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AFLATOXIN REDUCTION IN MAIZE

External Project Review

Project Number 931-1181

April 8-9, 1980

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SUMMARY

Maize strains from all over the world are being examined to identify genetic factors that reduce growth and invasion of maize kernels by aflatoxin-producing fungi or inhibit the synthesis of aflatoxin by these fungi. This research at the University of Missouri has been stymied by the lack of methods that will identify resistant maize selections.

The research team has obtained cooperators and cooperation on a worldwide basis. They have evaluated between 500 to 1,000 selections of maize, but have been unable to identify resistant material experimentally, although evidence indicates that field resistance is present. Experiments were carried out in seven countries (LDCs) in 1979 and 20 will be selected from 33 for further plantings in 1980. Six significant research papers and one review publication have been authored or coauthored by the members of the research team during the period of 1977-1979.

The technique for field inoculation evolved during several years of research before initiation of the project, but still requires further refinement and modification to become effective. A unique, innovative, laboratory screening method has been developed that shows promise, if perfected, to permit screening of corn lines and crosses in the off-season for resistance. The capabilities of the research team is not in question, since their slow progress resulted from an administrative decision to build a P-3 laboratory for the aflatoxin analyses. This delayed the analytical data for two years or more before they could identify the ineffectiveness of their inoculation techniques. Analyses of their first year's samples would have brought their problem to the surface.

The Review Team was unanimous in recommending a two-year, unfunded extension of this project. Refinement and modification of the inoculation techniques was recommended with emphasis on amount and concentration of the inoculum, degree of injury, and adequacy of the moist chamber. There needs to be a reassessment of sampling size and sampling procedures as well as the development of uniformity trials. A review of the aflatoxin level criterion for resistance in corn kernels needs to be made. International cooperation and ties need to be further strengthened and fostered by travel in both directions and organizing a workshop in a year or so. The development of an anuual report as well as publication of research accomplishments in normal scientific channels is suggested. Another progress review is suggested for August-September, 1981.

INTRODUCTION

1. Composition of Team:

   A. Dr. Urban L. Diener, Team Leader

   Department of Botany, Plant Pathology and Microbiology, Auburn University, Auburn, Alabama, 36830. Involved in aflatoxin research since 1963. Authored or coauthored over 40 publications in aflatoxin and mycotoxin research. Chairman of Task Force of the Council for Agricultural Science and Technology (CAST) that developed report on "Aflatoxins and Other Mycotoxins: An Agricultural Perspective."

   B. Dr. Alfred Manwiller

   Department of Agronomy, Clemson University, located at Pee Dee Experiment Station, Florence, South Carolina. Involved in corn disease and insect breeding for resistance research for over 30 years; recent research includes the evaluation of corn for resistance to aflatoxins.

   C. Dr. D. N. Moss

   Head, Department of Crop Science, Oregon State University, Corvallis, Oregon. Member of Research Advisory Committee (RAC) to A.I.D. Primary research effort in crop physiology.

   D. Mr. Richard Hughes

   Agriculture and Rural Development Office. Latin America Bureau, A.I.D. Background in animal science and economic and social development; 24 years experience with A.I.D., including seven mission assignments in Latin America, Near East, and Middle East as Food and Agricultural Officer.

2. Charge to the Review Team:

   A. Review the Project Statements; the implementing contract as amended; the Project Evaluation Summary (PES); the Project Identification Document (PID) March 30, 1977; the Project Authorization and Request for Allocation of Funds (PAF); the RAC Minutes; and the latest technical reports.

   B. Assess and evaluate the following aspects of the Project:
      a. The project goals and objectives as they address the development of well-adapted maize lines resistant to the growth of *Aspergillus flavus* and *A. parasiticus* and/or inhibition of aflatoxin synthesis.

      b. Planned results of the project.

      c. Assumptions in relation to anticipated end-of-product status (EOPS).

      d. Adequacy and correctness of the overall project design as well as specific areas of research and methodology that include:

(1) screening and identifying available maize collections for resistance to invasion by Aspergillus flavus and/or inhibition of aflatoxin synthesis; (2) inheritance for resistance studies; (3) environmental factors influencing aflatoxin production in the field; (4) cyclic breeding program; (5) development of inbred lines and hybrid crosses; and (6) developing breeding material suitable for corn improvement in LDCs worldwide.

3. Procedure:

A. Preparation. A team of four scientists conducted an external review on April 8, 9, 1980 at the Agronomy Department, University of Missouri, Columbia. The project manager, Dr. Mark Smith, had provided the following documents for the Aflatoxin Reduction in Maize Review in March.

a. Current contract - AID/ta/c-1451, with Univ. of MO, 9-30-77.

b. Original Project Statement for period 10/1/77 - 9/30/80.

c. Environmental Threshold Decision - 5/16/77.

d. Approval of "Aflatoxin reduction in maize" project by RAC Review Subcommittee, D.N. Moss, Chmn. - 7/14-15/77.

e. PES - Project Evaluation Summary, Keith M. Byergo - 12/10-13/78.

f. PID - Project Identification Document transmittal.

g. Publications developed during AID "Aflatoxin Reduction in Maize" project. Note Publications 1 to 6, Page 20.

h. General background publication concerning aflatoxin in corn and closely-related publications by UM research team. Note Publications 7 to 11, Page 20 and 21.

i. 1979 International Environment-Maize Genotype Study - Protocol.

B. The project Manager, Dr. Mark Smith, was present and the following representatives of the University of Missouri participated:

Dr. Wendell McKinsey, Assistant Dean, College of Agriculture.

Dr. E. C. A. Runge, Chairman, Department of Agronomy.

Dr. Marcus S. Zuber, Professor, Department of Agronomy.

Dr. Oscar H. Calvert, Professor, Department of Plant Pathology.

Dr. Arthur L. Karr, Asst. Professor, Department of Plant Pathology.

Dr. Jack R. Wallin, Res. Associate, USDA, Dept. of Plant Pathology.

Dr. Larry L. Darrah, Res. Geneticist, USDA, Dept. of Agronomy.

Dr. Louis Holroyd, UMC Safety Coordinator.

Ms. Candice A. Gardner, Graduate Asst., Dept. of Plant Pathology.

Mr. Paul Linnen, Graduate Student, Dept. of Plant Pathology.

Dr. Dan Mertz, Plant Physiologist, U.M.C.

Mr. Scott Angle, Graduate Student of Dr. Wagner.

C. The formal review followed this AGENDA.

Tuesday April 8, 1980

8:30 AM	Welcoming Remarks - Dean McKinsey & E.C.A. Runge
8:50 AM	Introduction of Review Team - Mark Smith
9:00 AM	Scope of the Evaluation - Urban L. Diener
9:15 AM	General Scope of the Project - M. S. Zuber
10:00 AM	Break
10:15 AM	Research Related to Aspergillus flavus mycelia growth and aflatoxin levels; other research - A. L. Karr and Paul Linnen
11:45 AM	Lunch
1:15 PM	Half-kernel method; Cargill exotic material; Guatemala maize collection - J. R. Wallin and C. A. Gardner
3:00 PM	Safety Problems related to aflatoxin and tour of the P-3 laboratory facilities - L. Holroyd and A. L. Karr
4:30 PM	Tour of analytical facilities - A. L. Karr

Wednesday April 9, 1980

8:30 AM	Survey results with exotic strains of maize grown in Florida, South Carolina, and Missouri - L. L. Darrah
9:00 AM	Diallel inheritance study; effect on aflatoxin levels by varying ratios of inoc/uninoc. kernels; comparison of inoculation methods, dates of inoc. with A. flavus - O. H. Calvert
9:45 AM	Uptake of aflatoxin by seedlings from soil; translocation in the ear - Dan Mertz
10:15 AM	Break
10:30 AM	Aflatoxin degradation and transformation in soil - S. Angle
10:40 AM	Int. Environ-Genotype study in maize re:toxins - M. S. Zuber
11:15 AM	Proposed future direction of the project - Discussion by UMC researchers and interchange with review team.
12:00 PM	Lunch
1:30 PM	Discussion of project with PI and then Development of Team's Recommendations by the Review Team. The review team had met on the evening of April 8, 1980 to discuss the progress and points needing further clarification by the researchers.

