SPECIAL GUEST EDITOR SECTION

Sampling, Sample Preparation, and Analytical Variability Associated with Testing Wheat for Deoxynivalenol

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The variability associated with testing wheat for deoxynivalenol (DON) was measured using a 0.454 kg sample, Romer mill, 25 g comminuted subsample, and the Romer Fluoroquant analytical method. The total variability was partitioned into sampling, sample preparation, and analytical variability components. Each variance component was a function of the DON concentration and equations were developed to predict each variance component using regression techniques. The effect of sample size, subsample size, and number of aliquots on reducing the variability of the DON test procedure was also determined. For the test procedure, the coefficient of variation (CV) associated with testing wheat at 5 ppm was 13.4%. The CVs associated with sampling, sample preparation, and analysis were 6.3, 10.0, and 6.3%, respectively. For the sample variation, a 0.454 kg sample was used; for the sample preparation variation, a Romer mill and a 25 g subsample were used; for the analytical variation, the Romer Fluoroquant method was used. The CVs associated with testing wheat are relatively small compared to the CV associated with testing other commodities for other mycotoxins, such as aflatoxin in peanuts. Even when the small sample size of 0.454 kg was used, the sampling variation was not the largest source of error as found in other mycotoxin test procedures.

Guest edited as a special report on "Sampling Agricultural Commodities for Aflatoxins" by Thomas B. Whitaker.

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Deoxynivalenol, sometimes called DON or vomitoxin, is a naturally occurring mycotoxin produced by several fungal species in the genus *Fusarium* (1). *Fusarium* infects wheat and other small grains such as barley and corn. DON toxicosis is associated with feed refusal, vomiting, and reproductive problems in animals (2). DON is most often associated with cool and wet environmental conditions where *Fusarium* tends to thrive.

The U.S. Food and Drug Administration (FDA) currently has the following DON advisory limits: one part per million (ppm) on consumer-ready wheat products such as flour and bran; 10 ppm on grains destined for ruminating beef and feedlot cattle (over 4 months of age), swine, and chickens; and 5 ppm on grain and grain products for all other animals (3). Advisory limits were established by FDA as guidelines for industry to use as a standard in the management and reduction of DON contamination in grain and grain products and does not automatically mean FDA will take regulatory action if limits are exceeded. Because of the FDA advisory limit, grain producers and processors test grains for DON to determine if levels are less than the FDA advisory limits. The USDA Federal Grain Inspection Packers and Stockyard Administration (GIPSA) provides official DON testing programs for grain producers and processors.

The test procedure used to measure DON in small grains is similar to that used to measure other mycotoxins in other grains. The test procedure consists of 3 steps: (*1*) the sampling step, where a random sample (test sample) is taken from the lot; (*2*) the sample preparation step, where the entire test sample is comminuted in a mill or grinder and a subsample is removed from the comminuted test sample. Grinding and subsampling are collectively called the sample preparation step; (*3*) the analytical step, where DON in the subsample is solvent extracted, purified, and quantitated.

The variability associated with each of the 3 steps contributes to the total variability associated with the DON test pro-

cedure and makes it difficult to estimate the true DON concentration of a bulk lot with a high degree of confidence. As a result, it is difficult to accurately classify lots into categories required by management strategies and regulatory activities. If the variability of the DON test procedure could be reduced, the lot concentration could be estimated with more confidence, which means that producers and processors would suffer smaller economic losses because fewer lots would be misclassified by the testing program. It is important to evaluate the performance of a DON sampling plan and to design sampling plans that reduce misclassification of producer's lots. The variability and distributional characteristics associated with a DON test procedure need to be determined so that methods to design and evaluate DON sampling plans can be developed.

Previous studies demonstrated that the variability among aflatoxin test results for corn, peanuts, and cottonseed is very large (4–6). However, a recent DON study with wheat (7) indicated that the variability among probe sample concentrations may be much less than among aflatoxin test results for peanuts and other commodities. Less variability among samples with the same number of kernels suggests that the distribution among wheat kernels contaminated with DON may differ from the distribution among peanut kernels contaminated with aflatoxin (8).

A DON test procedure must be designed to have the lowest variability that resources will allow. Therefore, the objectives of this study are to measure the variability of the sampling, sample preparation, and analytical steps of a test procedure used to measure DON in wheat, and to show how to decrease the variability of the test procedure and achieve more precise results. Efforts to describe the distribution among DON test results will be conducted in a separate study.

