Aflatoxin in White Corn Under Loan. V. Aflatoxin Prediction from Weight Percent of Bright Greenish-Yellow Fluorescent Particles

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ABSTRACT

Prediction equations for total aflatoxin from weight percent of bright greenish-yellow fluorescent (BGYF) particles and kernels of unground corn were dependent on originating farm. Ratios of G_1 to B_1 and B_2 to B_1 indicated differences in fungal populations between farms. Based on farm-to-farm differences in fungal contamination, differences in prediction models, and the imprecision of estimated aflatoxin level, BGYF is unsatisfactory as a precise quantitative predictor of aflatoxin level over a wide area. Differences in fungal metabolities between farms appear to be the major problem in developing a widely applicable procedure.

Bright greenish-yellow fluorescence (BGYF) has been used as a qualitative indicator of *Aspergillus flavus* Link ex Fries infection and possible aflatoxin contamination of corn (Fennell et al 1973; Rambo et al 1976; Shotwell et al 1972, 1975a). BGYF also has been used as a presumptive test for aflatoxin in corn marketing channels with some reservation concerning usefulness (Anonymous 1972, Lillehoj et al 1976a, Muhm and Jacobson 1975).

Aflatoxin surveys have shown BGYF associated with all aflatoxin-positive samples, but only half of the BGYF positives were confirmed to have aflatoxin at the level of 10 ppb or more (Lillehoj et al 1975a). Aflatoxin (>2 ppb) was detected in 152 samples, 51%, whereas 73% of the samples showed BGYF in freshly harvested South Carolina corn (Lillehoj et al 1975b). A quantitative relationship between percent of ears showing BGYF and log (B_1 +1), where B_1 is the ppb aflatoxin B_1, was developed based on *A. flavus*-inoculated ears from field experiments in Florida and South Carolina (Lillehoj et al 1976b). The relation differed between the two states.

Shotwell et al (1975a) studied the occurrence of aflatoxin and BGYF in 10-lb unground corn samples. BGYF particle counts were observed in unit intervals of 0–20 and greater than 20. Of 1,283 samples, 569 contained at least one BGYF-positive particle. Of these, 55% had measurable aflatoxin. For samples containing more than 20 positive particles, 94% were aflatoxin-positive; 12% of the BGYF-negative samples were aflatoxin-positive (1–3 ppb).

These results suggest the possible development of a precise prediction of aflatoxin based on BGYF. However, differences in growing conditions, in infectivity of the *Aspergillus* strains, in aflatoxin production between strains, and in the level of contamination can contribute to inconsistent results (Hara et al 1973, Hesseltine et al 1976, Northolt et al 1977, Shotwell 1975b). In some cases a combination of *A. flavus* and *A. parasiticus* Speare is indicated by the observation of aflatoxin G_1, which is not produced by *A. flavus*. Calvert et al (1978) described the production of aflatoxins B_1 and G_1 and the association with inocula prepared with different proportions of spores of the two species. Variation in the G_1/B_1 ratio was correlated with ratios of *A. flavus* to *A. parasiticus* spores in the inocula used to inject corn ears.

In 1973, with the cooperation of the Agricultural Stabilization and Conservation Service, the Northern Regional Research Center determined the aflatoxin content of truckloads of white corn delivered at Diehlstadt, MO. These data provide the basis for a quantitative estimate of aflatoxin. Using the weight of corn particles demonstrating BGYF, the objective was to examine the possibility of precise prediction of aflatoxin.

Quantitative models for predicting aflatoxin from BGYF were tested, and possible explanations for wide variation in results were examined.

METHODS

Samples were taken from truckloads of CCC white corn delivered at an elevator in southeast Missouri. They were identified by county of origin, a farm loan number, and a truckload number. Corn, as sampled, could represent a single field or a mixture of several different fields if corn from the same farm had been combined for storage and handled again at delivery.

One probe sample of about 10 lb was divided into 5-lb aliquots using a Boerner divider. The weight in grams of BGYF particles in one of these 5-lb unground samples was then determined by the Missouri State Inspection Service (Shotwell et al 1975a and b). Our data are based on an unground sample, but the current recommendation is to coarse grind the sample. Of course, cracking increases the problems of obtaining the BGYF weight (Lillehoj et al 1976c), since BGYF particles often disintegrate into many small particles. The total sample weight was also determined. A second 10-lb sample was taken with a continuous sampler as the truck was unloaded. The particle count (0 to >20) data discussed earlier (Shotwell et al 1975a) was determined. This 10-lb sample was then

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ground and a 50-g portion was assayed for aflatoxins B_1, B_2, G_1, and G_2 by the CB method (Shotwell et al 1975b).

