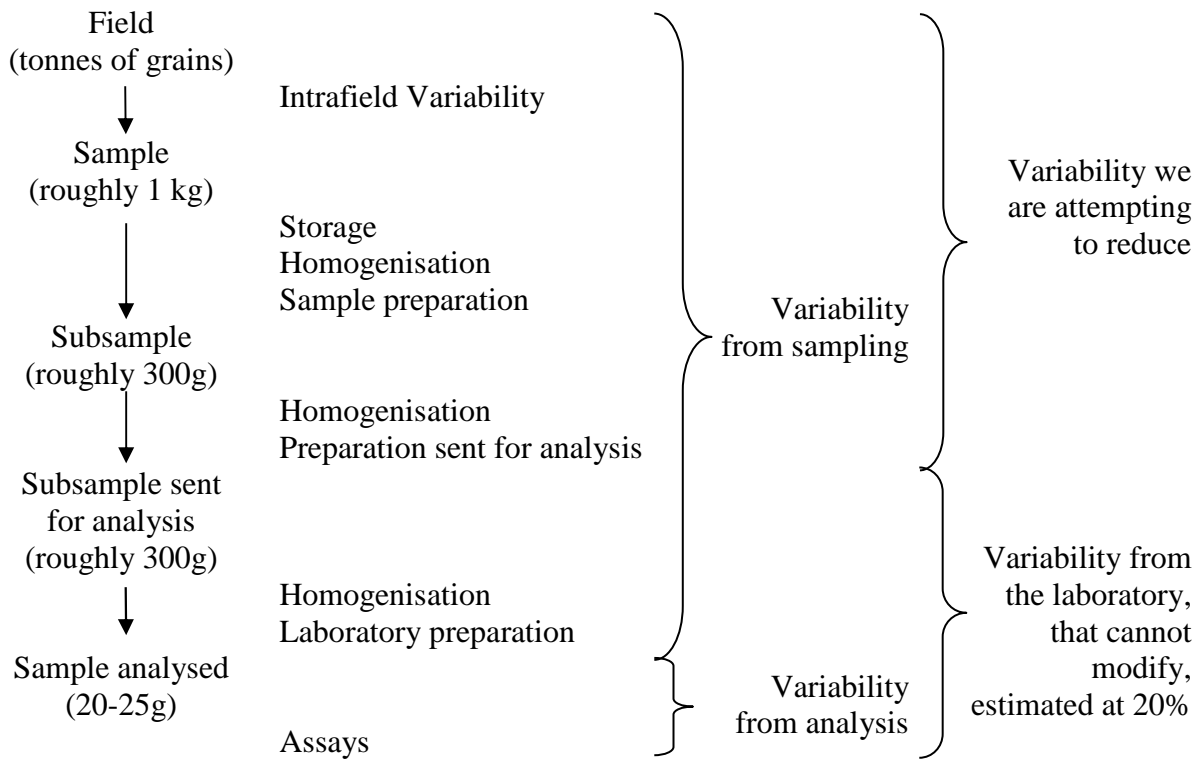
459 Figure 1:
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Type B trichothecenes: Deoxynivalenol (R1 = OH, R2 = H, R3 = OH, R4 = OH)
Nivalenol (R1 = OH, R2 = OH, R3 = OH, R4 = OH)

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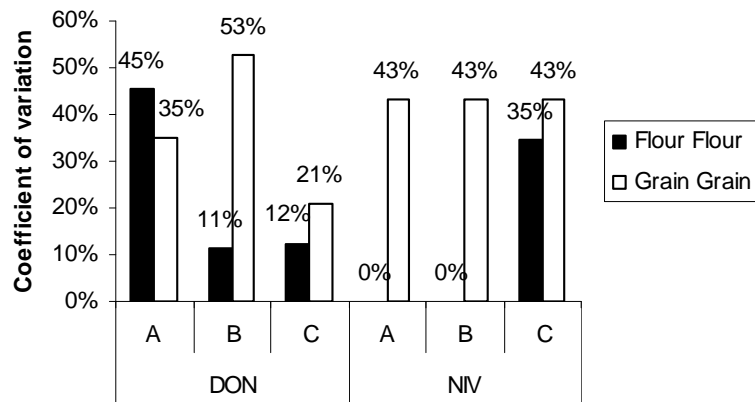
463 Figure 2:
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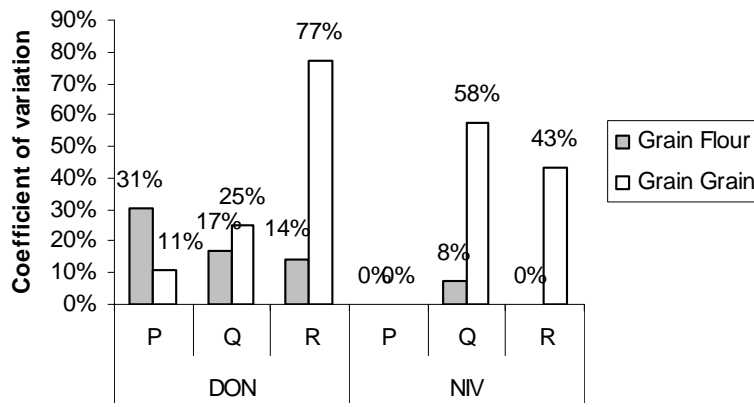
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467 Figure 3a:

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482 Figure 3b:



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