Experimental

Variability Estimates

The sources of error associated with the DON test procedure are shown in Figure 1. The total variability is a function of the sampling, sample preparation, and analytical variability. Assuming independence among the random errors for each step of the DON procedure, the total variance (σ_t^2) is assumed to be equal to the sum of the sampling variance (σ_s^2), sample preparation variance (σ_{sp}^2), and analytical variance (σ_a^2).

$$\sigma_t^2 = \sigma_s^2 + \sigma_{sp}^2 + \sigma_a^2 \quad (1)$$

The variance model shown in Equation 1 was described in detail by Whitaker et al. (9). It is also assumed that sample selection and sample preparation methods are random in nature and no biases are introduced in the application of the DON test procedure. Experimental estimates of the true variance, σ^2, are denoted by S^2.

Because analytical procedures are required to measure DON in the comminuted subsample, the sampling and sample preparation variances cannot be measured directly, but can be estimated indirectly. Experiments were designed to measure the total variance (S_t^2), combined sample preparation and analytical variance (S_{spa}^2), and the analytical variance (S_a^2). The combined sample preparation and analytical variances (S_{spa}^2) are defined in Equation 2:

$$S_{spa}^2 = S_{sp}^2 + S_a^2 \quad (2)$$

If the total variance, combined sample preparation, and analytical variance are known, then analytical variance, the sampling variance, and sample preparation variance can be determined by subtraction using Equations 1 and 2.

As a result,

$$S_{sp}^2 = S_{spa}^2 - S_a^2 \quad (3)$$

and

$$S_s^2 = S_t^2 - (S_{sp}^2 + S_a^2) \quad (4)$$

or

$$S_s^2 = S_t^2 - S_{spa}^2 \quad (5)$$

Experimental Design

Total variance.—A 20 kg bulk sample was taken from each of 24 commercial lots of wheat suspected of DON contamination and destined for further processing. Each bulk sample was riffle divided into thirty-two 0.454 kg (1 lb) samples. Each 0.454 kg sample was ground with a Romer mill (Romer Labs, Union, MO) with the grinding plates set to give the finest degree of grind. The adjustable gate on the Romer mill was set to automatically give ca 25 g subsample from the 0.454 kg sample during comminution. The DON in the 25 g subsample was extracted and quantitated using the Romer DON FluoroQuant™ (Romer Labs) analytical method with a fluorometer (10). The total variance (S_t^2) associated with testing DON in each of the 24 lots was estimated from the 32 DON measurements per lot. A total of 24 × 32 or 768 samples were analyzed for DON to measure total variability.

Sample preparation plus analytical variance.—Twenty comminuted samples were selected from the 768 comminuted

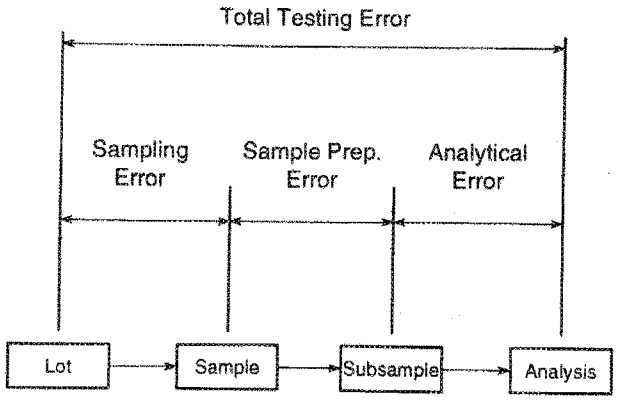

Figure 1. Partitioning the total variance associated with a mycotoxin test procedure into sampling, sample preparation, and analytical variance components.

Table 1. Deoxynivalenol (DON) test results measured in 32 samples of wheat from each of 24 lots; DON test procedure reflects 0.454 kg sample, Romer mill, 25 g subsample, and Romer Fluoroquant analytical method