The Review Team Leader and Project Manager discussed the format of the report on the evening of April 9, 1980. The Team Leader was charged with the responsibility of writing a first draft of the team report, distributing it for review, and revising a final report and forwarding it to the project manager by April 30, 1980.

OVERVIEW OF THE PROJECT

The aflatoxins are one group among the mycotoxins that are toxic, secondary fungal metabolites produced by Aspergillus flavus and A. parasiticus during growth on food and feed products. Aflatoxins have proven to be potent carcinogens in domesticated and experimental animals and at sublethal levels can greatly reduce feed efficiency and weight gains of domestic animals. These toxins cause liver and hepatic changes that are cancerous.

The epidemiological data from several tropical and subtropical countries point rather conclusively to a close association between high aflatoxin levels and high human liver cancer. Uganda, Mozambique, India, Taiwan, Kenya, Swaziland, and Thailand have supplied direct evidence for liver cancer and Reye's syndrome.

Aflatoxins occur in a wide variety of agricultural commodities including maize , peanuts, and cotton. Aflatoxin contamination in maize has been detected throughout the world with particularly high levels occurring in tropical and subtropical maize-producing countries, which include a number of the less developed nations as well as southern United States. Although aflatoxin contamination was considered a post harvest, stored grain problem at one time, recent experimental data showed that the toxins are produced extensively in preharvest maize. Since maize constitutes a major part of the human diet in many LDCs, the toxins may be deleteriously affecting the health of millions of people. Also, since A. flavus and A. parasiticus are worldwide in distribution and most frequent in warm humid areas, it is clear that a high potential human health hazard exists where maize matures under such conditions and is widely used for human food.

Means for lowering the incidence of aflatoxin contamination in corn are urgently needed. Since many of the other fungal-caused problems in maize have been controlled through breeding, it appears likely that plant breeding also can provide an effective control of the human health hazard of aflatoxin in corn.

This project proposes to search among the maize strains from different geographical areas in the world for genetic factors that either reduce the fungal growth by these two Aspergillus species or inhibit their synthesis of aflatoxin. Resistant maize types would then be used in breeding programs to produce agronomically desirable strains having a reduced likelihood of supporting A. flavus growth or of containing aflatoxin.

STATUS OF PROGRESS OF THE PROJECT

Financial status of the project is reviewed in the two accompanying tables 1 and 2. Table 1 shows the University of Missouri resources allocated to the project and those supported by U.S. A.I.D. funds. Table 2 summarizes the expenses projected through 9/30/80 ($58,000) plus the funds expended through 9/30/79 ($147,736.54) in carrying out the research activities of the project.

The funds remaining for this project(A.I.D.-$208,182.46) would be budgeted for the two years through 9/30/82 by the investigators to complete the project. These projected budgets will be presented later in the review report.

Table 1. Resources Allocated to Maize Aflatoxin Project, 1979-1980

	Research Years				Resources			
Personnel	Professional		Technical		Salary		Operations	
	UMO	USAID	UMO	USAID	UMO	USAID	UMO	USAID
Zuber*	0.25	0.25			50%	50%	75%	25%
Darrah, USDA	0.90	0.10			100%		90%	10%
Wallin, USDA	0.85	0.15			100%		75%	25%
Karr	0.25	0.75			100%		75%	25%
Calvert	0.85	0.15			100%		75%	25%
Loonan, USDA			0.70	0.30	100%			
Wibbenmeyer			0.10	0.90		100%	10%	90%
Johnson			0.25	0.75		100%		
Conrad			0.50	0.50	100%			
Pennington			0.80	0.20		100%		
Linnen**			0.05	0.45		100%		
Gardner**			0.25	0.25	100%			
Ulmer			0.50	0.50	25%	75%		

*Half-time faculty. ** Based on half-time assistantships

Table 2. Budget for Maize-Aflatoxin Project - AID/ta-C-1451 (1977-1980)

Budget for grant period through 1980	$ 413,919.00
Expenditures to October 10, 1979	147,736.54
Remainder	266,182.46
Projected expenses through September 1980	
Salaries	28,000.00
Fringe benefits	7,000.00
Supplies	11,000.00
Equipment	2,000.00
Subcontracts	7,000.00
Travel	2,000.00
Misc. (Medical exam, etc.)	900.00
Total 1979-1980	58,000.00
Money remaining from grant October, 1980	208,182.46

STATUS OF PROGRESS OF THE PROJECT

Tuesday April 8, 1980

8:30 AM Welcoming Remarks - Dean W. McKinsey, Dr. E. C. A. Runge

8:50 AM Introduction of Review Team - Dr. Mark Smith, Project Mgr.

9:00 AM Scope of the Evaluation - Urban Diener, Team Leader

9:15 AM General Scope of the Project - Dr. M. S. Zuber, PI

The first reports of serious aflatoxin problems were from southeast MO in 1971, when two carloads of white corn destined for Denton, Texas were picked up by FDA. The elevator operator went bankrupt when stuck with 50,000 bushels of contaminated corn. Truckloads of corn were lined up for 2-3 miles waiting to use the elevators drying facilities. During the incubation period, 25,000 bushels out of 1½ million were above 100 ppb of aflatoxin. E. B. Lillehoj and the No. Reg. Res. Ctr. collaborated in a series of investigations of the problem. Aflatoxin occurred in other years usually associated with areas with stress conditions; mostly a southern problem but has been found in southern Iowa.

The objectives of the project was to look for complete resistance to the fungus and aflatoxin production. In soybeans the inhibitor to A. flavus appears to be in the living seed. They enlarged the project to the international scope through Pioneer International, but found they could not bring aflatoxin-contaminated materials back into the USA; this problem was resolved in June 1979.

The approach was international in scope with Thailand, Philippines, Haiti, Colombia, Costa Rica, Mozambique, and Malawi agreeing to plant corn and return samples for analysis, in 1979. So. Reg Res. Ctr., Alex Ciegler, cooperated in grinding the corn. Ground corn is mailed to D. B. Sauer at Manhattan,Kansas USDA Lab. to inspect and evaluate for insects. It comes to Washington,D.C. by diplomatic pouch, thence to AID, Frank Cooper sends it on. They are using CIMMYTs yield trials with six populations of varieties grown everywhere. The program is expanding to more countries with about 33 possibilities from which to select about 20 countries. Varieties involved are a sampling of tropical and subtropical lines and crosses utilizing about 2.5 kilos of corn from each replication.

