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AGENCY FOR INTERNATIONAL DEVELOPMENT

PROJECT PAPER FACESHEET

1. TRANSACTION CODE: A (A = ADD, C = CHANGE, D = DELETE)

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2. DOCUMENT CODE: 3

3. COUNTRY/ENTITY: TA Bureau Research RDA #28

4. DOCUMENT REVISION NUMBER: Original 16p

5. PROJECT NUMBER (7 digits):

6. BUREAU/OFFICE: A. SYMBOL: TAB; B. CODE: 08

7. PROJECT TITLE (Maximum 40 characters): AFLATOXIN REDUCTION IN MAIZE

8. ESTIMATED FY OF PROJECT COMPLETION: 79

9. ESTIMATED DATE OF OBLIGATION: A. INITIAL FY 77; B. QUARTER 4 (Enter 1, 2, 3, or 4); C. FINAL FY 79

10. ESTIMATED COSTS ($000 OR EQUIVALENT $1 -)

A. FUNDING SOURCE	FIRST FY 77 B. FX	C. L/C	D. TOTAL	LIFE OF PROJECT E. FX	F. L/C	G. TOTAL
AID APPROPRIATED TOTAL	265		265	415		415
(GRANT)	(265)	( )	(265)	(415)	( )	(415)
(LOAN)	( )	( )	( )	( )	( )	( )
OTHER U.S. 1.						
OTHER U.S. 2.						
HOST COUNTRY						
OTHER DONOR(S)						
TOTALS	265		265	415		415

11. PROPOSED BUDGET APPROPRIATED FUNDS ($000)

A. APPROPRIATION	B. PRIMARY PURPOSE CODE	PRIMARY TECH. CODE C. GRANT	D. LOAN	E. 1ST FY 77 F. GRANT	G. LOAN	H. 2ND FY ___ I. GRANT	J. LOAN	K. 3RD FY 79 L. GRANT	M. LOAN
(1) IN	171 J	973		265				150	
(2)									
(3)									
(4)									
TOTALS									

A. APPROPRIATION	N. 4TH FY ___ O. GRANT	P. LOAN	Q. 5TH FY ___ R. GRANT	S. LOAN	LIFE OF PROJECT T. GRANT	U. LOAN
(1)					415	
(2)						
(3)						
(4)						
TOTALS						

12. IN-DEPTH EVALUATION SCHEDULED: MM 03 YY 79

13. DATA CHANGE INDICATOR. WERE CHANGES MADE IN THE PID FACESHEET DATA, BLOCKS 12, 13, 14, OR 15 OR IN PRP FACESHEET DATA, BLOCK 12? IF YES, ATTACH CHANGED PID FACESHEET.

X (1 = NO, 2 = YES)

14. ORIGINATING OFFICE CLEARANCE

SIGNATURE: Leon F. Hesser

TITLE: Leon F. Hesser
Director, Office of Agriculture

DATE SIGNED: MM 05 DD 10 YY 77

15. DATE DOCUMENT RECEIVED IN AID/W, OR FOR AID/W DOCUMENTS, DATE OF DISTRIBUTION: MM 05 DD 10 YY 77

PROJECT STATEMENT

A. PROJECT SUMMARY

1. Statistical

Project Title:	Reduction of Aflatoxin Incidence in Maize
Project Status:	New Contract
Contractor:	The Curators of the University of Missouri, on behalf of the Missouri Agricultural Experiment Station, Columbia, Missouri 65201
Principal Investigator:	Dr. Marcus S. Zuber
Duration:	Three years beginning October 1, 1977
Total Estimated Cost:	$415,000 for three years
Funding by Years:	First Year $125,000 Second Year 140,000 Third Year 150,000 (Funding in FY 77 265,000)

2. Narrative Summary

Aflatoxin has recently been discovered to be of wide occurrence in maize, particularly in warm, humid areas. Since this mycotoxin is known to be a potent carcinogen in animals and to have lethal effects when ingested in sufficient quantity, it is suspected of being very dangerous to human health. 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 *Aspergillus* species or inhibit the synthesis of aflatoxin. Resistant maize types would then be used in breeding programs to produce agronomically desirable strains having reduced likelihood of containing aflatoxin.

