Variability Associated with Testing Cottonseed for Aflatoxin[1]

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ABSTRACT

The sampling, subsampling, and analytical variance associated with testing cottonseed for aflatoxin were estimated by use of 4.54 kg samples, 100 g subsamples, and the Velasco method of analysis. Regression analysis indicated that each of the above variance components is a function of the concentration of aflatoxin in the populations tested. Functional relationships are presented to determine the sampling, subsampling, and analytical variance for any size sample, subsample, and number of analyses.

INTRODUCTION

Before aflatoxin concentrations can be accurately estimated in cottonseed lots, the variability associated with each stage of the aflatoxin testing procedure must be evaluated. In the testing of cottonseed lots for aflatoxin, a sample of cottonseed is drawn from the lot, the sample is dehulled, the kernels are comminuted in a mill, and a subsample is analyzed for aflatoxin. As illustrated in Figure 1, the total error associated with aflatoxin test results is composed of at least three error components: sampling, subsampling, and analysis. An aflatoxin test result, $\bar{x}$, may be represented as follows:

$$x = \mu + \alpha + \beta + \gamma \quad (1)$$

where μ = the true aflatoxin concentration of the population, α = random error due to sampling with expected value zero and variance $\sigma^2_{\bar{x}(s)}$, β = random error due to subsampling with expected value zero and variance $\sigma^2_{\bar{x}(ss)}$, and γ = random error due to analysis with expected value zero and variance $\sigma^2_{\bar{x}(a)}$.

The notation $\sigma^2_{\bar{x}}$ (neglecting subscripts, s, ss, and a for component identification) indicates the variance of a population of $\bar{x}$ values obtained by sampling a parent population of individual items where variance among the population items is σ^2. The variance σ^2, by definition, is related to $\sigma^2_{\bar{x}}$ by the following equation:

$$\sigma^2_{\bar{x}} = \sigma^2/n \quad (2)$$

[1]Paper 4821 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, NC 27607.

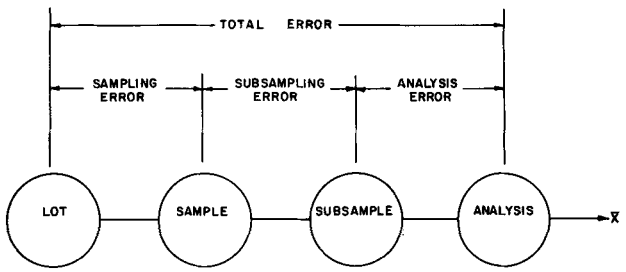

FIG. 1. Typical steps used to estimate the aflatoxin concentration $\bar{x}$ and the associated variance components.

where n is the number of individual items drawn from the population for which $\bar{x}$ is evaluated. By assuming both stochastic and functional independence among the random errors in equation 1, the following variance relationship is obtained:

$$\sigma^2_{\bar{x}(t)} = \sigma^2_{\bar{x}(s)} + \sigma^2_{\bar{x}(ss)} + \sigma^2_{\bar{x}(a)} \quad (3)$$

where $\sigma^2_{\bar{x}(t)}$ is the total variance associated with the aflatoxin test result.

The objective of this study was to quantify empirically the sampling, subsampling, and analytical variance associated with testing cottonseed for aflatoxin.

EXPERIMENTAL PROCEDURE

Method of Analysis

The total variance $\sigma^2_{\bar{x}(t)}$, combined subsampling and analytical variance $\sigma^2_{\bar{x}(ssa)}$ where

$$\sigma^2_{\bar{x}(ssa)} = \sigma^2_{\bar{x}(ss)} + \sigma^2_{\bar{x}(a)} \quad (4)$$

and the analytical variance $\sigma^2_{\bar{x}(a)}$ were estimated by direct measurements. Once $\sigma^2_{\bar{x}(t)}$, $\sigma^2_{\bar{x}(ssa)}$, and $\sigma^2_{\bar{x}(a)}$ are known, the remaining variance components for sampling $\sigma^2_{\bar{x}(s)}$ and subsampling $\sigma^2_{\bar{x}(ss)}$ were determined indirectly by use of the summation property shown in equations 3 and 4. Estimates of $\sigma^2_{\bar{x}}$ and μ by experimental values are denoted by $s^2_{\bar{x}}$ and $\bar{\bar{x}}$, where $\bar{\bar{x}}$ is the average of observed $\bar{x}$ values.

