JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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

AGNES CHAMPEIL,* JEAN-FRANCOIS FOURBET, AND THIERRY DORÉ

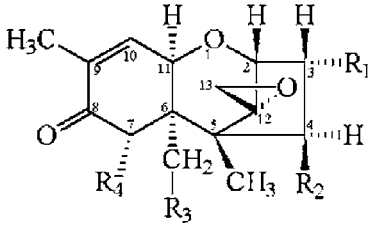
UMR d'Agronomie INRA/INA P-G, Centre INRA Versailles-Grignon, 78850 Thiverval-Grignon, France

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. Analyses were performed with the aim of formulating guidelines for grain sampling to increase the reliability of grain mycotoxin measurement in agronomic experiments. Two toxins in wheat samples, deoxynivalenol and nivalenol, were targeted. With a nested linear mixed model, it was estimated that the uncertainty of nivalenol determination was low (±15 μg/kg), whereas that for deoxynivalenol determination was higher (±38 μg/kg). It was 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, it was shown that heads can be harvested manually for agronomic experiments provided that sampling is representative (evenly distributed over the entire plot area). Finally, delaying the assay until after harvest was found to affect the results obtained and should therefore be avoided.

KEYWORDS: 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 edema, 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 be present at high concentrations in only <0.5% of the peanut crop, and concentrations may be as high as 1,000,000 μg/kg in 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.



Type B trichothecenes: DON (R1 = OH, R2 = H, R3 = OH, R4 = OH)
NIV (R1 = OH, R2 = OH, R3 = OH, R4 = OH)

Figure 1. Chemical structures of type B trichothecenes.

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 (*12*). Nine agronomic treatments were duplicated in this design, and two growing seasons were used (2001/2002 and 2002/2003). Thirty-six plots were

* Corresponding author (telephone +33130815420; fax +33130815425; e-mail champeil@grignon.inra.fr).

10.1021/jf049374s CCC: $27.50
Published on Web 09/04/2004

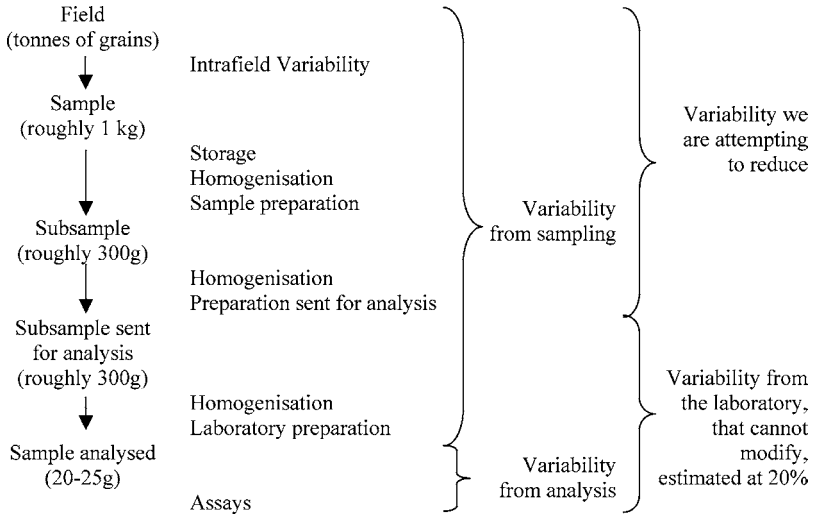


Figure 2. Steps of the grain sampling procedures.

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, and I, N, and Q were cropped under an integrated direct drilling system, whereas plots 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 2002/2003 (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 homogenized at least three times, in a mixer/divider. A small quantity of each sample was taken (20−25 g) 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 μg/kg for trichothecenes, and the quantification limit (q_l) was twice the detection limit (60 μg/kg). For the purpose of this study, mycotoxin contents below d_l or q_l 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 (no. 1−12) were completely ground, and three flour subsamples of roughly 300 g 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 12 remaining samples, three grain subsamples of roughly 300 g each were taken. The subsamples from samples 13−15 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 converted to flour only after subsampling. The subsamples from samples 16−24 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 characterize 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 characterizes 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:

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

Y_{ijk} is the measured toxin content; μ the general mean toxin content; α_i a variable for the *i*th sample, which has a fixed effect; $(A_i)_j$ a variable for the *j*th preparation, which has a random effect because the toxin content of the *j*th preparation depended on the toxin content of the sample A_i; and ϵ_{ijk} the standard error, relating to *k*, 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 equation

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

where *n* is the number of subsamples.