The approach to testing the corn breeding lines and crosses was by an inoculation simulating wounding of corn about 20 days after silking. Several methods had been used and were tested. (a) Hypodermic needle was inserted through the husk and a suspension of A. flavus spores were injected directly into a kernel or two. Since there was little or no lateral invasion of other kernels, the method did not contaminate a significant number of kernels for much of an evaluation. (b) A method of inoculation was finally developed using a pin board - a two by 12 to 15 pin row was finally adopted. The technique involved pulling down the husk,injuring a double row of kernels with the pin board, and then inoculating with about 1.5 ml of a suspension of a million spores per ml. The husk was then replaced, secured with rubber bands, and covered with a paper sack stapled shut to make a good moist chamber but not a heat trap. Mats of A. flavus mycelia developed on the injured kernels. It was apparent from the comments that the method was too severe, contaminating all varieties, and failing to obtain consistent results.

10:15 AM Aflatoxin Analysis Laboratory - Arthur L. Karr and J. Wibbenmeyer.

This laboratory was unable to carry out its functions until the P-3 Lab dictated by the UMC Safety Committee was complete. If this laboratory had been able to conduct the analyses the first year, the deficiencies in the inoculation method for separating resistant from susceptible corn breeding lines would have probably surfaced immediately. This laboratory is located at the Environmental Trace Substances Research Center. They are equipped to quantitate aflatoxin by minicolumn, TLC, HPLC, and other methods. The $110,000 lab has manual glove boxes, Bioguard Hoods, negative pressure airflow, and high airflow chemical type hoods.

Aflatoxin samples are ground, extracted with acetone, filtered into $(NH_4)_2SO_4$ solution, partitioned against hexane, then twice against chloroform, the chloroform is removed, and the amount of aflatoxin is visually estimated by comparison to standards by TLC and long-wave UV light. These procedures handle the seasonal and large sample load produced by the project, with the lab having a capacity of 50 to 60 samples per day. Utilization of Sep-pak C-18 cartridges to replace the solvent extraction steps should improve the cleanup procedure and shorten the method.

This group uses immuno- and affino-chemical techniques for quantitation of fungus growth in corn kernels. Immunochemical techniques use antiserum to A. flavus to measure the level of fungus antigens. The affinochemical technique uses jack bean lectin, rocket electrophoresis to measure the level of lectin receptors in corn. The latter procedure is described here in detail.

Sample Preparation. Surface fungus growth is removed from the corn by shaking in a slurry of rock polishing grit, hence measurement will be for the fungus inside the kernel. The dried kernels are ground and placed in water. The suspension is heated at 100 C as the lectin receptors are soluble in hot water. Insoluble material is removed by centrifugation and the level of lectin receptor in the supernatant liquid is measured by rocket electrophoresis (into lectin containing agar). Minimum detection levels of this procedure are near 10 ng of antigen (receptor). Variation in a series of identical samples, including extraction etc. is about 15%.

Relation of fungus growth to aflatoxin accumulation in the field.

Corn was inoculated in the field using the pin-board procedure. Samples were analyzed for both aflatoxin and lectin receptor. An 80-fold range of lectin receptor was found to be present in the samples. Through the lower half of this range the level of receptor was found to be a reliable indicator of aflatoxin levels. In the upper half of the growth range an increase in fungus growth (i.e., high receptor levels) was associated with decreasing aflatoxin levels. Conclusion. This technique is a reliable predictor of those samples that have low aflatoxin levels. The advantage of the technique is that it will exclude corn lines, in which high fungus growth (with other associated problems) but low aflatoxin accumulation occurs.

1:15 PM Epidemiology of A. flavus and resistance in corn - J. R. Wallin and C. A. Gardner.

The decapped corn kernel technique for identifying spores of A. flavus has been used to challenge corn lines with the fungus in the laboratory. Corn kernels are decapped with a razor blade and the germ portion inverted, cut side down, on strips of double-stick tape pressed on moist filter paper in petri dishes. Tape was sprayed with an aqueous suspension of A. flavus spores before sticking to the filter paper. Cultures are incubated 8 days at 28 C, at which time susceptible kernels are overgrown with mycelium. The plates are removed, the infected kernels counted, exposed to black light, and the number of kernels fluorescing are recorded. The fluorescing kernels in each plate are then analyzed for aflatoxin content.

Samples of composite PrMo2, 212 US inbreds, 7 single crosse of B73 x Mo17, 14 single crosses of A634 x Mo17, 30 accessions from Guatemala, a criollo maize from Mexico, and 8 seed lots of perennial teosinte, Zea diploperennis, from Jalisco, Mexico, have been screened by the decapped corn kernel method.

A few plants from kernels of PrMo2 looked promising, were grown in the field, and are under further investigation to determine their response to invasion by A. flavus. Six inbreds of the 212 tested look promising, diallels of these were made, grown in the field, inoculated, and will be evaluated for aflatoxin production. Kernels of these diallels were also subjected to the decapped kernel method in the laboratory. However, none of the 21 single crosses were low in aflatoxin content. Six Guatemalan criollos rated comparatively low in aflatoxin by decapping and are under investigation as top crosses with a few corn belt inbreds. The lowest levels of aflatoxin have been found in the diploid teosinte and criollo corn collected in Jalisco, Mexico, November, 1979.

The true aflatoxin content within the kernels may have been obscured by the fact that in the past the fungus mat has not been polished off of the kernels before grinding and aflatoxin analysis as described by Dr. A.L. Karr. Hence, the analyses have included the aflatoxin contained in the mycelium external and outside the kernel. This aspect is presently under investigation.

The Cargill collection of exotic maize populations has been studied since 1977. Some individuals from these populations rated comparatively low in aflatoxin levels by the decapped kernel method. However, those promising lots used and planted in the field were not particularly resistant to the pinboard method of inoculation.

There has been no correlation of promising low aflatoxin-producing lines identified by the decapped kernel method with the results obtained by the field inoculation-pinboard technique. Whether overkill or too severe an inoculation technique that overcomes possible practical field resistance by one or both methods has to be determined.

Although it is a long way from modern hybrids back to teosinte and some flint-type corns and Guatemalan corns, if resistance can be identified and concentrated by breeding it can be transferred by crossing techniques. The key to all progress appears to be the development of methods for identifying germplasms with resistance to either A. flavus invasion or aflatoxin formation in corn kernels.

3:00 PM Safety Problems Related to Aflatoxin Research - Dr. L. Holroyd.

Aflatoxin and A. flavus must be approached from all angles to protect the technicians and scientists. In the field and laboratory, masks are used where appropriate. The field version is an expensive "space age" appearing helmet with air filtering control for the wearer to prevent intake of A. flavus spores. The P-3 facilities have glove boxes, Bioguard negative pressure hoods and the standard chemical hoods with high exhaustion in terms of CFM. Solvents and hazardous waste disposal such as contaminated corn and corn cobs are burned in an incinerator. Protocols are needed to reassure and satisfy the employees and the employer, although the bench scientist is considered liable for his technicians and other assistants.

The personnel conducting the analytical procedures is highly competent and fully aware of the need and methods for working with hazardous and dangerous chemicals as well as the mycological hazards of spores of toxin-producing fungi. These facilities are adequate and completely equipped for modern analytical chemical methods.

The tours of the facilities of the P-3 laboratory and the analytical chemistry area for mycotoxins were informative and instructive.

Wednesday April 9, 1980

8:30 AM Survey Results with Exotic Strains of Corn - L. L. Darrah.

