fungal growth and the toxin levels. In another investigation, extracts of kernels of particular corn lines inhibited the in vitro formation of aflatoxin without restricting the growth of the fungus (15). Preliminary characterization of the active agent showed that the material was a relatively small peptide or number of peptides. The potential for small peptides or other host plant compounds to exert a protective effect in seeds in terms of invasion by pests raises an important possibility for plant breeders who are interested in identifying resistance to insects and fungi in agricultural commodities.

It is apparent that we are on the verge of an era that will focus the full impact of crop breeding on the identification of genetic characters for protection of our major crops from mycotoxin contamination. Presence of undesirable fungal metabolites in food and feed commodities represents a unique facet of the larger problem of the genetic vulnerability of crop plants. Characterization of a genetic repository that can be used to control accumulation of toxin substances in the edible portions of plants is a critical task and a real challenge for the next few decades.

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Bright Greenish-Yellow Fluorescence and Aflatoxin in Recently Harvested Yellow Corn Marketed in North Carolina[1]

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ABSTRACT

Corn kernels that exhibited bright greenish-yellow fluorescence (BGYF) under long-wave ultraviolet light were hand-picked from samples of yellow corn produced in eastern North Carolina. The BGYF kernels from 113 4-kg samples contained an average of 8665 parts per billion (ppb) aflatoxin compared to an average of 46 ppb in the non-BGYF kernels. A regression analysis between the ppb aflatoxin concentration and the wt % BGYF kernels in 2,304 4.5-kg samples produced the regression equation: ppb in sample = 197 (wt % BGYF). The correlation coefficient for the analysis was 0.90. Testing programs to reduce aflatoxin concentrations in purchased lots of corn based on either the BGYF method or the AOAC chemical assay method were compared. The average aflatoxin concentration in lots accepted by the AOAC method was 4 ppb, 10 ppb or 18 ppb when an acceptance level of $\leq$ 20 ppb, $\leq$ 50 ppb or $\leq$ 100 ppb, respectively, was used. For the BGYF method, the average aflatoxin concentration in accepted lots was 10 ppb, 16 ppb or 22 ppb when an acceptance level of $\leq$ 0.10% BGYF, $\leq$ 0.25% BGYF or $\leq$ 0.50% BGYF, respectively, was used. Approximately the same percentage of lots were accepted by both methods when either the low, medium or high acceptance level was used.

INTRODUCTION

A bright greenish-yellow fluorescence (BGYF) under long-wave ultraviolet (UV) light has been associated with the presence of aflatoxin in cottonseed, corn and pistachio nuts (1-3). Examination of corn for BGYF has been proposed as a rapid screening method to detect aflatoxin-contaminated lots at time of marketing. Previous studies with the BGYF method indicate that when there are no BGYF particles in 4.5-kg samples of cracked corn, probability is very low that the sample contains aflatoxin. On the other hand, the aflatoxin content of samples with BGYF particles may range from none to very high concentrations (4,5).

Marketing tolerances for aflatoxin concentrations of 20 parts per billion (ppb) or more, depending on the intended use for the corn, have been used in southeastern U.S. For a BGYF screening method to be practical in the Southeast, it must be a dependable, quantitative estimator of aflatoxin concentrations ranging from 20 to 100 ppb in commercial lots of corn. Studies on white corn produced and stored on farms in Missouri in 1971 indicated that wt % of BGYF particles was not a satisfactory quantitative estimator for aflatoxin (6). The objective of this study is to determine the relationship between the wt % BGYF kernels and aflatoxin concentration in 4.5-kg samples taken from North Carolina farm lots of yellow corn within a week after harvest.

PROCEDURE

During the corn harvest seasons of 1977 and 1978 a sample

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weighing ca. 4.5 kg was collected from each of 2,387 lots of recently harvested corn from eastern North Carolina farms. An additional 4-kg sample was collected from each of 250 of the lots sampled in 1977. The samples were collected within 1 week after harvest. Within 12 hr after collection, the samples were placed in cold storage (5 C) until they could be processed. The samples were taken during a 3-week period, including the peak harvesting time, and were collected throughout the major corn production areas of eastern North Carolina.

The wt % of BGYF kernels in each sample was determined by hand-sorting the kernels under long-wave UV light. Sorting was accomplished by passing the sample over a 15-cm-wide inspection belt. The single-kernel layer of corn on the inspection belt was illuminated with 2 long-wave UV lights (General Electric, F15T8-BLB, 15 W) mounted in a standard fluorescent lighting fixture suspended 30 cm above the belt. All other light was excluded from the sorting room. When kernels with the characteristic BGYF were detected, the belt was stopped and the BGYF kernels were removed. When the operator was not sure about the fluorescence of a kernel, the kernel was cut open and only those kernels with typical BGYF were removed. No attempt was made to stir the kernels on the belt to detect BGYF that was not initially exposed to ultraviolet light. However, close observation indicated that very few BGYF kernels were missed by the sorting procedure.