Cottonseed samples were passed through a Bauer attrition mill with the blades set to crack the hulls of the seed. The seed were then passed over a small beater to separate the kernels from the hulls. Because aflatoxin doesn't contaminate the hulls (1), they were discarded. The cottonseed kernels were comminuted with a mill similar to that used for peanuts in most aflatoxin laboratories (2). A 1/16 in. diameter screen was used in the mill for cottonseed as compared to a 2/16 in. screen typically used for peanuts. A sieve analysis indicated that 99.8% of the comminuted sample passed through a 1410 (No. 14) micron sieve, while 41.7% of the sample passed through a 841 (No. 20) micron sieve. The Velasco method (3) was used for analysis, and fluorescent intensities were quantified by densitometric procedures. All analyses were made in the same laboratory. Kernel counts indicated an average of 19,031 kernels/kg.

Total Variance

The total variance $\sigma^2_{\bar{x}(t)}$ is defined as the variance among aflatoxin determinations on replicated samples from the same lot of cottonseed. The estimated total variance $\sigma^2_{\bar{x}(t)}$ was computed from data obtained in a previous study (4). For that study, 41 "sublots" weighing ca. 90.8 kg (200 lb) each were drawn from 41 commercial lots of cottonseed contaminated with aflatoxin. Each sublot was divided with a riffle divider into 20 samples of ca. 4.54 kg (10 lb) each.

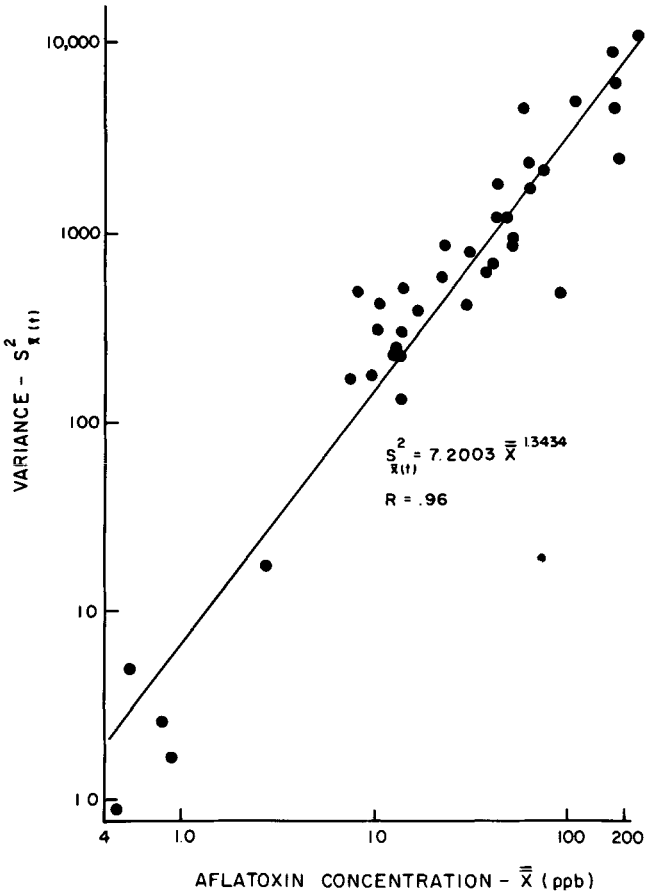

FIG. 2. Relationship between the total variance $s^2_{\bar{x}(t)}$ and the aflatoxin concentration $\bar{\bar{x}}$ in parts per billion.