	Sample																																	
Lot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Mean
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04	0.07	0.08	0.17	0.18	0.02
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.07	0.08	0.16	0.22	0.24	0.25	0.28	0.04
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.05	0.06	0.06	0.12	0.13	0.16	0.16	0.17	0.20	0.29	0.05
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.07	0.10	0.28	0.30	0.38	0.46	0.05
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.05	0.05	0.05	0.08	0.08	0.08	0.08	0.10	0.12	0.12	0.14	0.15	0.16	0.17	0.20	0.30	0.35	0.64	0.09
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.07	0.07	0.13	0.16	0.19	0.21	0.27	0.34	0.41	0.49	0.69	0.10
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.08	0.08	0.12	0.15	0.15	0.16	0.17	0.18	0.19	0.26	0.38	0.54	0.72	0.74	0.13
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.02	0.04	0.05	0.05	0.06	0.06	0.09	0.12	0.12	0.13	0.19	0.19	0.20	0.22	0.44	0.59	0.71	0.79	0.13
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.05	0.06	0.09	0.12	0.13	0.14	0.15	0.17	0.20	0.20	0.22	0.23	0.24	0.25	0.31	0.32	0.33	0.38	0.40	0.52	0.53	0.16
21	0.00	0.00	0.00	0.00	0.06	0.08	0.09	0.13	0.14	0.15	0.17	0.18	0.19	0.20	0.24	0.25	0.27	0.28	0.29	0.29	0.29	0.29	0.29	0.31	0.36	0.46	0.54	0.56	0.58	0.58	0.64	0.65	0.27
24	0.89	1.30	1.30	1.50	1.50	1.60	1.60	1.70	1.70	1.70	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.90	2.00	2.00	2.00	2.00	2.10	2.10	2.20	2.20	2.20	2.20	2.30	2.30	2.70	2.70	1.88
2	1.40	1.50	1.70	1.70	1.80	1.80	2.00	2.00	2.10	2.10	2.10	2.20	2.20	2.20	2.30	2.30	2.30	2.30	2.40	2.40	2.40	2.50	2.50	2.60	2.60	2.70	2.70	2.70	2.80	2.90	3.10	3.60	2.31
4	2.20	2.20	2.30	2.50	2.50	2.60	2.60	2.70	2.70	2.70	2.70	2.80	2.80	2.80	2.80	2.80	2.90	2.90	3.00	3.00	3.10	3.10	3.10	3.10	3.10	3.30	3.30	3.40	3.50	3.60	3.70	4.40	2.94
23	2.20	2.60	2.70	2.80	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90	3.00	3.00	3.00	3.00	3.10	3.10	3.10	3.20	3.30	3.40	3.40	3.50	3.60	3.70	3.70	3.80	3.80	3.80	3.90	4.10	3.19
3	2.70	2.80	2.90	3.00	3.30	3.30	3.40	3.40	3.50	3.50	3.50	3.50	3.60	3.60	3.60	3.60	3.60	3.70	3.70	3.70	3.70	3.80	3.90	3.90	4.00	4.10	4.20	4.30	4.30	4.40	4.40	4.70	3.68
20	3.30	3.60	3.70	3.70	3.70	3.70	3.80	3.80	3.80	3.90	3.90	4.10	4.10	4.20	4.20	4.20	4.20	4.30	4.30	4.30	4.30	4.40	4.50	4.50	4.60	4.60	4.60	4.80	5.10	5.20	5.30	6.10	4.28
14	4.10	4.60	4.70	4.90	4.90	5.20	5.20	5.20	5.30	5.30	5.40	5.40	5.40	5.40	5.50	5.60	5.60	5.80	5.80	5.80	5.90	6.00	6.10	6.20	6.20	6.30	6.30	6.60	7.20	7.20	7.30	7.60	5.75
7	4.50	5.20	5.60	5.60	5.60	5.70	5.70	5.80	5.90	5.90	6.00	6.00	6.00	6.00	6.10	6.10	6.10	6.20	6.40	6.50	6.60	6.70	6.70	6.80	6.80	6.80	7.00	7.00	7.00	7.30	7.50	7.90	6.28
8	7.10	7.10	7.20	7.20	7.40	7.50	7.60	7.70	7.80	7.80	7.90	7.90	8.00	8.00	8.10	8.10	8.20	8.20	8.20	8.20	8.30	8.40	8.40	8.70	9.10	9.10	9.30	9.70	9.90	10.00	10.00	10.00	8.32
6	6.40	7.40	7.50	7.70	7.70	7.80	7.90	8.00	8.00	8.00	8.00	8.10	8.10	8.30	8.30	8.50	8.60	8.80	8.80	8.80	8.80	9.00	9.00	9.00	9.10	9.10	9.20	9.40	9.40	9.60	9.80	10.00	8.50
5	7.30	7.50	7.60	7.70	7.70	7.70	7.80	7.90	8.00	8.00	8.10	8.10	8.10	8.10	8.20	8.30	8.50	8.60	8.60	8.70	8.90	8.90	9.00	9.20	9.20	9.20	9.60	9.70	9.70	9.90	10.00	11.00	8.59
1	7.10	7.70	7.70	7.70	7.80	8.10	8.20	8.20	8.20	8.30	8.40	8.40	8.50	8.50	8.50	8.80	8.80	9.00	9.00	9.00	9.00	9.10	9.20	9.30	9.50	9.60	9.60	9.80	9.90	10.00	10.00	10.00	8.78
10	7.90	8.00	8.20	8.40	8.50	8.60	8.80	8.90	8.90	8.90	9.10	9.10	9.10	9.10	9.30	9.40	9.40	9.50	9.50	9.50	9.90	9.90	10.00	10.00	10.00	10.00	10.00	10.00	11.00	11.00	11.00	13.00	9.50
19	12.00	12.00	13.00	13.00	13.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	16.00	16.00	16.00	16.00	17.00	14.38

wheat samples described above to provide samples with a wide range in DON concentration. Eight 25 g subsamples were taken from each of the 20 comminuted samples with a riffle divider. The Romer Fluoroquant method was used to measure DON in 4 aliquots taken from each subsample extract. The combined sample preparation and analytical variance (S^2_{spa}), and the analytical (S^2_a) variance were estimated from the $20 \times 8 \times 4$ or 640 DON measurements in the nested design using SAS procedures (11). Using Equations 3 and 5, the sampling and sample preparation variances were estimated.