The results of aflatoxin analysis of exotic strains of corn grown in Florida, South Carolina, and Columbia, Missouri were reviewed by Dr. Darrah. Lines tested consisted of 20 races (Exp. 35); 51 lines of Florida Helminthosporium maydis-resistant synthetic (Exp. 36); conducted in Florida in 1978. Aflatoxin analyses of this material has only been recently completed.

In South Carolina, 124 S. Carolina lines were evaluated for aflatoxin production (Exp. 37), while another 40 lines of Jarvis and Indian Chief S2 lines were also screened for toxin production (Exp. 38) in 1978.

In Missouri, 240 exotic testcrosses ex Stuber were tested for aflatoxin production in 1978.

Statistical analyses of all data showed high variation between the two replications in Exp. 35 in Florida. Significance was found only between the two highest (most susceptible) lines at 30,000 ppb and the two lowest at about 4500-5000 ppb aflatoxin. The data from Exp. 36 is not much different. This pattern of data continues from South Carolina, Exp. 37, and 38, in the latter the range seemed wider from highs to lows in aflatoxin. The 240 Regional Exotic Testcrosses in Missouri showed highs of 60,000 ppb and lows about 2,000 ppb. Whether the extremes indicate true differences in aflatoxin susceptibility and resistance is the question? Certainly they are too high to be acceptable in the field. It was most unfortunate that these analyses were not made in the winter of 1978-79, so that adjustments and experimentation on the variables involved in the inoculation could have been initiated a year earlier. However, hindsight is always 20:20.

Wednesday April 9, 1980

9:00 AM Aflatoxin Investigations with Hatch,Mo-298 Funds - O. H. Calvert

To identify genetic resistance in corn to aflatoxin production by Aspergillus flavus and A. parasiticus.

I. Hypothesis: The genome of the corn plant plays a definitive role in determining the formation of aflatoxin in the corn kernel. Aflatoxin production in both species of fungi is genetically controlled as reported by Papa & Foudin of the Univ. of Georgia.

Data published in 1978 supports this hypothesis. The results presented leave much to be desired due to the large errors involved in aflatoxin analysis, which have been accepted. The difference between 1,685 ppb and 5,773 ppb is probably not significant unless an exceptionally high number of replications were involved and most variables were under very precise control.

II. Hypothesis: The genetic mechanism is being expressed as a substrate phenomenon (e.g. chemical composition - sugars, amino acids) or an active resistance mechanism (e.g. antifungus compounds, hypersensitivity mechanical barrier) or an as yet unknown mechanism.

The approach to testing this hypothesis is to test intact, mature corn kernels of different lines for their ability to resist aflatoxin contamination and/or fungus invasion. The same lines are also being tested to determine differences in aflatoxin production when kernels are first autoclaved and (a) left intact, or (b) ground into meal before inoculation.

Methods have been developed to rehydrate living seed and to inoculate them with the fungi. Strains are being collected from various sources of Aspergillus flavus and A. parasiticus that produce varying amounts of aflatoxin in YES broth in vitro.

Corn lines are being tested as to the level of aflatoxin produced in ng/g, to determine if this stable quantitative character in corn lines can be detected in the laboratory or whether this is primarily a field phenomenon.

After dividing the lines into high, intermediate and low aflatoxin producers in the laboratory by inoculation, the lines will be evaluated in field studies and the degree of correlation noted. If successful, this inoculation evaluation technique would permit screening two generations per year, one in the lab and one in the field.

9:45 AM Uptake and translocation of aflatoxin from the soil - Dan Mertz

The uptake of radiolabelled aflatoxin from the soil by lettuce seedlings was described. Translocation of aflatoxin from the internode into the developing ear was also confirmed in these experiments. Although these are superficial initial studies, they indicate an area of inquiry not previously pursued that has implications for human health and possible injury to plant growth and reproduction.

10:30 AM 1979 Int. Environ.-Maize Genotype Study On Aflatoxins - M. S. Zuber

Cooperators: E. B. Lillehoj, SRRC, USDA
A. Ciegler, SRRC, USDA
R. L. Paliwal, CIMMYT
M. S. Zuber, Univ. of Missouri
L. L. Darrah, SEA, USDA, Univ. Mo.
E. W. Sprague. CIMMYT

Field Cooperators: M. Torregroza, Colombia
C. Grand-Pierre, Haiti
S. Jinahyon, Thailand
S. Bokde, Mozambique
A.L.P. Sinclair, Costa Rica
D.R.B. Manda, Malawi
E. Serrano, Philippines

Objectives:
1. Determine the maize-genotype environment interaction effect on fungus infection and toxin accumulation in preharvest maize grain samples.
2. Confirm preliminary findings that toxin levels in preharvest maize kernels are under genetic control.
3. Identify maize genotypes with minimum kernel rots and with least kernel injury by insects.
4. Establish environmental influence on the interaction between insect and fungus predators of developing maize ears.

Material: Maize trials conducted in various countries by CIMMYT will be available for sampling.

Locations: In 1979, the initial study will be restricted to seven countries.

1. Colombia, S.A.
2. Costa Rica, C.A.
3. Haiti, Caribbean
4. Mozambique, Africa
5. Malawi, Africa
6. Thailand, Asia
7. Philippines, Asia

It has been arranged to move the collected samples through the Foreign Agricultural Service via diplomatic Air Pouch from the country involved to the U.S. Mr. John DeCourcy,FAS, has informed the appropriate authorities in U.S. embassies of the study. Corn samples will be submitted by cooperators to the Agric. Attache, in U.S. Embassies except in Mozambique it will go the Economic Officer, State Dept., and in Malawi to AID.

The entries for sampling from the Int. Exp. Variety Trial (EVT13) conducted by CIMMYT in 1979 are:

Entry no.	Strain name	Entry no.	Strain name
2	La Maquina 7827	10	Suwan 7726
3	Sete Lagaos 7728	12	Sta Cruz Parillo (1) 7835
4	Across 7728	14	Tocumen (1) 7735

Sampling procedure: The cooperator in each of the seven countries will obtain ca 5 kg of shelled mature maize grain from each of the six entries. From replications 1 and 2, combine and obtain 2.5 kg of grain and from reps. 3 and 4, obtain the other 2.5 kg. Samples must be dried as rapidly as possible after harvest to 12% grain moisture. Each sample bag should include a tag inside and outside of the bag with the entry number, replication number, and country of origin.

Information for the field cooperators: The 2-2.5 kg samples from each country for each of the six designated entries can best be obtained and will be most representative by bulking replications 1 and 2 for one 2.5 kg sample, and replications 3 and 4 for the second 2.5 kg sample of the same entry. Information to be provided from each location includes: (1) cultural practices (including planting date, fertilizer applied, and any pesticides applied to the growing crop), (2) meteorological description of the growing season (rainfall minimum), (3) elevation (meters) of the planting site, (4) prevalent insects that have fed on the growing crop, and (5) flowering and harvest dates.

All samples should be in double cloth bags for shipment to the USA. No insecticide or fungicide should be applied to the post harvest grain samples.

Information for the Agricultural Attache or AID Representative

You will be furnished the name and location of the cooperator in the country where you will be asked to obtain the maize grain samples. It would be desirable for you to contact the cooperator before the crop is harvested.

Sample Processing

The seventy-two 2.5 kg whole kernel samples will be forwarded through the the U.S. Agricultural Attache FAS to:

John DeCourcy, USDA, FAS, Grain and Feed Division,
14th & Independence, Washington, D.C. 20250

After receipt by John DeCourcy, the samples will be forwarded to:

Mr. Frank Cooper, USDA, PPQ, APHIS,
Federal Building Rm 635A, Hyattsville, MD 20782

After clearance by APHIS, the samples will be sent to Dr. E. B. Lillehoj, where they will be analyzed for fungi and toxins.