The hulls were cracked and discarded, leaving ca. 2.27 kg (5 lb) of kernels per sample. Twenty 100 g subsamples, each representing the 2.27 kg (5 lb) sample of kernels, were comminuted in a mill and were analyzed for aflatoxin. The total variance $s^2_{\bar{x}(t)}$ and the average aflatoxin concentration $\bar{x}$ of the 20 test results were calculated for the 41 sublots.

Subsampling and Analytical Variance

The subsampling and analytical variance components $\sigma^2_{\bar{x}(ss)}$ and $\sigma^2_{\bar{x}(a)}$ were estimated jointly by use of a non-balanced two-stange design (5). This design provides estimates of the combined subsampling and analytical variance $\sigma^2_{\bar{x}(ssa)}$ and of the analytical variance $\sigma^2_{\bar{x}(a)}$ from an analysis of variance routine. The subsampling variance $\sigma^2_{\bar{x}(ss)}$ is computed by subtraction.

The subsampling variance $\sigma^2_{\bar{x}(ss)}$ is defined as the variance among the aflatoxin concentrations on replicated subsamples taken from a sample of aflatoxin-contaminated cottonseed; the analytical variance $\sigma^2_{\bar{x}(a)}$ is defined as the variance among aflatoxin determinations on equal aliquots of extract taken from the blender after the extraction step specified in the Velasco method.

In this study, 13 samples (ca. 3.3 kg each) of cottonseed kernels were taken from a contaminated lot. Each sample was divided into 15 subsamples weighing 200 g each. The subsamples were blended 3 min with 1,200 ml of acetone and water. For five of the subsamples, the blended solution was divided into four aliquots which were analyzed for aflatoxin; for each of the remaining 10 subsamples, only one

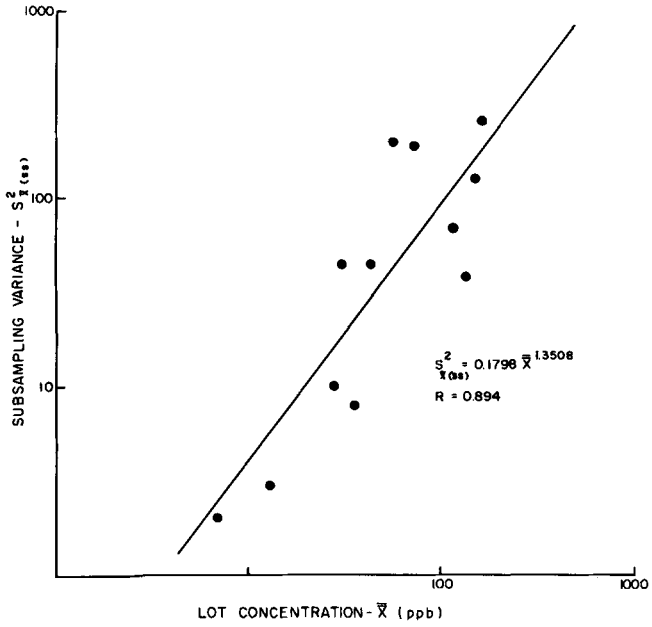

FIG. 3. Relationship between the subsampling variance $s^2_{\bar{x}(ss)}$ and the aflatoxin concentration $\bar{\bar{x}}$ in parts per billion.

aliquot was analyzed. By an analysis of variance of the 30 test results of each sample, the values of $s^2_{\bar{x}(ss)}$, $s^2_{\bar{x}(a)}$, and the aflatoxin concentration $\bar{x}$ were computed. It was assumed that the subsample concentration was equal to the sample concentration. The 13 samples provided 13 estimates of $s^2_{\bar{x}(ss)}$, $s^2_{\bar{x}(a)}$, and $\bar{\bar{x}}$.