The sampling variance, S^2_s, is specific for a 0.454 kg sample; the sample preparation variance, S^2_{sp}, is specific to grinding the sample with a Romer mill and using a 25 g comminuted subsample for extraction; the analytical variance, S^2_a, is specific to the Romer Fluoroquant method (12). DON measurements are reported in ppm.

Results

Total Variance

The 32 DON measurements used to measure total variance are shown in Table 1 for each of the 24 lots. For each lot, DON test results are ranked from low to high. The lot concentrations were estimated by averaging the 32 DON measurements for each lot. The estimated lot concentrations ranged from 0.02 to 14.38 ppm. The 24 lots are also ranked by lot concentration from low to high. With this presentation in Table 1, the range of DON test results for each lot is easily seen. For example, sample test results for lot 21 ranged from 0.00 to 0.65 ppm and the 32 DON sample values averaged 0.27 ppm.

The estimated total variance, coefficient of variation (CV), and DON concentration are shown in Table 2 for each of the 24 lots. The lots are ranked from low to high concentration. A full-log plot of total variance (S^2_t) versus DON concentration (C) is shown in Figure 2. Consistent with other mycotoxin studies, the variability among DON test results appears to increase with DON concentration. Because the plot in Figure 2 is approximately linear in a full-log plot, a regression equation of the form

$$S^2_t = a\, C^b \qquad (6)$$

where a and b are constants determined from the regression analysis was used to relate variance, S^2, to DON concentration, C. From the regression analysis, Equation 6 becomes

$$S^2_t = 0.117\, C^{0.817} \qquad (7)$$

with a correlation coefficient of 0.91 in the log scale. The standard error associated with the exponent (0.817) is 0.035. Regression Equation 7 is also shown in Figure 2 with the variance data.

Sample Preparation Plus Analytical Variance

The data in the nested design were analyzed with an SAS procedure (11) that determines the combined sample preparation and analytical variance (S^2_{spa}) and the analytical variance (S^2_a). The combined sample preparation and analytical variance, analytical variance, and sample concentration are shown in Table 3 for each of the 20 samples. The samples are also ranked from low to high concentration. The combined sample preparation and analytical variance and the analytical variances in Table 3 appear to increase with DON concentration and are plotted versus DON concentration in full-log plots in Figures 3 and 4, respectively. From the regression analysis, the combined sample preparation and analytical variance and the analytical variance equations are shown in Equations 8 and 9, respectively.

$$S^2_{spa} = 0.083\, C^{0.913} \qquad (8)$$

$$S^2_a = 0.028\, C^{0.783} \qquad (9)$$

The correlation coefficients associated with the regression in Equations 8 and 9 are 0.97 and 0.88, respectively. The standard error associated with the exponents in Equations 8 and 9 are 0.056 and 0.097, respectively. Regression Equations 8 and 9 are also shown in Figures 3 and 4, respectively.

Table 2. Total variance and coefficient of variation (CV) associated with measuring deoxynivalenol in wheat lots; test procedure reflects 0.454 kg sample, Romer mill, 25 g subsample, and Romer Fluoroquant analytical method

Lot No.	Lot concentration, ppm	Total variance	CV, %
12	0.02	0.00	261.8
13	0.04	0.01	209.9
17	0.05	0.01	168.1
11	0.05	0.01	231.1
9	0.09	0.02	145.2
16	0.10	0.03	180.9
15	0.13	0.04	161.7
18	0.13	0.04	164.9
22	0.16	0.02	98.3
21	0.27	0.04	72.7
24	1.88	0.15	20.2
2	2.31	0.22	20.1
4	2.94	0.21	15.6
23	3.19	0.19	13.7
3	3.68	0.22	12.9
20	4.28	0.34	13.5
14	5.75	0.66	14.1
7	6.28	0.50	11.2
8	8.32	0.79	10.7
6	8.50	0.62	9.3
5	8.59	0.76	10.2
1	8.78	0.61	8.9
10	9.50	1.05	10.8
19	14.38	1.27	7.9