For each truck, the data were the weight percent BGYF based on an unground 5-lb sample (measurement X) and the total aflatoxins estimated from analysis of a 50-g subsample of corn from a 10-lb ground sample (measurement Y). The relative standard deviation for B_1 based on 52 pairs of subsamples from 52 different 10-lb samples was 37%. When both X and Y were zero, the truck was omitted from further consideration.

The data for X and Y were used to determine constants in three models for estimating aflatoxin from percent weight of BGYF. Two models were linear. One used a straight line through the origin; the other used a straight line through a nonzero intercept. In the third model, which was exponential, a nonlinear estimation procedure was used to avoid the problem of defining the logarithm of zero values. The models are simple and consistent with procedures for establishing a standard prediction equation for an assay method. Computations were made for each farm, with the number of observations depending on the number of truckloads delivered. Standard statistical analysis and a nonlinear model-fitting subroutine were used for computation.

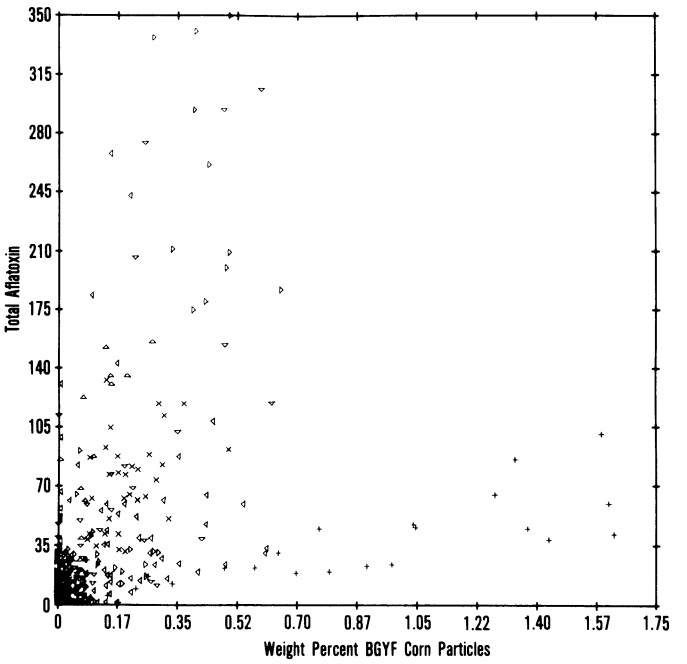

Fig. 1. Association of total aflatoxin to weight percent bright greenish-yellow fluorescent (BGYF) corn particles for 6 counties. + = county 31, × = county 69, > = county 133, ∨ = county 143, < = counties 155 and 201, ∧ = county 207.

TABLE I
Distribution of Test Results for Truck Samples Examined for BGYF[a] and Aflatoxin

	BGYF Result			
	+	+	−	
	Aflatoxin Result			
County	+ (%)	− (%)	+ (%)	No. of Samples
1	72	16	12	32
2	80	13	7	46
3	58	27	15	110
4	42	13	45	119
5	46	22	32	133
6	57	7	36	42
Overall percent	54	18	28	
Number of samples	259	88	135	482

[a]BGYF = bright greenish-yellow fluorescence.

RESULTS

The distribution by county of test results from 482 truckloads positive for BGYF and/or aflatoxin (X and Y measurements) is shown in Table I. Clearly, there is wide variation between geographic areas in association of BGYF (from the unground 5-lb samples) with positive aflatoxin (from chemical assay of 10-lb samples). Conversely, a negative BGYF result was associated with a positive aflatoxin assay in 7–45% of the loads within a county. There is an internal check on the BGYF data, since both 5-lb and 10-lb samples were examined. A total of 347 of the 5-lb samples were BGYF-positive, whereas 569 of the corresponding 10-lb samples were BGYF-positive. This difference is to be expected with a doubling of sample size. The probability of positive BGYF with the 5-lb sample is 0.27 (347/1,283). If the sample size is doubled, the probability of a positive is $1 - (1 - 0.27)^2 = 0.46$. The observed proportion of positives with the 10-lb sample was 0.44 (569/1,283). The two samples were examined at two different locations, and the BGYF data on the 10-lb sample was based on coarsely ground material. The agreement in the observed proportion of BGYF between the two series appears to be satisfactory.

A plot of total aflatoxin (Y) vs percent BGYF (X) for all positive trucks is shown in Fig. 1. Data are plotted with different symbols for each county. No single equation relates BGYF and aflatoxin level. Data from county 31 show a fairly consistent trend, with points (+) representing 27 truckloads from one farm in the county. For the other counties there is no consistent relation.