RESULTS AND DISCUSSION

Total Variance

The average aflatoxin concentration $\bar{\bar{x}}$ and the total variance $s^2_{\bar{x}(t)}$ for each sublot are shown in Figure 2. The results indicated that the total variance may be a function of the aflatoxin concentration $\bar{\bar{x}}$. Cochran (6) reported that studies suggested the relationship to be

$$s^2_{\bar{x}} = A\bar{\bar{x}}^B \tag{5}$$

where A and B are constants independent of $\bar{\bar{x}}$. If equation 5 is appropriate, the plot in Figure 2 should look approximately linear on a ln-ln graph. The regression equation for the plot in Figure 2 is

$$\ln\left[s^2_{\bar{x}(t)}\right] = 1.9741 + 1.3434 \ln\left[\bar{\bar{x}}\right] \tag{6}$$

with a correlation coefficient of 0.961 in the ln scale. Equation 6 can be transformed to give

$$s^2_{\bar{x}(t)} = 7.2003\ \bar{\bar{x}}^{1.3434} \tag{7}$$

A plot of regression equation 7 is also shown in Figure 2.

Subsampling Variance

The average aflatoxin concentration $\bar{x}$ and the subsampling variance $s^2_{\bar{x}(ss)}$ for each of the 13 samples are plotted in Figure 3. The increase in $s^2_{\bar{x}(ss)}$ with $\bar{\bar{x}}$ indicates that $s^2_{\bar{x}(ss)}$ may be a function of $\bar{x}$ as was $s^2_{\bar{x}(t)}$. Regression equation 5 was fitted to the data by use of ln values. From the regression analysis, the expression

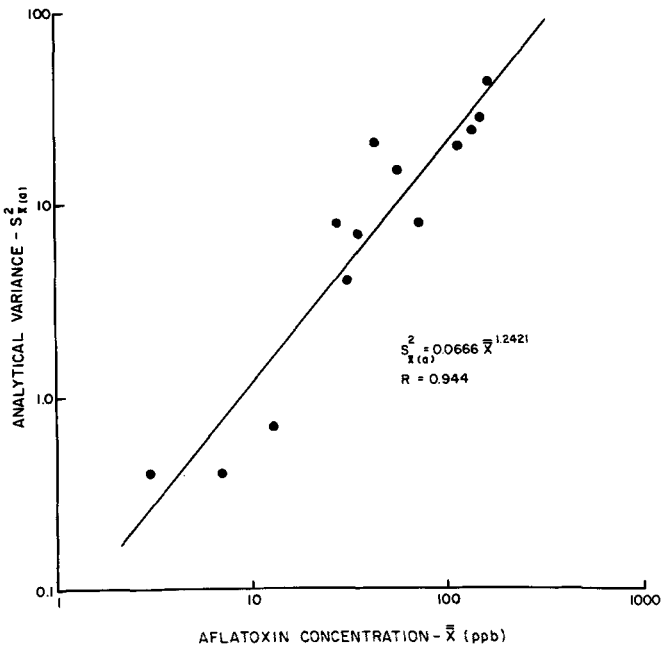

FIG. 4. Relationship between the analytical variance $s^2_{\bar{x}(a)}$ and the aflatoxin concentration $\bar{\bar{x}}$ in parts per billion.

$$\ln s^2_{\bar{x}(ss)} = -1.7195 + 1.3508 \ln[\bar{\bar{x}}] \quad (8)$$

is obtained with a correlation coefficient of 0.894 in the ln scale. Equation 8 can be transformed to give

$$s^2_{\bar{x}(ss)} = 0.1798\,\bar{\bar{x}}^{1.3508} \quad (9)$$

Figure 3 contains a plot of equation 9.

Analytical Variance

The average aflatoxin concentration $\bar{\bar{x}}$ and the analytical variance $s^2_{\bar{x}(a)}$ for each of the 13 samples are plotted in Figure 4. As for the two previous variances, regression equation 5 was fitted to the data, giving

$$\ln[s^2_{\bar{x}(a)}] = -2.0791 + 1.242.\ \ln[\bar{\bar{x}}] \quad (10)$$

with a correlation coefficient of 0.944 in the ln scale. Equation 10 can be transformed to give

$$s^2_{\bar{x}(a)} = 0.0666\bar{\bar{x}}^{1.2421} \quad (11)$$

Figure 4 contains a plot of equation 11.