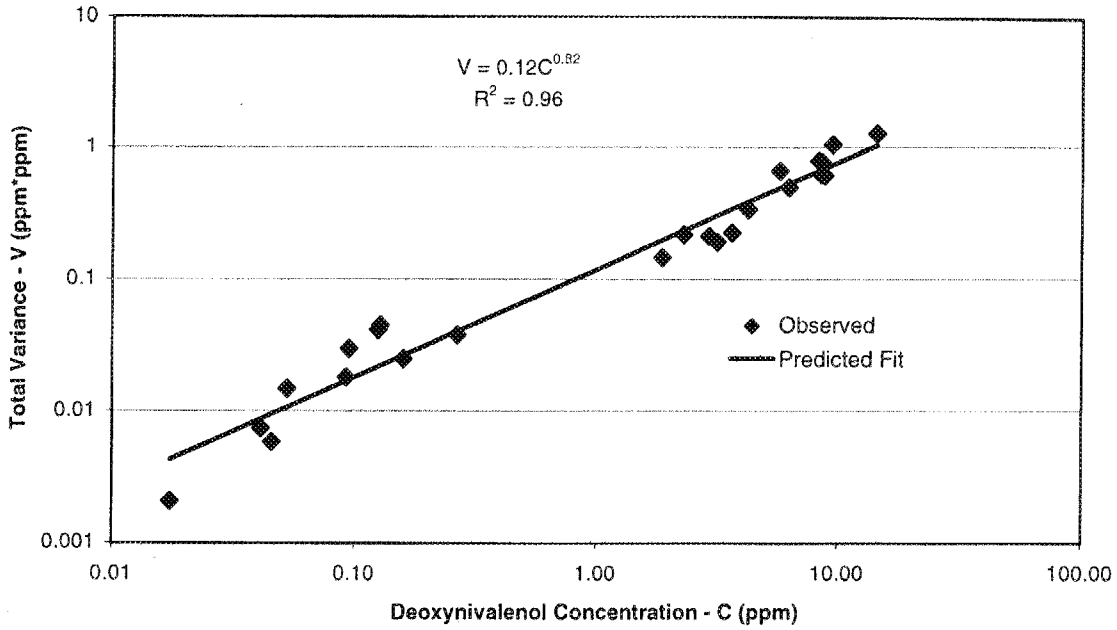

Figure 2. Full-log plot of total variance versus deoxynivalenol (DON) concentration when wheat is tested for DON. DON test reflects 0.454 kg sample, Romer mill, 25 g subsample, and Romer Fluoroquant analytical method.

Table 3. Combined sample preparation and analytical variance and the analytical variance associated with testing wheat samples for deoxynivalenol; test procedure reflects Romer mill, 25 g subsample, and Romer Fluoroquant analytical method

Lot No.	Sample No.	Sample concn., ppm	Combined sample prep. & analytical variance	Analytical variance
11	22	0.03	0.00	0.00
17	22	0.09	0.01	0.00
18	21	0.11	0.02	0.01
24	21	1.84	0.10	0.05
23	22	2.91	0.08	0.02
3	21	3.44	0.12	0.03
3	22	3.84	0.21	0.16
14	22	6.12	0.40	0.07
7	21	6.87	0.31	0.03
3	8	7.59	0.92	0.20
6	22	8.03	0.41	0.07
5	22	8.32	0.98	0.48
1	23	8.64	0.69	0.07
10	23	9.94	1.19	0.38
19	22	12.41	0.88	0.36
19	14	12.78	1.32	0.18
19	15	13.75	0.95	0.46
19	7	13.91	1.07	0.22
19	16	14.75	1.03	0.42
19	21	14.75	0.97	0.29

The exponent values (b coefficient in Equation 6) in Equations 7, 8, and 9 were reasonably close in magnitude. It would be simpler mathematically when adding and subtracting Equations 7, 8, and 9 to obtain sampling and the sample preparation variances, if all exponents in Equations 7, 8, and 9 were the same value. Based upon the similarity of the estimated exponent values in Equations 7, 8, and 9, and the standard error associated with each estimate, it was decided to fit the total, combined sample preparation and analytical, and analytical variance equations to the data in Tables 1 and 2 and force the b coefficient in Equation 6 to be the same value for all 3 regression equations. The resulting regression equations for total, combined sample preparation and analytical, and analytical variances are shown in Equations 10, 11, and 12 respectively:

$$S_t^2 = 0.118\ C^{0.833} \quad (10)$$

$$S_{spa}^2 = 0.092\ C^{0.833} \quad (11)$$

$$S_a^2 = 0.026\ C^{0.833} \quad (12)$$

Using Equations 3 and 4, the sample preparation variance and the sampling variance were estimated by subtraction and are given by Equations 13 and 14, respectively.

$$S_{sp}^2 = 0.066\ C^{0.833} \quad (13)$$

$$S_s^2 = 0.026\ C^{0.833} \quad (14)$$

The variances in Equations 12, 13, and 14, are specific for the DON test procedure used in this study (0.454 kg sample of wheat, Romer mill, 25 g comminuted subsample, and Romer Fluoroquant analytical method).