We used all of 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, ϵ_{ij}, which characterizes the modeling error, was associated with the maximum value of variability for the assay. The maximum measurement uncertainty can therefore be expressed as

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

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−C and P−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) interpreparation variance, that is, the variance of $(A_i)_j$ and (ii) intrapreparation variance, that is, the ratio between the difference between interpreparation variances [those of

Table 1. Mycotoxin Levels Determined for Three Subsamples per Sample, with Each Sample Corresponding to a Plot[a]

preparation when sampling	sample sent for analysis	sample	plot	deoxynivalenol, μg/kg			nivalenol, μg/kg		
				subsample 1	subsample 2	subsample 3	subsample 1	subsample 2	subsample 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

[a] No result indicates that no analysis was carried out; <d_l = mycotoxin not detected (<30 μg/kg); <q_l = mycotoxin level lower than the quantification limit (<60 μg/kg).

$(A_i)_j$] and the standard error (those of ϵ_{ijk}) and the number of subsample mycotoxin content values (k). We investigated whether there was an interpreparation effect or an intrapreparation 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 Harvest Procedure. Three in-field sampling procedures were investigated (one mechanical and two manual harvest methods) and compared on three plots (P−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 control 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 10 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 1 m × 2 adjacent rows from each experimental plot (900 g−1 kg). The harvested heads were dried at 80 °C for 48 h, and the glumes and rachis were separated from the grains. Grains from all of 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 ∼300 g) were sent for mycotoxin analysis.

Effect of Grain Storage Procedure. The effect of the grain storage procedure was investigated on several plots. In each case, subsamples of ∼300 g were collected for mycotoxin analysis. Five kinds of storage process were tested: storage at room temperature for 8 months (plots A−C); at 4 °C for 2 months (plots H−J); at −20 °C for 2 months (plots H−J); at room temperature for 2 months (plots H− J and M−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 chi^2 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 ϵ_{ij} values 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 ϵ_{ij} (according to Bartlett's test with $\alpha \leq 35\%$) for nivalenol. For deoxynivalenol content, 8 samples (no. 1, 9, 14, 16−18, 20, and 23) presented ϵ_{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 of the samples, whereas that for deoxynivalenol was estimated using the 16 samples for which no significant inequality was observed in the variances of ϵ_{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−C, the standard deviations of deoxynivalenol and nivalenol measurements were lower after the flour−flour

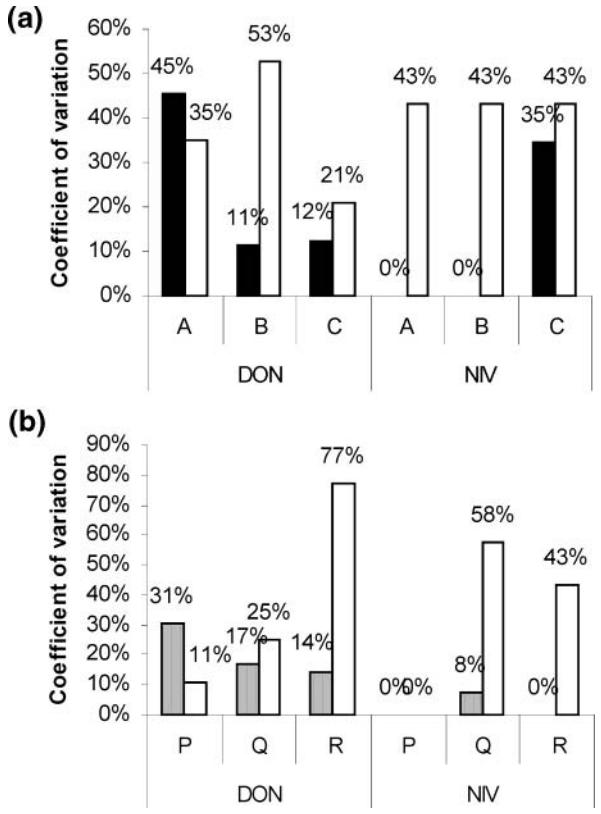


Figure 3. Effect of sample preparation on the variability of mycotoxin contamination measurement for plots A–C (**a**) and P–R (**b**): (**a**) (shaded bars) flour–flour procedure, (white bars) grain–grain procedure; (**b**) (shaded bars) grain–flour procedure, (white bars) grain–grain procedure.