Dr. E. B. Lillehoj, USDA, SEA, FR, SRRC,
1100 R. E. Lee Blvd., P. O. Box 19687
New Orleans, LA 70179

Future Outlook

In 1980, this study is to be expanded to about 20 of a possible 33 countries (South temperate zone and tropics they are planted in our fall and winter)

Opaque-2, high lysine type both hard and soft kernels will be grown in at least 10 countries (locations).

Data will be acquired relative to the CBC black light, system shaker.

The primary sources of knowledge of corn and handling indicated in the Central America trip to Guatemala and other common market countries of C.A. is with commercial industries involved with animal feeds.

PROBLEMS and ISSUES POSED by the PROJECT MANAGER

1. *Is the assumption that aflatoxin is a serious health problem in maize-growing LDCs worldwide valid and supported by research data?*

Yes. Epidemiological data from Taiwan, Uganda, and Thailand as cited in the background for this project indicate part of the human health hazard of aflatoxin. However, data from Mozambique and India show direct correlations for the aflatoxin levels with human liver carcinoma and in some cases there have been good correlations of aflatoxin in the diet with Reye's syndrome in children. In 1975, over 100 people died and another 300 were afflicted from the high level of aflatoxin consumed from locally grown corn in India.

2. *Is the assumption that preharvest reduction of aflatoxin will significantly reduce aflatoxin consumption in foods and feed valid and supported by research data? Will postharvest buildup of aflatoxin in storage negate preharvest aflatoxin reduction?*

Yes. Growing corn lines that resist field invasion by the fungus and/or subsequent buildup of aflatoxin in the kernel from biochemical resistance would be the most effective way to control aflatoxin formation in LDCs or USA. HOWEVER, it would not probably remove the necessity of proper harvesting, drying and storage of corn after harvest. Improper drying or rewetting in poor storage facilities would be very likely result in A. flavus invasion and aflatoxin production in corn or any other grain, seed, feed, or processed food.

3. *Is the assumption that resistance breeding is a valid approach to preharvest aflatoxin reduction (i.e. reduction of fungus growth or toxin synthesis) valid and supported by research data?*

Yes. This is the most efficient way to control any plant disease. Observations were numerous that A.flavus grew poorly if at all on the kernels of some corn varieties and thus the germplasm for breeding for resistance would not be hard to find. Germplasm resistant to invasion by Fusarium species has been found and utilized in corn varieties to decrease losses due to Fusarium ear rots in corn. However in this research identifying resistant lines has been extremely difficult to date. This fact was not identified the first year because the aflatoxin analyses waited for the P-3 Lab. High coefficients of variation are and always have been the rule in aflatoxin analyses. Failure to get reproducible results may involve several factors. The investigators are dealing with highly variable material, since most individual kernels produce different levels of toxin and the individual spores that initiate fungus invasion may have different toxin producing potentials.

4. *Are the research techniques being used to inoculate corn plants and seeds with A. flavus and A. parasiticus proper to assess resistance to fungus growth and inhibition of aflatoxin synthesis?*

Yes and No. Both the half-kernel technique and the pin-board inoculation method afford great potential in theory to differentiate between resistant and susceptible corn varieties or inbreds. In practice, the investigators have been notably unsuccessful in identifying resistance. Once the aflatoxin analyses were in, they immediately recognized the problem as "overkill". This single word in this context denotes that the techniques are too severe so that practical field resistance if present cannot be distinguished from susceptibility. This may

be the result of the interplay of several variables in the techniques. (1) The load of inoculum (over one million spores per ear) used in the field may be flooding the protective mechanisms in the varieties having some level of practical field resistance. (2) The injury mechanism of the pin-board may be too severe, since it severely damages the kernels and the fungus proliferates most vigorously on the nutrients freed from the kernel. (3) The chamber for holding the moisture high under the shuck on the ear of corn may be overly favorable for fungus growth and not permit normal daily changes in moisture levels to occur. (4) The analyses per ear and per variety may be drawn from too small a sample of kernels to eliminate the wide variability between kernels and the wide variability between infecting spores (toxin-producing ability) to identify low levels of resistance. (5) Genetic resistance may involve several genes that may not all be present in any present corn line under test. Nevertheless, greater sensitivity must be introduced into this method by evaluating the several variables until an effective inoculation method is developed. (6) The aflatoxin level criterion appears open to question. Differences between resistance and susceptibility will require data varying from less than 100 ppb for resistance to the high figures of 1,000 to 40,000 ppb in susceptible lines. It is questionable in the team leader's mind as to whether the techniques used have identified any resistance due to the high inoculum load etc. Obviously, the half kernel technique of Wallin and Loonan to make a significant contribution to this project must correlate with the field technique. This method may also need refinement as genetic differences in varieties it has identified have not been confirmed in the field. However, it could be that only the field test is too severe. However, evaluations and adjusting of the procedures in the half-kernel technique should be studied further, also.

It should be kept in mind especially by the plant pathologists that A. flavus is more a saprophyte than an aggressive plant pathogen. For this reason alone, the techniques may have to be adjusted or varied to make them work. However, Fusarium in many instances is saprophytic and resistance to fusaria in corn have been utilized by the breeder.

5. *Is research on persistence of aflatoxin in the soil germane to the overall project objectives?*

No. This research raises some interesting points that should be pursued, but they do not appear to be pertinent to this program.

6. *Are linkages with LDCs, International Agricultural Research Centers, and other aflatoxin research efforts in the U.S. adequate?*

Yes. At this time, the investigators appear to have working relationships established with CIMMYT and to a number of LDCs. They are certainly adequate for this stage of the project.

7. *What is the most recent medical assessment of the correlation between aflatoxin and human and animal health (i.e. carcinoma)?*

As noted in reply to the first question, more and more data is accumulating to the effect that where options in human food are few, there are direct relationships between high aflatoxin levels in food and mortality and carcinoma in humans. The correlation has been both experimentally and in reality proven in domestic and experimental animals with aflatoxin and other mycotoxins.

8. *Are the Univ. of Missouri staff and facilities adequate to accomplish the project objectives in a reasonable period of time?*

Yes. The principle investigator is a scientist of the highest integrity and has been a plant breeder-geneticist for many productive years. He has good support in Dr. Darrah, who shows great potential and is also highly competent. Collaboration with the plant pathologists, Dr. Calvert and Wallin, is good and effective working relationships and interaction can be seen. The facilities and capability of the personnel analyzing toxin-contaminated materials are excellent. The facilities and support from the central administration is quite good as anticipated in a land-grant institution.

The investigators are quite realistic about their lack of progress and are possibly more pessimistic than the review team. They had recognized the need to refine the inoculation techniques before this review. In view of the slowdown for the P-3 Lab, it is most fortunate and credit to the PI, group, and institution that adequate funds still remain to make the changes and still accomplish the objectives of the project. However, it is well to remember that breeding-type research is always a long range research approach and is seldom accomplished or completed in 3 to 5 years. However, significant breakthroughs can be made to assure the ultimate accomplishment of the goals set forth.