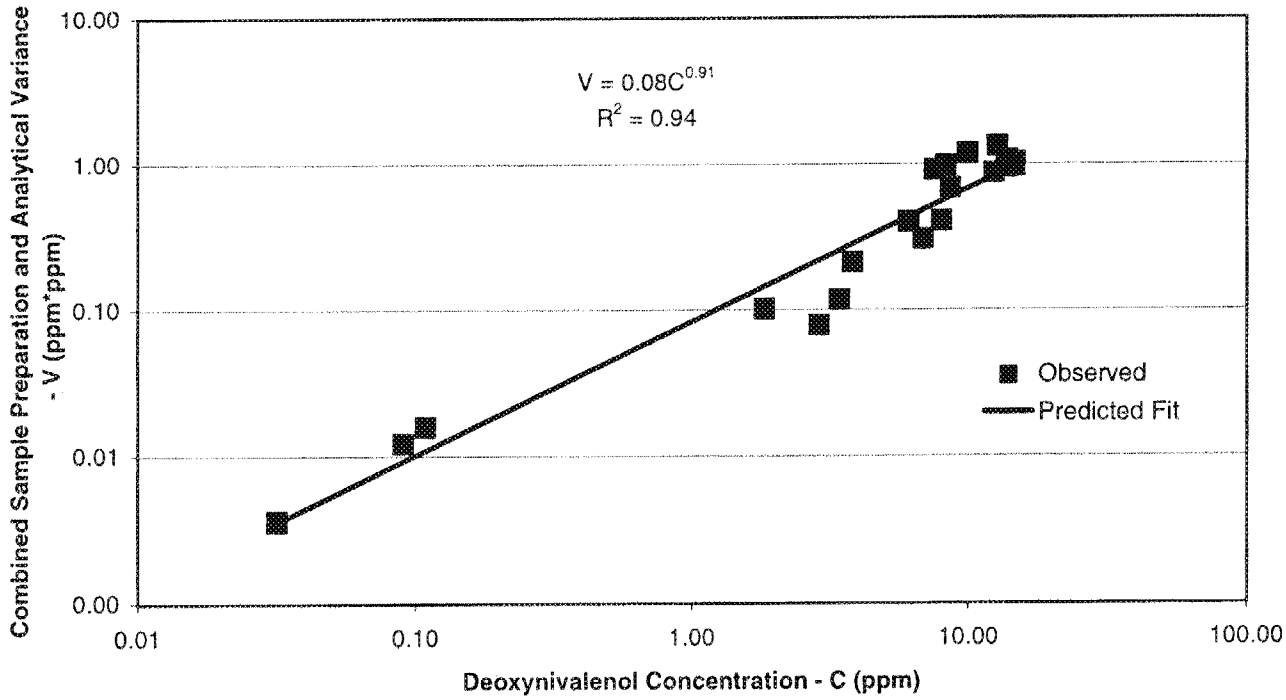

Figure 3. Full-log plot of combined sample preparation and analytical variance versus deoxynivalenol (DON) concentration when wheat is tested for DON. Variance reflects Romer mill, 25 g subsample, and Romer Fluoroquant analytical method.