B. RESEARCH PURPOSE, OBJECTIVES, AND EXPECTED PRODUCTS

1. Purpose

This project is intended to provide basic information and source materials which can then be used to increase the supply of high-

quality human food in maize-eating developing countries, by reducing or eliminating a serious health hazard now frequently occurring in maize.

2. Objectives

The research will have two major specific objectives:

a. Identify maize genotypes with resistance to kernel rots caused by *Aspergillus flavus* and/or *A. parasiticus*.

b. Search for genetic factors that inhibit the biosynthesis of aflatoxins following fungal growth of these *Aspergillus* species.

3. Expected Products

a. Knowledge of the effectiveness of genetic screening for resistance to *Aspergillus* growth and to toxin production.

b. Plant genetic stocks resistant to growth of *Aspergillus* species.

c. Genetic stocks carrying factors which inhibit aflatoxin synthesis even when some growth of *Aspergillus* fungi occurs.

C. SIGNIFICANCE AND RATIONALE FOR THE RESEARCH

1. The Development Problem

Aflatoxins are a group of toxic, secondary fungal metabolites or mycotoxins produced by *Aspergillus flavus* ex Fr. and *A. parasiticus* Speare during growth on feed or food products. Aflatoxins occur in a wide variety of agricultural commodities (8) including maize (*Zea Mays* L.), peanuts (*Arachia hypogaea* L.), and cotton (*Gossypium* spp.). Until recently aflatoxin contamination of maize was considered a post-harvest, stored grain problem but recent surveys showed that the toxins occurred in preharvest maize (3, 16).

Aflatoxins have been shown to be potent carcinogens in animals and at sublethal levels can dramatically reduce the feed efficiency and general health of domestic animals. Typically, the toxins cause liver changes which are routinely characterized as precancerous (5).

Aflatoxin contamination has been detected in maize grown throughout the world with particularly high levels occurring in those hot, humid, maize-growing nations which include a number of the developing nations. Since maize constitutes a major part of the human diet in many of these developing countries, the toxins can be deleteriously affecting the general health of millions of people.

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 for this dangerous human health hazard.

2. State-of-the-Art

a. Acute Toxicity. Most reports of acute aflatoxin poisonings come from areas which also have suspected problems of chronic aflatoxicoses. These include reports from Taiwan (9), Uganda (11), and Thailand (4). In a number of individual cases people died after eating food containing high levels of aflatoxin B_1. Some individuals died as soon as 6 hours after ingesting the contaminated food. In the case in Uganda (11), an autopsy and histopathological examination revealed changes which were typical of those observed in laboratory animals suffering from acute aflatoxin poisoning. In all cases only individuals eating the contaminated food became ill.

More direct evidence involving acute aflatoxin poisonings in man comes from case studies of Reye's syndrome in Thailand. This "disease" of adolescent children is epidemic in parts of Thailand. It is characterized by a short prodrome of several hours followed by vomiting, hypoglycemia, convulsions, hyperammoniemia, and coma, usually ending in death 24-48 hours after onset. In one case a 3-year old Thai boy died after eating rice containing high levels of aflatoxin. An examination indicated the child had died of Reye's syndrome. Additional specimens collected from 23 Thai children who died of Reye's syndrome showed aflatoxin in the bodies of 22 of the 23 children. Finally, it has been shown that macaques (but not rats) which are acutely poisoned with aflatoxin develop symptoms quite similar to those that define Reye's syndrome (4).

b. Chronic Toxicity. Considerable presumptive evidence links consumption of sublethal levels of aflatoxin with the development of liver tumors in man. For example a recent report of A. Ciegler, NRRC, ARS (Peoria, Illinois) states that over 1,000 cases of hepatoma were found in a town of 50,000 residents in Costa Rica. Maize (70-90 per cent of which was aflatoxin contaminated) constituted the major portion of the resident's diet. It is likely that the continual ingestion of aflatoxin contributed to the unusually high number of liver tumors found in the population.

More complete evidence is available from long term studies carried out in Uganda, Kenya, Swaziland, and Thailand (1, 6, 10, 12, 13, 14, 15). In the study of Kenya (10), it was possible to relate

the incidence of primary liver cancer (from data in the Kenya Cancer Registry) to the level of aflatoxin consumed in the diet for people in different areas of the country. Relating these data yielded a regression line $y = 19.06 \log_{10} X - 10.16$ with $r^2 = 0.87$ for 4 degrees of freedom ($0.05 > P > 0.02$). Even more interesting was the fact that data collected in Thailand (relating the incidence of liver cancer to the level of aflatoxin in consumption) fit the regression line drawn from data collected in Kenya. The agreement between data in these two studies, the results of the other studies cited above, and the clear demonstration of the carcinogenicity of aflatoxins in experimental animals strongly suggests that these compounds cause liver tumors in human populations.