9. *Given the current project and budget expenditures to date, should an unfunded extension of the project be considered for completion of the work outlined in the current project paper? (Also should a new project paper be developed to more accurately outline the future direction of the project?)*

Yes. Although little specific progress has been made to date, this is probably because of overoptimism that resistant genotypes in maize would be easily and quickly identified. Despite the setbacks, the research team is in place and this negative experience has had an integrating effect and improved the interactions between members of the team in bringing them to realize that each persons contribution is needed and essential for team success. They are agreed that the major effort must be in refining the experimental techniques as elucidated elsewhere in these comments. AID needs to review the project later to determine that meaningful progress is being made. It was agreed in the final session that both budgets for the next two years beyond September 30, 1980 and a work plan for those two years would be developed and become a part of this report. The researchers are competent and dedicated. Regardless of this fact, there is never any guarantee that every research project will gain all its ends or accomplish all of its objectives.

The extension as well as the original project can still identify sources of resistance,although an acceptable resistant variety in 2 years is probably impossible. However, valuable genetic information on aflatoxin resistance can be gained that will be useful to corn breeders everywhere.

PUBLICATIONS DURING THE PROJECT

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2. Fennell, D.I., W.F. Kwolek, E.B. Lillehoj, G.L. Adams, R.J. Bothast, M.S. Zuber, O.H. Calvert, W.D. Guthrie, A.J. Bockholt, A. Manwiller, and M.D. Jellum. 1977. Aspergillus flavus presence in silks and insects from developing and mature corn ears. Cereal Chem. 54:770-778.

3. Lillehoj, E.B., O.H. Calvert, W.F. Kwolek, and M.S. Zuber. 1979. Aflatoxin variation among corn samples with varying ratios of Aspergillus flavus-inoculated/noninoculated kernels. J. Assoc. Off. Anal. Chem. 62:1083-1086.

4. Lillehoj, E.B., D.I. Fennell, W.F. Kwolek, G.L. Adams, M.S. Zuber, E.S. Horner, M.W. Widstrom, H. Warren, W.D. Guthrie, D.B. Sauer, W.R. Findley, A. Manwiller, L.M. Josephson, and A.J. Bockholt. 1978. Aflatoxin contamination of corn before harvest: Aspergillus flavus association with insects collected from developing ears. Crop. Sci. 18:921-924.

5. Lillehoj, E.B., W.F. Kwolek, M.S. Zuber, O.H. Calvert, E.S. Horner, N.W. Widstrom, W.D. Guthrie, G.E. Scott, D.L. Thompson, W.R. Findley, and A.J. Bockholt. 1978. Aflatoxin contamination of field corn: Evaluation of regional test plots for early detection. Cereal Chem. 55:1007-1013.

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BACKGROUND PUBLICATION ON AFLATOXIN IN CORN

7. Zuber, M.S., and E.B. Lillehoj. 1979. Status of the aflatoxin problem in corn. J. Environ. Qual. 8:1-5.

PUBLICATIONS BY PROJECT PERSONNEL PRIOR TO THE PROJECT

8. Lillehoj, E.B., W.F. Kwolek, A. Manwiller, J.A. DuRant, J.C. LaPrade, E.S. Horner, J. Reid, and M.S. Zuber. 1976. Aflatoxin production in several corn hybrids grown in South Carolina and Florida. Crop Sci. 16:483-485.

9. Lillehoj, E.B., W.F. Kwolek, E.E. Vandegraft, M.S. Zuber, O.H. Calvert, N. Widstrom, M.C. Futrell, and A.J. Bockholt. Aflatoxin production in Aspergillus flavus inoculated ears of corn grown at diverse locations. Crop Sci. 15:267-270. (1975).

10. Lillehoj, E.B., and M.S. Zuber. 1975. Aflatoxin problem in corn and possible solutions. Proc. 30th Annu Corn Sorghum Res. Conf. pp 230-250.

11. Zuber, M.S., O.H. Calvert, E.B. Lillehoj, and W.F. Kwolek. 1976. Preharvest development of aflatoxin B_1 in corn in the United States. Phytopathology 66:1120-1121.

RECOMMENDATIONS OF THE REVIEW TEAM - APRIL 8,9, 1980

1. A two-year unfunded extension of the project "Aflatoxin Reduction in Maize" AID Project No. 931-1181

2. Reassessment of validity of techniques used to identify resistance in corn lines. The inadequacy of inoculation methods did not become apparent until the aflatoxin analyses were done, which had been delayed until the P-3 laboratory was constructed.

   Improvement in Methods.

   A. Field inoculation techniques need to be evaluated for several variables.

      (a) Inoculum concentration and amount, (b) Degree of kernel injury,

      (c) Moist chamber technique, (d) Aflatoxin level significant to denote resistance.

   B. Uniformity Trials.

   C. Sampling Procedures.

   D. Review methodology with other aflatoxin researchers.

   E. Reassess the aflatoxin level criterion for resistance in corn kernels.

3. Strengthen IARC cooperation (i.e. CIMMYT) by traveling to cooperating states, countries, and bring in CIMMYT representatives to Columbia.

4. Organize a Workshop in about one year.

5. Publish an Annual Report and Scientific Accomplishments in Journals.

6. Progress Review about August-September, 1981.

7. Request Seed Storage Facility on Cost-Sharing Basis in Budget.

DIRECTION OF PROJECT FOR THE NEXT TWO YEARS (1980-82)

Current results suggest that differences may exist among maize genotypes for aflatoxin levels as the result of either natural or artificial inoculation with Aspergillus flavus. However, it has been difficult to demonstrate consistent differences among genotypes from year to year or location to location. The failure to show repeatability may be due to the extremely high error which results from the inoculation and sampling procedures.

Many factors have been suspected to contribute to the large error especially when artificial inoculation with A. flavus has been used. Since the fungus is a very weak pathogen it cannot penetrate the seed coat. Thus, insects that feed on immature kernels act as vectors by carrying spores of the fungus to the site where a break in the kernel seed coat has occurred by insect feeding. An inoculation method that involved a row of pins was used to injure several rows of kernels. The injury was made about 20-25 days post-flowering followed by spraying the injured kernels with A. flavus inoculum. This method appeared to simulate natural infection by insect vectors. Inoculated ears were harvested at about 30 days post-inoculation and dried rapidly at 60C for five days. Only infected kernels were analyzed for aflatoxin. The net result was very high levels of aflatoxin with extreme fluctuations among samples of the same genotypes. The high levels of aflatoxin and their erratic fluctuation among replications and locations may be swamping differences due to genotypes, under the assumption that aflatoxin levels are under genetic control.

Some of the factors that may contribute to the large errors encountered include:

I. Variability among kernels on the same ear
   A. Genetically (F_2 kernels on F_1 plants), heterogenous as in exotic strains
   B. Genetically alike such as inbred lines selfed etc., but aflatoxin levels differ due to (II) mentioned below
II. Method of inoculation
   A. Depth and degree of kernel injury
   B. Differing amounts of conidia concentration
      1. Number
      2. Amount of inoculum applied
   C. Kind of inoculation
      1. Hypodermic syringe
      2. Pinboard
      3. No injury
      4. Silk inoculation
      5. Air brush
      6. Immature ear sections (in vitro)
III. Sample size
   A. Number of infected kernels to best estimate aflatoxin level
      1. For individual ears, kernels alike genetically
      2. Kernels heterogeneous genetically
   B. Total infected kernels per plot (no/kernels x no of ears)
   C. Number of replications per entry (no/ears x no ears/plot x no of plots)

IV. Effect of the environment
   A. Stress Natural (i.e. drought during grain-filling period).
   B. Induced stress (i.e. leaf removal, etc.)