Three models

$$Y = RX \quad (1)$$
$$Y = CX^D \quad (2)$$
$$Y = A + BX \quad (3)$$

were examined for predicting aflatoxin (Y) based on the percent BGYF (X). Values for R, C, D, A, and B were estimated by least squares methods. Data from 482 truckloads were grouped on the basis of farm, with 59 farms showing one or more loads either with BGYF-positive or aflatoxin-positive samples. This grouping was used to examine farm-to-farm differences.

A summary of results for 33 farms where five or more truckloads were delivered is shown in Table II. The mean levels by farm and the simple linear correlation of BGYF and total aflatoxin (equation 3) are shown in columns 3 to 5. For 12 of 33 farms the correlation was significant, and constants A and B in the estimating equation (3) are shown. For comparison, the slope of the equation for those cases where the correlation was not significant is displayed. Variation between slopes associated with different farms was highly significant. Results of equations 1 and 3 were similar. Values for R ranged from 0 to 1915 with an overall mean of 287. For the power model $Y = CX^D$ (equation 2), D ranged from 0 to 2.81. The precision was approximately that of the linear model $Y = A + BX$, so constants for equations 1 and 2 are omitted from the table. The relation between BGYF and aflatoxin is highly dependent on the particular farm. The overall standard deviation of the aflatoxin value (Y) for a fixed BGYF (X) was 29 ppb. However, this value ranged from 5 to 91 depending on the farm. Thus, the approximate 95% limits for predicted aflatoxin would be given by an average factor of at best ±59 but could range from ±10 to ±182 depending on the farm. This variability suggests that an estimate of ppb aflatoxin based on BGYF is too imprecise for practical use.

To explain why farm-to-farm differences occur, the ratio of G_1 to B_1 was investigated. There were 24 samples from eight farms that contained aflatoxin G_1. Number of samples and mean G_1 level are also shown in Table II. The ratio G_1/B_1 showed highly significant variation between farms (Table III). This result suggests that *A. parasiticus* occurs in varied amounts in these selected farms. Also, the standard deviation in G_1/B_1 ratio between trucks was 0.14 (15 d.f.), a value that compares well with precision estimate of 0.13 for the G_1/B_1 ratio in Calvert et al (1978). Presence of G_1 provides strong evidence of *A. parasiticus* contamination since *A. flavus* does not produce G_1. Significant variation in G_1/B_1 ratio suggests varying proportions of the two species.

We also examined the ratio of B_2/B_1 for each truckload. The

number of samples containing both B_1 and B_2 and the mean ratio are shown in Table IV. Variation between farms was highly significant and suggests that differences in the synthesis of B_1 and B_2 are dependent on farm. The ratio varied from 0.090 to 0.216. Many differences exceed the least significant difference conservatively based on 5 values per mean. There was no correlation of this ratio with BGYF results.

DISCUSSION

Three equations were determined for predicting total aflatoxin per sample based on the weight percent of BGYF particles. Highly significant variation in estimated equation constants between farms indicates the difficulty inherent in a prediction process. For each farm the association was positive. Clearly, at a high enough level of BGYF, all predicted aflatoxin levels will be above 20 ppb. Thus, a qualitative prediction based on some minimum BGYF may be feasible. However, precise quantitative estimation of aflatoxin based on BGYF is not feasible. Great diversity is shown between farms. For example, a truckload from one farm yielded no BGYF particles, yet the mean aflatoxin level was 46.9 ppb and coarse grinding of the 10-lb sample did yield BGYF fragments. A total of 14 samples originating at farms 15 and 22 contained no BGYF, yet aflatoxin was observed at mean levels of 20 and 47 ppb, respectively. Based on the coefficients in the equation Y = A + BX, estimates of aflatoxin would range from 38.5 to 1121 times the percent BGYF. Thus, 0.2% BGYF particles in a sample would yield an estimated total aflatoxin of from 7.6 to 224 depending on the originating farm.

Wide differences between farms suggest that BGYF reflects strain and species differences in production of aflatoxin. For farm 7, for example, data yielded a negative slope with the model Y = A + BX. An inspection of the plotted points revealed two clusters at aflatoxin levels of 325 and 190 ppb, with more BGYF at the 190 ppb level. This suggests that within farms different BGYF-to-aflatoxin relations exist. Further evidence suggesting different aflatoxin contamination between farms is provided by examination of G_1/B_1 ratios for 21 samples containing G_1. Highly significant variation in ratios between farms indicates differences in the fungal species that infect the corn. Variation in G_1 production between *A. parasiticus* strains is a possible explanation. B_2/B_1 ratios also varied between farms. Although *Aspergillus* strains were not isolated and identified, evidence of differences in the BGYF vs aflatoxin relation between farms, variation in G_1/B_1 ratios, and the B_2/B_1 ratios strongly suggest fungal heterogeneity between farms.