Cancer may not be the only consequence of man's chronic exposure to aflatoxin. Some evidence exists that aflatoxin ingestion may be a factor in a disease known as Indian childhood cirrhosis. This is a widely occurring disease with a peak of incidence at 3 years. There is direct evidence implicating aflatoxin ingestion with this disease. Indian children being fed a plant protein supplement developed symptoms reported to be identical to those seen in cases of Indian childhood cirrhosis (2). The protein supplement was found to contain 300 mg/kg aflatoxin.

c. Occurrence of Aspergillus and Aflatoxin on Maize. Aflatoxin at low levels has been reported from commercial maize samples in the U. S. for a number of years. A detailed survey was undertaken in 1971, and results as reported by Anderson et al (3) indicated that the toxin occurred much more frequently, and in higher concentrations, than had been thought likely. Also, where this had previously been considered to be a postharvest storage problem, these studies (3) clearly showed that the major infection by *Aspergillus* and production of the toxin occurs on the maize ear, in the field, before the grain is fully mature. 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.

d. Genetic Resistance. Many cases of successful breeding of maize for genetic resistance to plant diseases are known. Breeding for resistance to *Aspergillus* species has not previously been a specific research objective because the fungus was not considered to be a major disease hazard to the maize crop. Clear evidence of major genetic resistance to a closely-related toxin-producing fungus (*Pencillium oxalicum*) has been available for many years (7). The work of Anderson et al (3), Zuber (17), and others clearly indicates that genetic variability exists in maize for susceptibility to aflatoxin production.

D. PLANS TO DEVELOP LINKAGES AND TO FACILITATE UTILIZATION OF RESULTS

1. CIMMYT and IITA

Germ plasm collections and breeding stocks will be obtained from CIMMYT and IITA as well as other major maize germ plasm programs. Results of screening trials will be communicated to CIMMYT, and IITA, along with germ plasm samples of resistant types identified. Personal visits will be paid to international center headquarters and major breeding stations and center personnel will be invited to visit laboratories and field research facilities of the contractor.

2. Major Regional and Country Programs

Direct cooperation with the national maize program of Guatemala (advised by CIMMYT) is planned. Short-day types of maize which cannot be grown to maturity in the U. S. will be tested in Guatemala, through cooperation with ICTA and its CIMMYT-advised national maize program.

3. Commercial Seed Firms

Cooperation will be established with one or more major commercial seed firms which have operations in developing countries. Pioneer Hi-Bred International has already agreed to cooperate through its Latin American research program and also by furnishing breeding stocks for testing. Cooperation will take several forms, including growing of strains for inoculation and sampling, furnishing exotic breeding material for tests, and inclusion of identified resistant material in further breeding programs.

4. Other U. S. Institutions

Special cooperation will be maintained with the USDA-ARS Northern Regional Laboratory at Peoria, Illinois, particularly to further refine analytical methods for the rapid, accurate detection of aflatoxin. The Northern Regional Laboratory has done outstanding work on the mycology of Ascomycete fungi (*Aspergillus* is an Ascomycete) and on metabolic products of *Aspergillus*, *Penicillium* and related fungi, for more than 40 years. Close cooperation will be maintained with maize breeders and pathologists at other U. S. experimental stations, particular those in areas where *Aspergillus* is likely to be prevalent.

5. Dissemination and Application of Results

Information obtained from the research and any resistant breeding stocks discovered will promptly be made available to all

interested maize breeders, including both public and commercial agencies and firms. The established working cooperation with CIMMYT, ICTA, other experiment stations, and commercial firms will make this dissemination and application particularly effective.

E. MANAGEMENT CONSIDERATIONS

No unusual management considerations are expected to arise. The proposed contractor is an established agricultural research institution, experienced in international development and in contract work with A.I.D. Management requirements for AID/TAB are expected to be minimal.