In 1980, several experiments will be conducted with the objective of obtaining answers to many of the above questions.

Also, an interregional study to determine the effect of endosperm types on naturally occurring infection of A. flavus and subsequent preharvest aflatoxin levels in maize will be conducted in 1980. Corn will be grown at 14 locations in the U.S. Protocol for the study is attached.

In another study of the same material, we will determine kernel hardness to determine if this characteristic is related to observed kernel rot infection.

Corn inbred lines have been observed to differ in the amount of zinc accumulated in the grain. Previous in vitro research in India with high-lysine maize (0-2) suggested that high levels of zinc enhanced aflatoxin levels. Field and in vitro studies are planned to determine if a relationship exists between zinc levels and aflatoxin-susceptibility in corn.

An international study to determine the Environment-Maize Genotype interaction on naturally occurring toxins was initiated in 1979. A copy of the protocol is attached. Six entries in the CIMMYT International Maize Variety Trials (EVT 13) are being sampled in seven different countries. Thus far, grain samples have been received from three of the seven countries. In 1980, the study will be expanded to include 20 countries located in either tropical or subtropical regions of the world.

Specific Plans

Dr. O. H. Calvert

A control or prevention of aflatoxin B_1 contamination of corn has been sought without success. Results have been highly variable following the spraying of corn with conidia of A. flavus after mechanical injury of kernels. These routine inoculations are now perceived as being harsh and too time-consuming. Natural field resistance to aflatoxin B_1 production in maize has possibly been overlooked.

The inoculation methods proposed here are to uncover the expression of host resistance and to shorten the time necessary to carry them out. Field inoculations will be made by inoculating the silk with an array of A. flavus strains that produce aflatoxin B_1, B_2, G_1 and/or G_2 in varying amounts. Aflatoxin production within the mycelium of these fungi is under genetic control and it is assumed to be the same with the host if it can be identified in the host-pathogen association. This may be accomplished by a new inoculation method.

Further, this project proposes to speed up the search for the genetic control in the host by testing various corn inbred and hybrid strains in the laboratory. Mature kernels are rehydrated and inoculated without injuring

the pericarp with conidial suspensions. New crosses and inbreds have to originate in the field. But the use of field only experiments to locate sources of genetic controls results in only one set of results per year. In addition, field experiments are acted upon by a variety of environmental factors. By going to a repeatable laboratory method the finding of a source of resistance may be speeded up several fold.

The present method of inoculation will be evaluated by varying degree and method of injury, inoculum concentration, amount applied, and alternative types of moist chambers.

Dr. Art Karr

The effect the position of the fungus in the kernel has on fungal growth rate and on aflatoxin accumulation will be determined. The F_1 seeds from the reciprocal cross between a high reaction and a low reaction (in terms of aflatoxin accumulation) inbred lines will be used. Each kernel will be cut into 5 slices. The slices will be separated into groups based on their position in the kernel. The slices will be hydrated and inoculated with A. flavus. At regular times, groups of slices will be removed and analyzed for aflatoxin content and for fungal growth (the latter by immuno-electrophoresis). The determination of whether fungal growth and aflatoxin production differ in the endosperm and embryo regions is important to the project for several reasons. First, it will permit a determination of whether eventual levels of aflatoxin will vary depending on the depth to which the inoculum is introduced. Second, it will provide a valid control of certain selection procedures (such as the half kernel method) that may be measuring only resistance in the endosperm tissue.

Using these data, an investigation will be initiated into the basic biological differences in the interaction between the fungus and those tissues that permit or restrict fungal growth. This will first be carried out at the light microscopic level using traditional stain technology and labeled con-canavilin A. Such a study is attractive because it is an inexpensive means of providing some of the basic information needed to support the decisions made in the breeding project.

Dr. Jack Wallin

A three-pronged attack in the search for genetic resistance to Aspergillus flavus and minimal levels of aflatoxin will involve:

(1) The unwounded exotic flint corns obtained from Mexico and Guatemala will be tested by inoculation with cultures of A. flavus in the laboratory. Those corns demonstrating the greatest tolerance or resistance will be used for further testing by the decapped kernel method in petri dishes. After the flinty types are studied, other types will be challenged.

(2) Those accessions showing tolerance to A. flavus will be screened by the decapped kernel method in petri dishes to determine if they are chemically constituted toward the production of low levels of aflatoxin assuming that this constitution is genetically controlled. Kernels from

those populations that fail to fluoresce will be removed from the plates, planted in the greenhouse, then later, transplanted to the field. Mature plants will be inoculated with *A. flavus* and their reaction noted. Some method of wounding will be used as this is the mode of entry for the fungus.

(3) Study of the response of *Zea diploperennis*, the perennial teosinte, to *A. flavus* and monitoring of the amount of aflatoxin produced in the seeds by the fungus will be continued.

TITLE: Determine the effect of endosperm types on natural *Aspergillus flavus* infection and subsequent preharvest aflatoxin levels in maize.

OBJECTIVES:

1. Determine the effect on endosperm type on natural occurring *A. flavus* infection and subsequent aflatoxin levels.
2. Compare specific endosperm types with their normal counterparts in susceptibility to *A. flavus* and aflatoxin levels.
3. Determine if endosperm types and their normal counterparts interact with the environments provided by different locations.
4. Study the association of kernel hardness of different endosperm types on *A. flavus* infection and aflatoxin levels.

COOPERATORS:

1. Earl Horner, Gainseville, FL
2. Neil Widstrom, Tifton, GA
3. Al Manwiller, Florence, SC
4. Don Thompson, Raleigh, NC
5. O. H. Calvert, Columbia, MO
* 6. L. L. Darrah, Columbia, MO
7. Tony Bockholt, College Station, TX
8. Dennis West, Knoxville, TN
9. William Findley, Wooster, OH
10. Dave Sauer, Manhattan, KS
11. John Henshaw, Baton Rouge, LA
12. Gene Scott, State College, MS
*13. Stan King, State College, MS
*14. Urban Diener, Auburn, AL
15. Herman Warren, Lafayette, IN

METHODS:

1. Plots will consist of two rows of 20 plts, replicated twice at each location; i.e., 80 k/entry needed (100 k will be sent).
2. Ten plants in each of two rows per entry will be either self or sib pollinated since most of the endosperm mutants are recessive any normal pollen will give a normal kernel regardless of the recessive endosperm type. Three days post pollination, the shoot bags or ear bag will be removed to allow the chance for natural infection by *A. flavus* to occur.
3. At harvest the 10 hand-pollinated ears will be harvested.
4. Harvested ears will be scored for insect damage on a plot basis.
5. Harvested ears will be dried at 60°C for 5-7 days and shipped to Southern Regional Research Center in New Orleans, LA, for aflatoxin determination.