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−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 deviations were 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−C and P−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−C and P−R. There was also an interpreparation effect on nivalenol contamination ($\alpha = 0.01$) for plots P−R: mycotoxin contamination levels were higher for grain samples than for flour samples except for nivalenol contamination in plots P−R, for which the opposite result was obtained.

Effect of Harvest and Sample Storage Methods. All of 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 as 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.

Table 2. Mycotoxin Contamination Determination According to Harvest Method[a]

		plot			
		P	Q	R	Bonferroni
deoxynivalenol, μg/kg	quadrat harvest	350	600	240	a
	hundred harvest	180	550	160	b
	mechanical harvest	190	550	110	b
nivalenol, μg/kg	quadrat harvest	70	60	90	a
	hundred harvest	<q_l	70	60	ab
	mechanical harvest	<q_l	80	<q_l	b

[a] <q_l = mycotoxin level lower than quantification limit (<60 μg/kg).

Table 3. Mycotoxin Contamination According to Type of Storage[a]

	Plot	0 months no conservation	2 months ambient temp	2 months 4 °C	2 months -20 °C	8 months ambient temp
Deoxynivalenol, μg/kg	H	230	240	230	230	
	I	800 a	500 b	630 b	600 b	
	J	140	140	120	110	
	A	150				220
	B	310 a				240 b
	C	600				370
	M	<d_l	<q_l			
	N	1100 a	600 b			
	O	600	340			
Nivalenol, μg/kg	H	<q_l	<q_l	<q_l	<q_l	
	I	110 a	70 b	90 b	90 b	
	J	70	50	<q_l	50	
	A	60				<q_l
	B	440 a				<q_l b
	C	<d_l				<q_l
	M	<d_l	<d_l			
	N	200 a	<q_l a			
	O	<q_l	60			

[a] No result indicates that no analysis was carried out; <d_l = mycotoxin not detected (<30 μg/kg); <q_l = mycotoxin level lower than the quantification limit (<60 μg/kg). a and b: results of Bonferroni correction applied on the three triples H–J, A–C, and M–O.

Deoxynivalenol levels seemed to be lower when measured 2 and 8 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 test ($\alpha = 0.05$) performed on whole plots. A similar trend was observed for nivalenol contamination but was found to be nonsignificant (chi^2 test with $\alpha = 0.10$) for plots with the four types of storage tested. For 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 test ($\alpha = 0.05$) showed that nivalenol contamination after 8 months of storage was lower than that with no storage, but no difference was observed between 2 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, and 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 con-

stitutes 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 μg/kg up to a minimum nivalenol concentration of 60 μg/kg (the quantification limit), and the measurement uncertainty was <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 analyze 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 minimize errors. Similar results have been obtained for aflatoxin in shelled peanuts (*28*) and for deoxynivalenol in wheat (*25*, *30*). Indeed, the trend toward 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 analyzed 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 10 samples of 100 heads seemed to give better results than analyses of the heads in 9 quadrats made up of 2 rows $\times$ 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 to be 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 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 6 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 to be advisable to sort and grind samples immediately after harvest and, if this is not possible, to minimize the time between harvest and analysis.

These results, which are of potential value for agronomic research, are also likely to be useful for the harmonization of mycotoxin-sampling plans (*28*). They may also contribute to the standardization of maximum limits, which currently differ among countries (*35*), and thereby facilitate international trade (*28*, *36*).

ACKNOWLEDGMENT

We 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.