BGYF as a predictor of aflatoxin level depends on an assumed consistent relation between the two variables. This relation, how-

TABLE II
Summary of BGYF[a]–Aflatoxin Data by Farm

Farm	Number of Samples	Mean % BGYF × 100	Mean Total Aflatoxin	Correlation[b] (r)	$Y^c = A + BX^d$ A	B
1	27	72.31	28.74	0.83**	0.90 +	38.502
2	7	4.85	5.14	0.91**	−3.28 +	173.734
3	30(5)[e]	19.52	63.70(12.4)[f]	0.49**	34.11 +	151.580
4	9	13.27	46.33	0.57		169.21[g]
5	7	3.48	35.14	0.88**	−1.92 +	1065.583
6	36(3)	8.14	9.39(3.3)	0.56**	3.11 +	77.08
7	12(2)	44.42	257.00(8.0)	−0.32		−249.53
8	15	1.47	2.27	−0.48		−114.82
9	10	4.59	13.80	0.51		134.23
10	16	3.53	11.63	0.72**	4.11 +	212.88
11	5	2.38	3.48	−0.57		−89.81
12	7(3)	9.79	60.71(16.7)	0.95**	−49.08 +	1121.88
13	10	3.80	8.90	0.12		37.46
14	15	0.46	12.40	0.05		50.34
15	5	0	20.00	0		0
16	9	4.04	8.22	0.33		70.90
17	6	0.16	8.50	0.42		435.74
18	5(1)	31.46	61.40(4)	0.07		13.69
19	10	20.18	151.80	0.85**	19.38 +	656.34
20	8(5)	17.64	60.38(27.6)	0.97**	10.86 +	280.68
21	10(1)	19.35	33.10(5)	0.60		123.55
22	9	0	46.89	0		0
23	30(1)	20.18	14.80(1)	0.81**	1.36 +	66.63
24	11	6.96	2.09	0.42		16.12
25	23	0.96	12.74	0.17		184.65
26	6	8.85	32.33	0.76		169.55
27	10	12.56	108.90	0.40		692.51
28	12	4.88	14.58	0.66*	7.22 +	150.89
29	7	17.83	24.29	0.72		208.92
30	6	2.47	0	0		0
31	18(3)	7.45	68.11(12.3)	0.86**	22.30 +	614.77
32	12	3.65	13.83	0.44		175.16
33	6	0.54	2.67	0.25		70.47

[a]BGYF = bright greenish-yellow fluorescence.
[b]**Significant at 0.01 level.
*Significant at 0.05 level.
[c]Y = Aflatoxin $B_1 + B_2 + G_1 + G_2$.
[d]X = Weight percent BGYF particles.
[e]Number of positive G_1 samples.
[f]Mean G_1 level.
[g]Slope of equation (nonsignificant correlation).

TABLE III
Mean G_1/B_1 Ratio for Nine Farms Containing G_1 Contamination

Farm	Number of Samples	Mean G_1/B_1 Ratio
3	5	0.18
6	3	0.17
7	2	0.04
12	3	0.24
18	1	0.15
20	5	0.55
21	1	0.63
23	1	0.08
31	3	0.12
Total Samples	24	Mean 0.26
SD = 0.14	(15 degrees of freedom)	
LSD[a] = 0.30		

[a]Least significant difference assuming 2 values per mean.

TABLE IV
Mean B_2/B_1 Ratio for Farms with Five or More Samples B_2-Positive

Farm	Number of Samples	Mean B_2/B_1 Ratio
1	21	.098
3	27	.135
5	7	.186
6	15	.108
7	12	.174
9	6	.176
10	5	.090
12	6	.218
16	5	.148
18	5	.174
19	5	.166
20	8	.166
21	5	.163
22	8	.127
23	18	.106
25	12	.195
27	8	.106
28	8	.204
31	15	.186
32	7	.143
Total Samples	203	Mean .146
SD = 0.079	(209 degrees of freedom)	
LSD[a] = .087		

[a]Least significant difference assuming 5 observations per mean.

ever, depends on the origin of the samples. Variation between farms appears to reflect differences in fungal contamination. Evidence that these differences are real is based on wide variation in estimating equations and aflatoxin ratios between farms. Within a more uniform fungal population, aflatoxin can be predicted from BGYF, as evidenced by significant correlations within some farms, but even then, precision is unsatisfactory. Variations associated with measurement of grams of BGYF and with sampling variation at the truck, sample, kernel, and subsample levels are major contributors to the estimation problem. Because of imprecision and farm-to-farm variation in *A. flavus* and *A. parasiticus* contamination, the use of BGYF as a definitive test for quantitative estimation of aflatoxin over an area of many farms is unproven. An explanation of differences in fungal metabolites between farms would perhaps provide a basis for understanding the process of aflatoxin contamination.

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