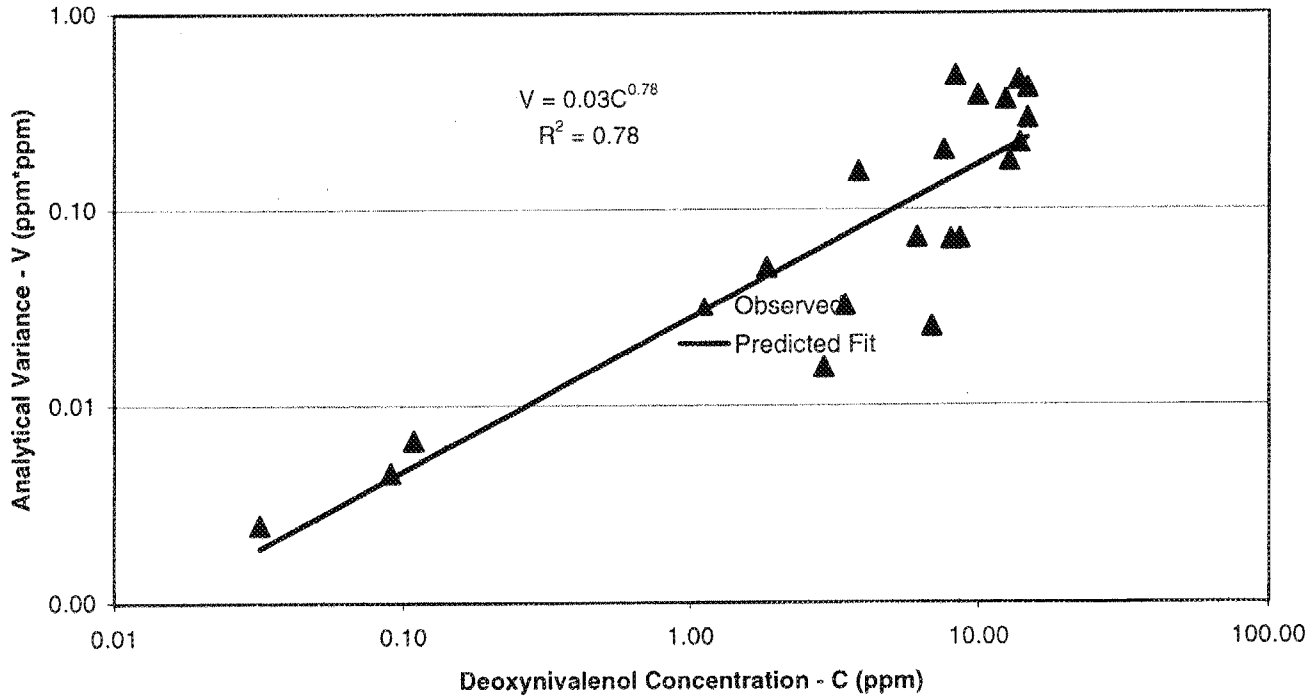

Figure 4. Full-log plot of analytical variance versus deoxynivalenol (DON) concentration when wheat is tested for DON. Variance reflects Romer Fluoroquant analytical method.

Reducing Variability

The total variance associated with the DON test procedure can be reduced by increasing sample size or the number of 0.454 kg sampling units, increasing subsample size or the number of 25 g subsampling units taken from the Romer mill, and analyzing multiple aliquots for DON by the Fluoroquant method. Equations 12, 13, and 14 can be modified to predict the effects of sample size, subsample size, and number of aliquots on reducing variability associated with each step of the DON testing procedure:

$$S_s^2 = \left(\frac{0.454}{ns}\right) 0.026\ C^{0.833} \quad (15)$$

$$S_{sp}^2 = \left(\frac{25}{nss}\right) 0.066\ C^{0.833} \quad (16)$$

$$S_a^2 = \left(\frac{1}{na}\right) 0.026\ C^{0.833} \quad (17)$$

where *ns* is the sample size in kg, *nss* is the subsample size in g when the sample is comminuted in a Romer mill, and *na* is the number of aliquots analyzed by the Romer Fluoroquant analytical method. Equation 15 was derived from the principle that if you double sample size (or double number of sample units), the sample variance is cut in half. This principle also applies to subsample size and number of aliquots quantitated (Equations 16 and 17). The total variance associated with the DON test procedure for a given sample size (*ns*), subsample size (*nss*), and number of aliquots (*na*) can be determined by adding Equations 15, 16, and 17 together.

$$S_t^2 = \left(\frac{0.454}{ns}\right) 0.026\ C^{0.833} + \left(\frac{25}{nss}\right) 0.066\ C^{0.833} + \left(\frac{1}{na}\right) 0.026\ C^{0.833} \quad (18)$$

For the DON test procedure used in this study, the variance and CV associated with a DON concentration of 5 ppm is shown in Table 4. For example, the CVs associated with sampling, sample preparation, and analysis are 6.3, 10.0, and 6.3%, respectively. The CV of 13.4% shown in Table 4 for the DON test procedure is relatively low compared to testing other commodities for other mycotoxins such as testing peanuts for aflatoxin (CV = 200%; 5). Variances in Table 4 associated with each step of the DON test procedure suggest that the best use of resources would be to increase the subsample size from 25 to 50 g, which would reduce the sample preparation variance from 0.252 to one-half the original value or to 0.126, which would make the sample preparation variance about the same magnitude as the sampling and analytical variability. The CV associated with a DON test procedure using a 0.454 kg sample, 50 g subsample comminuted in the Romer mill, and analyzing one aliquot by the Romer Fluoroquant method is shown over a range of DON concentrations in Figure 5. Because a different cost is associated with methods to reduce the variability of each step of the DON test procedure, it is important to consider cost as well as the expected reduction in variability when designing a DON test procedure.

Table 4. Variance and coefficient of variation (CV) associated with the deoxynivalenol (DON) test procedure using a single 0.454 kg sample, Romer mill, 25 g comminuted subsample, and a single aliquot quantitated by the Romer Fluoroquant method for a DON concentration of 5 ppm

Variability measure	Sampling	Sample preparation	Analysis	Total
Variance	0.099	0.252	0.099	0.450
CV, %	6.3	10.0	6.3	13.4
Variance ratio, %[a]	22.0	56.0	22.0	100.0

[a] Ratio of variance = sampling/total; sample preparation/total analysis/total.