LITERATURE CITED

(1) Le Boulc'h, V.; Bedouret, S.; Poyet, V.; Barchietto, T.; Hazouard, D.; De Paepe, I.; Seng, J. M. Un test innovant pour contrôler la qualité sanitaire des céréales: détection des champignons potentiellement producteurs d'une famille de mycotoxines, les trichothécènes. *AFPP—6ème Conférence Internationale sur les Maladies des Plantes*; Association Française de Protection des Plantes: Tours, France; 2000; Vol. 1, pp 451−458.

(2) Galtier, P.; Comera, C.; Oswald, I.; Puel, O. Mycotoxines: origines et toxicités. *AFPP—6ème Conférence Internationale sur les Maladies des Plantes*; Association Française de Protection des Plantes: Tours, France; 2000; Vol. 1, pp 77−86.

(3) Gilbert, J.; Tekauz, A. Review: Recent developments in research on *Fusarium* head blight of wheat in Canada. *Can. J. Plant Pathol.* **2000**, *22*, 1−8.

(4) Placinta, C. M.; D'Mello, J. P. F.; Macdonald, A. M. C. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Anim. Feed Sci. Technol.* **1999**, *78*, 21−37.

(5) Chelkowski, J. Fungal pathogens influencing cereal seed quality at harvest. In *Cereal Grain: Mycotoxins, Fungi and Quality in Drying and Storage*; Chelkowski, J., Ed.; Developments in Food Science 26; Elsevier: Amsterdam, The Netherlands, 1991; pp 53−56.

(6) Thouvenot, D. Moisissure et mycotoxine. In *Mycotoxines: un Prochain enjeu de Sécurité Alimentaire?*; Les Cahiers de Recherche 2; CERAAF: Blois, France, 2002, pp 14−17.

(7) Maurin, N.; Chenet, I. Espèces de *Fusarium* présentes dans les cultures de céréales en France. *Phytoma* **1993**, *453*, 20−22.

(8) Cassini, R. Facteurs favorables ou défavorables au développement des fusarioses et septorioses du blé. *Proceedings of the Meeting of Sections Cereals and Physiology*; Eucarpia: Dijon, France, 1970; pp 271−279.

(9) Parry, D. W.; Jenkinson, P.; McLeod, L. *Fusarium* ear blight (scab) in small grain cereals—a review. *Plant Pathol.* **1995**, *44*, 207−238.