MATERIALS:

	Entries	Source
1	High amylose class 5 (*ae*)	Custom Maize, 85588
2	High amylose class 7 (*ae*)	Custom Maize, 85473
3	Waxy (*wx*)	Custom Maize, 85070
4	Normal counterpart, dent	Custom Maize
5	High-lysine (*o-2*), Pioneer Brand L3369	Pioneer International

6	Normal counterpart, Pioneer Brand 3369A	Pioneer International
7	SC54 o-2 x SC76 o-2 , high lysine	Manwiller, South Carolina
8	SC54 x SC76, normal counterpart	" " "
9	SC54 fl-2 x SC76 fl-2	Manwiller, South Carolina
10	High oil 6.5%	Lester Pfister
11	Mo17 x B73 (near normal counterpart)	MFA Seed Division
12	Cargill Hybrid I, flint	Cargill, Grinnell, IA
13	" " dent	" "
14	Cargill Hybrid II, flint	Cargill, Grinnell, IA
15	" " dent	" "
16	Cargill Hybrid III, flint	Cargill, Grinnell, IA
17	" " dent	" "
18	Mo17 x H60	79:787,788 MO
19	Mo5 x Oh545	79:797,798 MO
20	MoSQA(H)C6	79:2307-2316 MO

ANALYTICAL LOAD:

20 entries x 12 locations x 2 reps = 480 aflatoxin determinations

ADDITIONAL INSTRUCTIONS:

1. Three labels for each entry marked with plot number, location number, and experiment number are included. Two of the labels are for plot envelopes and the third must be used at harvest for placing on the tag for identification.
2. We are asking that each plot be rated for insect damage on a 1 to 5 basis where one represents no injury and five, maximum injury.
3. Since hand pollinations are to be made in each plot, we would appreciate that the average pollination date be recorded.
4. Data sheets for recording the above data will be included in this mailing. The seed will be sent under separate cover.
5. Send the data sheets upon completion, to Dr. Lillehoj at the SRRC at New Orleans, LA, under separate cover from the samples.
6. Addressed, franked labels will be furnished for sending samples to SRRC at New Orleans. Boxes will be furnished upon request.

* Not part of this experiment

MSZ/1980

1979 International Environment-Maize Genotype Study

by

M. S. Zuber and E. Lillehoj

Cooperators:
E. Lillehoj, SRRC-USDA
M. S. Zuber, University of MO
E. W. Sprague, CIMMYT
A. Ciegler, SRRC-USDA
L. L. Darrah, SEA-USDA
R. L. Paliwal, CIMMYT

Field cooperators:
M. Torregroza, Columbia
A. L. P. Sinclair, Costa Rica
C. Grand-Pierre, Haiti
D. R. B. Manda, Malawi
S. Jinahyon, Thailand
E. Serrano, Philippines
S. Bokde, Mozambique

Objectives:
1. Determine the maize-genotype environment interaction effect on fungal infection and toxin accumulation in preharvest maize grain samples.
2. Confirm preliminary findings that toxin levels in preharvest maize kernels are under genetic control.
3. Identify maize genotypes with minimum kernel rots and with the least kernel injury by insects.
4. Establish environmental influence on the interaction between insect and fungal predators of developing maize ears.

Material: Maize trials conducted in various countries by CIMMYT will be available for sampling.

In 1979, the initial study will be restricted to seven countries:

1. Columbia, South America
2. Costa Rica, Central America
3. Haiti, Caribbean
4. Mozambique, Africa
5. Thailand, Asia
6. Philippines, Asia
7. Malawi, Africa

We have arranged through the Foreign Agricultural Service (FAS) to move the collected samples via diplomatic Air Pouch from the country involved to the U.S.. Mr. John DeCourcy, FAS, has informed the appropriate authorities in the U. S. Embassies of the study. Corn samples will be submitted by cooperators to the officials listed below for shipment to the U.S.:

Page 2

1.	Agricultural Attache U.S. Embassy	Bogatá D.E. Columbia
2.	" "	San José, Costa Rica
3.	" "	Santa Domingo, Domincan Republic
4.	" "	Bangkok, Thailand
5.	" "	Manila, Philippines
6.	Economic officer - State Dept.	Maputo, Mozambique
7.	Agency for International Development (AID)	Lilongwe, Malawi

We have chosen the entries listed below for sampling. These entries are from the International Experimental Variety Trial (EVT13) conducted by CIMMYT in 1979.

Entry no.*	Strain name
2	La Maquina 7827
3	Sete Lagoas 7728
4	Across 7728
10	Suwan 7726
12	Sta Cruz Parillo(1) 7835
14	Tocumen (1) 7735

Sampling procedure

The cooperator in each of the seven countries will obtain ca 5 kg of shelled mature maize grain from each of the above six entries.

From replications 1 and 2, combine and obtain 2.5 kg of grain and from reps 3 and 4, obtain the other 2.5 kg. Samples must be dried as rapidly as possible after harvest to 12% grain moisture. Each sample bag should include a tag inside and outside of the bag with the entry number, replication number, and the country of origin.

Information for the field cooperators

The 2-2.5 kg samples from each country for each of the six designated entries can best be obtained and will be most representative by bulking replications 1 and 2 for one 2.5 kg sample, and replications 3 and 4 for the second 2.5 kg sanple of the same entry. Information to be provided from each location includes (1) cultural practices (including planting date, fertilizer applied, and any pesticide applied to the growing crop), (2) meteorological description of the growing season (rainfall, minimum and maximum temperature), (3)

elevation (meters) of the planting site, (4) prevalent insects that have fed on the growing crop, and (5) flowering and harvest dates.

All samples should be in double cloth bags for shipment to the USA. No insecticide of fungicide should be applied to the post harvest grain sample.

Information for the Agricultural Attaché or AID representative

You will be furnished the name and location of the cooperator in the country where you will be asked to obtain the maize grain samples. It would be desirable for you to contact the cooperator before the crop is harvested.

Sample processing

The 72-2.5 kg whole kernel samples will be forward through the U.S. Agricultural Attaché FAS to:

John DeCourcy
USDA, FAS
Grain and Feed Division
14th and Independence
Washington, D.C. 20250

Subsequently after being received by John DeCourcy, he will forward the samples to:

Mr. Frank Cooper
USDA, PPQ, APHIS
Federal Building, Rm 635A
Hyattsville, Maryland 20782

After clearance by APHIS, the samples will be sent to Dr. Eivend Lillehoj, where they will be anlayzed for fungi and toxins.

Dr. Eivend Lillehoj
Southern Regional Research Lab
1100 Robert E. Lee Blvd.
P.O. Box 19687
New Orleans, Louisiana 70179

Table 3. Cost Estimates for Maize-Aflatoxin Project - AID/ta-C-1451

	1980-81	1981-82
	SALARIES AND WAGES	
1. Project supervisor	$ 7,500	$ 8,250
2. Technicians (Res. Specialist, Sr. Res. Tech., and Res. Tech.)	31,580	34,737
3. Graduate Research (1 half-time asst.)	6,600	7,200
4. Hourly labor	2,500	2,750
total salaries and wages	48,180	52,937
STAFF BENEFITS (16% of positions 1 and 2; 5.85% of position 4)	6,399	7,039
	SUPPLIES, EQUIPMENT, TRAVEL	
5. Travel and transportation	2,000	2,200
6. Equipment	1,000	**
7. Misc. supplies (analytical supplies, medical exams, etc.)	12,000	13,200
8. SUBCONTRACTS	1,500	1,650
TOTAL DIRECT COSTS	71,079.	77,026.
INDIRECT COSTS (53.99% of salaries and wages	26,012.	28,581.
ANTICIPATED EXPENDITURES	97,091.	105,607.
EXPENDITURES TO 3/31/80		192,538.07
TOTAL PROJECTED FOR 2-YR EXTENSION		202,698.00
TOTAL		395,236.07
EXPENDITURES 4/1/80 TO 9/30/80		18,682.93
TOTAL BUDGETED FOR GRANT		413,919.00