Sampling Variability

The sampling variance $s^2_{\bar{x}(s)}$ is evaluated by use of the summation property in equation 4, where $s^2_{\bar{x}(t)}$, $s^2_{\bar{x}(ss)}$, and $s^2_{\bar{x}(a)}$ are given by equations 7, 9, and 11, respectively. The subsampling variance, equation 9, reflects 200 g subsamples while the total variance reflects 100 g subsamples. Therefore, equation 9 must be adjusted to reflect 100 g subsamples before use of the summation property. By definition, doubling the subsample size halves the variance. Therefore, equation 9 becomes

$$s^2_{\bar{x}(ss)} = 0.3596\,\bar{\bar{x}}^{1.3508} \quad (12)$$

which represents the subsampling variance for 100 g subsamples. The sampling variance $s^2_{\bar{x}(s)}$ is computed to be

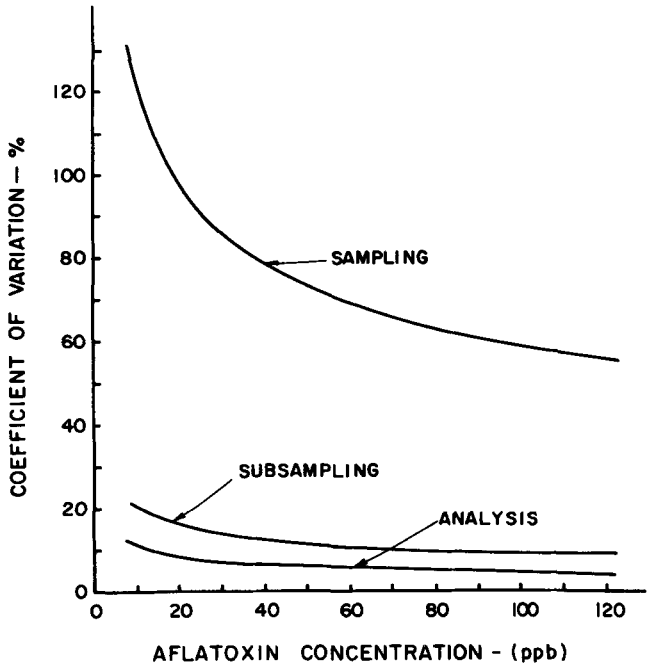

FIG. 5. Coefficient of variation characterizing sampling, subsampling, and analysis is shown as a function of aflatoxin concentration μ in parts per billion.

$$s^2_{\bar{x}(s)} = 7.2003\,\bar{\bar{x}}^{1.3434} - 0.3596\,\bar{\bar{x}}^{1.3508} - 0.0666\,\bar{\bar{x}}^{1.2421} \quad (13)$$

The variances can be used to determine the coefficient of variation (CV) associated with each step of the aflatoxin test procedure. The CV in percent is

$$CV = 100\,\sigma_{\bar{x}} / \mu \quad (14)$$

where $\sigma_{\bar{x}}$ is the standard deviation or the square root of the variance $\sigma^2_{\bar{x}}$. Substituting the square root of the appropriate variance in equation 9, 11, or 13 into equation 14 provides the CV associated with each step of the aflatoxin testing procedure. The coefficients of variation for sampling, subsampling, and analysis are shown in Figure 5.