(10) Champeil, A.; Doré, T.; Fourbet, J. F. Review—*Fusarium* head blight: Epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. *Plant Sci.* **2004**, *166*, 1389−1415.
(11) Dill-Macky, R.; Jones, R. K. The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. *Plant Dis.* **2000**, *84*, 71−76.
(12) Champeil, A.; Fourbet, J. F.; Doré, T. Influence of cropping system on *Fusarium* head blight and mycotoxin levels in winter wheat. *Crop Prot.* **2004**, *23*, 531−537.
(13) Teich, A. H. Epidemiology of wheat (*Triticum aestivum L.*) scab caused by *Fusarium* spp. In *Fusarium: Mycotoxins, Taxonomy and Pathogenicity*; Chelkowski, J., Ed.; Elsevier: Amsterdam, The Netherlands, 1989; pp 269−282.
(14) Lemmens, M.; Haim, K.; Lew, H.; Ruckenbauer, P. The effect of nitrogen fertilization on *Fusarium* head blight development and deoxynivalenol contamination in wheat. *J. Phytopathol.* **2004**, *152*, 1−8.
(15) Cromey, M. G.; Shorter, S. C.; Lauren, D. R.; Sinclair, K. I. Cultivar and crop management influences on fusarium head blight and mycotoxins in spring wheat (*Triticum aestivum*) in New Zealand. *N. Z. J. Crop Hortic. Sci.* **2002**, *30*, 235−247.
(16) Raimbault, J. M.; Orlando, D.; Grosjean, F.; Leuillet, M. Méthodes d'échantillonnage et de quantification des mycotoxines—Réussir à trouver des aiguilles dans une meule de foin. *Perspect. Agric.* **2002**, *278*, 32−35.
(17) Whitaker, T. B.; Wiser, E. H. Theoretical investigations into the accuracy of sampling shelled peanuts for aflatoxin. *J. Am. Oil Chem. Soc.* **1969**, *46*, 377−379.
(18) Armitage, D. Grain sampling methods to achieve consumer confidence and food safety. In *HGCA Research Review*; Home Grown Cereals Authority; London U.K., 2003.
(19) Hart, P.; Schabenberger, O. How to sample wheat to accurately determine vomitoxin levels. http://www.msue.msu.edu/imp/modab/26309701.htm, 1999; last accessed on Feb 18, 2004.
(20) Codex Alimentarius Commission. *Report of the 33rd Session of the Codex Alimentarius Committee on Food Additives and Contaminants*; ALINORM 01/12A; FAO/WHO: Rome, Italy, 2001; p 300.
(21) GIPSA. *Grain Fungal Diseases and Mycotoxin Reference*; GIPSA, Technical Services Division: Kansas City, MO, 1999; p 54.
(22) Feinberg, M. *La Validation des Méthodes d'Analyse—Une Approche Chimiométrique de l'Assurance Qualité au Laboratoire*; Masson: Paris, France, 1996; p 397.
(23) ISO. *Guide to the Expression of Uncertainty in Measurement*; 2001 ALINORM 01/23; 1993.
(24) Codex Alimentarius Commission. *Report of the 23rd Session of the Codex Committee on Methods of Analysis and Sampling*; ALINORM 01/23; FAO/WHO: Rome, Italy, 2001; p 51.
(25) Hart, L. P. Variability of vomitoxin in truckloads of wheat in a wheat scab epidemic year. *Plant Dis.* **1998**, *82*, 625−630.
(26) Commission Directive. Commission directive 98/53/EC of the 16 July 1998 on laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs. *Off. J. Eur. Communities* **1998**, *201*, 93−101.
(27) Commission Directive. Commission directive 2002/27/EC of the 13 March 2002 amending Directive 98/83/EC on laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs. *Off. J. Eur. Communities* **2002**, *75*, 44−45.
(28) Whitaker, T. B. Standardisation of mycotoxin sampling procedures: An urgent necessity. *Food Control* **2003**, *14*, 233−237.
(29) Whitaker, T. B.; Dickens, J. W. Variability of aflatoxin test results. *J. Am. Oil Chem. Soc.* **1974**, *51*, 214−218.
(30) Whitaker, T. B.; Hagler, W. M., Jr.; Giesbrecht, F. G.; Johansson, A. S. Sampling, sample preparation, and analytical variability associated with testing wheat for deoxynivalenol. *J. AOAC Int.* **2000**, *83*, 1285−1292.
(31) Vidal, D. R. Propriétés immunosuppressives des mycotoxines du groupe des trichothécènes. *Bull. Inst. Pasteur* **1990**, *88*, 159−192.
(32) Quillien, J. F. *Les Mycotoxines*; Institut National de la Recherche Agronomique (INRA), Centre de Réseaux pour l'Innovation en Agriculture et Agroalimentaire (CRIAA): Rennes, France, 2002, p 24.
(33) Mitterbauer, R.; Adam, G. *Saccharomyces cerevisae* and *Arabidopsis thaliana*: Useful model systems for the identification of molecular mechanisms involved in resistance of plants to toxins. *Eur. J. Plant Pathol.* **2002**, *108*, 699−703.
(34) Miller, J. D.; Young, J. C. Deoxynivalenol in an experimental *Fusarium graminearum* infection of wheat. *Can. J. Plant Pathol.* **1985**, *7*, 132−134.
(35) Food and Agriculture Organization. *Worldwide Regulations for Mycotoxins 1995—A Compendium*; FAO Food and Nutrition: Rome, Italy, 1997; p 64.
(36) FAO/WHO/UNEP. *Report of the Third Joint FAO/WHO/UNEP International Conference on Mycotoxins*; MYC-CONF/99/REPe; FAO/WHO/UNEP International: Rome, Italy, 1999; p 22.

Received for review April 19, 2004. Revised manuscript received July 23, 2004. Accepted July 23, 2004. This research was funded by the DGAL (French Ministry of Agriculture) and by INRA.

JF049374S