The total variance, calculated from Equation 18, can be used to estimate the range of DON test results expected when a contaminated wheat lot is tested. The total variance associated with the DON test procedure described in Table 4 for a lot at 5 ppm is 0.450 or a CV of 13.4%. A variance of 0.450 suggests that replicate DON test results on a lot at 5 ppm will vary from $5 - (1.96 \times 0.671)$ to $5 + (1.96 \times 0.671)$ or 5 ± 1.31 or from 3.69 to 6.31 ppm 95% of the time. This calculation assumes normal distribution of DON test results, which may or may not be the case. Studies with other mycotoxins suggest that the distributions are positively skewed (8, 12). DON test results in Table 1 suggest that the distribution among DON test results for a given lot is skewed. Table 1 shows that for a given lot, more than 50% of the DON test results are below the average of the 32 DON test results. This is particularly true for lots with low DON concentrations. Further studies are required to describe the distributional characteristics of DON test results for wheat.

Summary

For a 0.454 kg sample, Romer mill, 25 g subsample, and the Romer Fluoroquant analytical method, the coefficient of variation associated with testing wheat for DON was about 13.4% at 5 ppm, which is relatively low compared with other mycotoxin test procedures and other commodities. The CV associated with taking a 0.454 kg sample was 6.3%, which is approximately the same magnitude as the CV associated with sample preparation and analysis. It is assumed that sampling variability is solely due to the distribution among contaminated kernels and that there are no biases associated with the sample selection process. The small variability associated with the sampling step (relative to other mycotoxins and other commodities) is due in part to the kernel count of wheat per unit mass (about 30 kernels per gram). The kernel count per unit mass for wheat is about 10 times larger than that for shelled corn and 30 times larger than that for shelled peanuts.

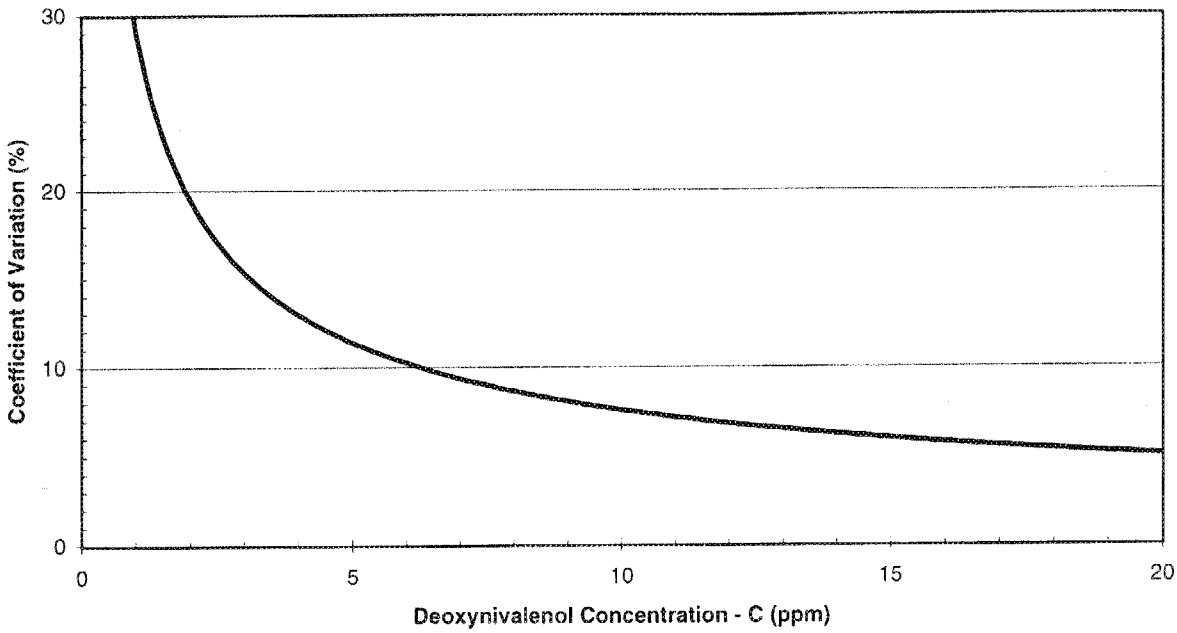

Figure 5. Coefficient of variation versus deoxynivalenol (DON) concentration when wheat is tested for DON. DON test reflects 0.454 kg sample, Romer mill, 50 g subsample, and Romer Fluoroquant analytical method.

The smaller variability also suggests that unlike aflatoxin, a larger percentage of kernels is contaminated, and the distribution of contamination among wheat kernels may be less skewed than for aflatoxin. Studies are currently being conducted to find a suitable theoretical distribution to accurately simulate the observed distribution among DON test results taken from the same lot of wheat. The variability and distributional information will be combined and methods will be developed to evaluate the performance of DON sampling plans for wheat.

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