Test 1

One hundred and thirteen of the 250 4-kg samples collected in 1977 contained BGYF kernels. The BGYF and non-BGYF (NBGYF) portions from each of these 113 samples were weighed, ground to pass a screen with 1-mm openings, and analyzed for aflatoxins using the AOAC Official First Action Method (CB Method) (7). (Modifications in the analytical procedure were necessary to accommodate the small samples of BGYF corn.) The relative weights and aflatoxin concentrations of the BGYF and NBGYF portions were used to calculate the aflatoxin concentration of the entire sample and the relative percentages of the total aflatoxin content of the entire sample that were confined to each portion. A portion of the finely ground NBGYF corn was pressed into a 10-cm diam. petri dish, and a count was made of the number of BGYF particles observed in the smoothly pressed surface of the ground corn.

Test 2

After the wt % BGYF kernels was determined, the BGYF portion was added back to each of the 2,387 samples of corn collected in 1977 and 1978. The 4.5-kg samples were ground to pass a no. 14 sieve. A 1-kg subsample of the coarsely ground material was ground to pass a no. 20 sieve and a 50-g subsample of the finely ground material was analyzed for aflatoxin using the aqueous acetone extraction procedure of Pons et al. (8). A linear regression analysis was performed on the data to determine the correlation between the wt % BGYF kernels and the ppb aflatoxin in the samples.

Comparison of the BGYF Method and a Chemical Analysis Method

The data set from test 2 was used to compare the efficacy of the wt % BGYF rapid screening method and a screening method based on aflatoxin analyses of 4.5-kg samples of corn by the AOAC Official First Action Method (7). To evaluate the chemical analysis method, variance estimates for aflatoxin tests on corn (9) were used in conjunction with a Monte Carlo technique to simulate aflatoxin tests on lots of corn by the AOAC Method. The Monte Carlo simulation technique has been reported for peanuts (10). The aflatoxin concentration for each sample from the test 2 data set was treated as a lot mean, and the simulated aflatoxin test results were used to determine the proportion of the lots "accepted" or "rejected" when an acceptance level of either $\leqslant$ 20 ppb, $\leqslant$ 50 ppb or $\leqslant$ 100 ppb was used. The average aflatoxin concentrations in the accepted and rejected lots were computed for each acceptance level.

To evaluate the BGYF method, the aflatoxin concentration for each sample in the test 2 data set was again treated as a lot mean and the lot was accepted or rejected when the wt % BGYF found in the sample was compared to an arbitrary acceptance level of either $\leqslant$ 0.10%, $\leqslant$ 0.25% or $\leqslant$ 0.50%. The average aflatoxin concentration of the accepted and of the rejected lots was computed for each acceptance level.

RESULTS AND DISCUSSION

Test 1

Average values for data from test 1 are given in Table I. A correlation analysis of the data showed a good correlation between wt % BGYF kernels and aflatoxin concentration in the samples (R = .78). The aflatoxin concentration in the BGYF kernels was ca. 188 times the concentration in the NBGYF kernels. The aflatoxin in the NBGYF kernels probably was confined to small or hidden BGYF portions of the kernels that were not removed by the sorting operation. The presence of BGYF in the NBGYF portion is indicated by the presence of BGYF particles in the finely ground NBGYF kernels. Due to the difficulty of detecting all of the BGYF in whole kernels, some researchers have recommended cracking the corn before examination for BGYF (5). Cracking, however, makes sorting for wt % determinations extremely difficult.

TABLE I

Determinations for the 113 Samples of Corn Used in Test 1

Average wt of samples	3924 g
Average aflatoxin concentration in NBGYF kernels	46 ppb
Average aflatoxin concentration in BGYF kernels	8665 ppb
Average aflatoxin concentration in total sample	79 ppb
Average wt % BGYF kernels in samples	0.38%
Average % of total aflatoxin in BGYF kernels	41.9%
Average % of total aflatoxin in NBGYF kernels	58.1%
Average no. of fluorescent particles/cm^2 of surface	1.41

Equation I is based on the premise that all of the aflatoxin in the samples of corn used in this study was confined to the BGYF fraction and that the average aflatoxin concentration in the BGYF fraction was 8,665 ppb.

$$\text{ppb in sample} = \%\ \text{BGYF in sample} \times (8{,}665\ \text{ppb})/100 \qquad [I]$$