The total variance associated with aflatoxin test results can be reduced by reducing one or more of the variance components in equation 3. One way the variance components can be reduced is to increase the quantity of material inspected. The effect of sample size upon the variance of the mean of n items $\sigma^2_{\bar{x}}|_n$ can be demonstrated by use of equation 2. Since the variance among the individual items of a population σ^2 is a fixed parameter, the variance of the mean of n items $\sigma^2_{\bar{x}}|_n$ varies inversely with the number of items drawn from the population. By evaluating σ^2 where

$$\sigma^2 = n\sigma^2_{\bar{x}}|_n \quad (15)$$

the variance of the mean of any quantity of material N can be estimated from the following expression

$$\sigma^2_{\bar{x}}|_N = (n/N)\sigma^2_{\bar{x}}|_n \quad (16)$$

From equation 16, the sampling variance for any given sample size ns becomes

$$\sigma^2_{\bar{x}(s)}|_{ns} = (2270/ns)\sigma^2_{\bar{x}(s)}|_{2270} \quad (17)$$

where $\sigma^2_{\bar{x}(s)}|_{ns}$ is the sampling variance for a sample of size

ns; $\sigma^2_{\bar{x}(s)}|_{2270}$ is the sampling variance of a 2,270 g sample given by equation 13. Therefore,

$$\sigma^2_{\bar{x}(s)\ ns} = (2270/ns)\ (7.2003\mu^{1.3434} - 0.3596\mu^{1.3508} - 0.0666\mu^{1.2421}) \quad (18)$$

A similar expression exists for the subsampling variance

$$\sigma^2_{\bar{x}(ss)}|_{nss} = (200/nss)\ \sigma^2_{\bar{x}(ss)}|_{200} \quad (19)$$

where $\sigma^2_{\bar{x}(ss)}|_{nss}$ is the subsampling variance for a given subsampling size nss; $\sigma^2_{\bar{x}(ss)}|_{200}$ is the subsampling variance associated with a 200 g subsample given by equation 9. Therefore,

$$\sigma^2_{\bar{x}(ss)}|_{nss} = (200/nss)\ (0.1798\mu^{1.3508}) \quad (20)$$

Since the subsample size may easily approach the size of the population (sample) from which it was taken, a finite population correction term is added to equation 20, giving

$$\sigma^2_{\bar{x}(ss)}|_{nss} = [35.96/nss\ (1 - (nss/ns)]\ \mu^{1.3508} \quad (21)$$

The analytical variance given by equation 11 is for the analysis of a 50 ml aliquot carried through all steps in the Velasco procedure subsequent to the blending step. Doubling the number of analyses (carrying two aliquots through the Velasco procedure) would halve the analytical variance $\sigma^2_{\bar{x}(a)}$. The effect of the number of analyses upon the analytical variance is given by the following equation:

$$\sigma^2_{\bar{x}(a)}|_{na} = (1/na)\ \sigma^2_{\bar{x}(a)}|_1 \quad (22)$$

where $\sigma^2_{\bar{x}(a)}|_{na}$ is the analytical variance for na analyses; $\sigma^2_{\bar{x}(a)}|_1$ is the analytical variance given by equation 11 associated with one analysis. Equation 22 becomes

$$\sigma^2_{\bar{x}(a)}|_{na} = (0.0666/na)\ \mu^{1.2421} \quad (23)$$

By adding equations 18,21, and 23 together, the total variance can be estimated for any given sample size, subsample size, and number of analyses.

The variances estimated in this study reflect the following: (a) 2,270 g sample averaging 43,200 kernels, (b) subsampling mill used to comminute the samples, (c) 200 g subsample, (d) Velasco method of analysis with densitometric quantification of aflatoxin intensities, and (e) use of one particular laboratory for analyses.

Even with clearly defined aflatoxin test procedures, variability in test results among different laboratories may be high (7-9). Since all measurements in this study were made in the same laboratory, the variances do not reflect differences in laboratories and may not be representative of all laboratories.

Since the experimentally determined variance components appear to be functionally related to the aflatoxin concentration, the assumption made concerning the nature of the random errors α, β, and γ in equation 1 may be open to questions. Other statistical models, such as the multiplicative model, have been investigated but have not provided a workable alternative.

However, the variance relationships presented in this article should indicate major sources of error in testing cottonseed for aflatoxin and provide insights concerning ways to reduce the total variability.

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[Received November 15, 1975]