As the average aflatoxin concentration in the samples was 79 ppb, solution of Equation I indicates that the equivalent average wt % BGYF in the samples was 0.91% rather than the 0.38% determined by hand-sorting of whole kernels. Equations II and III derive a relationship between the total amount of BGYF kernels in the sample and the amount of BGYF kernels removed by hand-sorting.

$$\%\ \text{BGYF in samples} = \frac{.91}{.38} (\%\ \text{BGYF determined by hand-sorting}) \qquad [II]$$

$$\%\ \text{BGYF in samples} = 2.39 (\%\ \text{BGYF determined by hand-sorting}) \qquad [III]$$

Substitution of Equation III into Equation I yields the following equation for the relationship between the average

TABLE II

Comparison of Efficacy of the BGYF Screening Method and the Chemical Assay Method to Detect Aflatoxin Contamination in 2304 Lots of Corn with an Average Aflatoxin Concentration of 66 ppb

Chemical assay method			
Aflatoxin concentration in sample when lot accepted (ppb)	< 20	< 50	<100
% of all lots tested that were accepted	60	73	82
Average aflatoxin concentration in accepted lots (ppb)	4	10	18
% of all lots tested that were rejected	40	27	18
Average aflatoxin concentration in rejected lots (ppb)	157	218	291
BGYF screening method			
Wt % BGYF kernels in sample when lot accepted	< .1	< .25	< .5
% of all lots tested that were accepted	59	72	81
Average aflatoxin concentration in accepted lots (ppb)	10	16	22
% of all lots tested that were rejected	41	28	19
Average aflatoxin concentration in rejected lots (ppb)	148	195	260

aflatoxin concentration of the samples and the average wt % BGYF kernels determined by hand-sorting:

ppb in samples = 207 (% BGYF determined by hand-sorting) [IV]

Test 2

If the premise that the aflatoxin in samples of corn is confined to the BGYF portion of samples is valid, a plot of aflatoxin concentration vs wt % BGYF kernels in the sample is linear with no intercept. Equation V is a linear regression equation with no intercept for 2,304 data points. The remaining 83 data points from the 2,387 samples used in the test were removed from the data set as outliers because their observed values deviated from the predicted values more than ± 2.5 standard deviations. The correlation coefficient for the data is 0.90.

ppb in sample = 197 (% BGYF determined by hand-sorting) [V]

The average aflatoxin concentration in the 2,304 samples was 66 ppb, and aflatoxin was not detected in 42% of the samples. The agreement between Equations IV and V is remarkable, because they were derived by different procedures. More studies are necessary to determine the validity of this apparent agreement. However, these results indicate that an estimation of wt % BGYF by hand-sorting may be an effective rapid screening method to estimate aflatoxin concentrations in samples of corn.

Comparison of the BGYF Method and a Chemical Analysis Method

A comparison of the efficacy of the BGYF screening method and a screening method based on aflatoxin analyses of 4.5-kg samples by the AOAC method is given in Table II. A comparison of results from acceptance levels of ⩽ 20 ppb, ⩽ 50 ppb or ⩽ 100 ppb for the chemical assay method with acceptance levels of ⩽ 0.10%, ⩽ 0.25% or ⩽ 0.50%, respectively, for the BGYF method indicate that both methods rejected approximately the same percentage of the lots tested. However, the difference in average aflatoxin concentration for the accepted lots shows that the same lots were not rejected by both methods. The ratios of the aflatoxin concentration in lots accepted by the BGYF method to the aflatoxin concentration in lots accepted by chemical assay are 2.5, 1.6 and 1.22, respectively, for the low, intermediate and high acceptance levels used in this study. These results indicate that the chemical assay method is more discriminating than the BGYF method, but the difference between the 2 methods diminishes when the acceptance level is increased. Both cost and efficacy of the 2 methods should be considered when choosing between them.

On the average, BGYF kernels contain high concentrations of aflatoxin; thus there probably is a better correlation of wt % BGYF with the aflatoxin concentration in a given sample than with the aflatoxin concentration in the lot represented by the sample. Therefore, the procedure used in this study to evaluate the BGYF method probably indicates lower concentrations in the accepted lots than would actually be the case. However, the authors do not believe that this bias in the procedure is large enough to significantly affect the validity of the comparison between the 2 methods.

The comparison given in Table II is based on a population of lots characterized by 2,304 samples collected in eastern North Carolina during 1977 and 1978 when the average aflatoxin concentration in all samples was 66 ppb. The relative concentrations of aflatoxin in lots accepted by either method would probably be affected by the distribution according to aflatoxin concentration of all lots tested. In addition, the correlation between wt % BGYF and aflatoxin concentration in corn might be affected by corn hybrid, growing conditions for the corn, error in wt % BGYF determination, and many other factors. Further research is needed to compare efficacy of the 2 methods under a variety of conditions other than those represented in this study.

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