The project will be evaluated by a technically qualified review team after approximately 18 months of operation. Project monitoring will be conducted on a continuing basis by a qualified project manager in TA/AGR/CP.

Contractor will clear all manuscripts proposed for publication with the project manager, and will submit an annual report within forty-five days of the close of each contract year.

F. BACKGROUND DESCRIPTION

As the information described under "state-of-the-art" above became available, the University of Missouri took up research on possible means of controlling aflatoxin incidence in maize by breeding.

During the past five years researchers at the University have been heavily involved in cooperative research on aflatoxin. The principal investigator has been coproject leader involving the Northern Central Research Center, several AES and ARS researchers located at 12 different locations in the U. S. The results of these studies have been published in 4 papers and several are in press. In addition, research at the University of Missouri on the development of screening techniques for detecting genetic control of aflatoxin in maize have progressed to the extent they are ready for large scale screening of genotypes.

Rapid methods for screening large numbers of kernels for genetic resistance from heterogeneous populations are necessary. The University of Missouri has developed the half-kernel assay method, where kernels from each genotype are cut horizontally into two halves (the cap half and the germ half) with a specially constructed minature circular saw. The cap halves are placed in a petri dish, cut side down on sticky tapes that have been covered with *A. flavus* conidia. The tapes are placed on Whatman filter paper in

petri dishes which are then moistened with water, covered, and incubated at 26.7 C for ten days. The presence of intense BGY fluorescence indicates intensive fungal growth and usually accompanying aflatoxin contamination, whereas the absence of BGY fluorescence suggests limited fungal growth and a possibility for no aflatoxin production. Germ halves that had cap halves with limited growth are propagated in the field and resultant kernels that are produced are then assayed in the same manner. This assay can potentially provide information about the genotype's ability to exclude or restrict the growth of the fungus or prevent the production of aflatoxin. The method allows (a) screening of a large number of genotypes and (b) at the same time the germ half of each test kernel can be used to produce a plant for subsequent field inoculation tests.

G. PROJECT DESIGN AND METHODS

1. Summary

a. A systematic screening program among the exotic germ plasm available in seed banks and USDA Plant Introduction Stations will be conducted in a search for resistance to fungal growth of Aspergillus species and/or inhibitors of aflatoxin synthesis.

b. Two screening methods will be used. One will involve the half kernel assay method and the second will employ field inoculation of ears using a pinboard-injury technique developed at the University of Missouri. Infected kernels will be analyzed for aflatoxin B_1 and G_1.

c. Identified germ-halves with resistance as indicated from the assay of the cap half will be planted to produce ears. Where feasible, ears will be inoculated with *A. flavus* and/or *A. parasiticus* and further evaluated for degrees of fungal growth and level of aflatoxin B_1. Kernels from the non-infected portion of inoculated ears will be used for additional propagation.

d. Identified resistant genotypes will be propagated in their area of adaptation. Exotic strains adapted to a short day will be grown either in winter nurseries, Central or South America. Later this will involve the cooperation of U. S. seed companies with established breeding programs in these areas or native maize breeders.

e. Genotypes with identified resistance to fungal growth or inhibitors of aflatoxin synthesis will be used:

(1) In inheritance studies;

(2) As donor parents in transferring desirable traits to adapted strains; and

(3) In cyclic selection programs (if resistance is polygenically inherited) for population improvement.

2. Specifics

a. Screening available maize collections will involve:

(1) Half-kernel assay of 30 kernels per strain.

(2) Propagate a plant from germ-half with resistance to fungal growth or absence of BCY fluorescence as indicated from the upper kernel half.

(3) Propagate in locations where the exotic strain is adapted (e.g. short-day adapted strains in Central America; long-day adapted strains in U. S. corn belt).

(4) Produce ears by either selfing or sibbing.

(5) Inoculate ears with *A. flavus* and/or *A. parasiticus*.

(6) Rate inoculated ears for degree of fungal growth.

(7) Return noninfected and infected kernels to Columbia, Missouri for further study.

(8) Repeat half-kernel assay on noninfected kernels.

(9) Determine aflatoxin levels of infected kernels.

(10) Select strains that support low levels of fungal growth and produce low levels of aflatoxin for further study.

b. Identified strains with resistance will be used in:

(1) Inheritance studies.

(2) Determining the effect of environment.

(3) Cyclic breeding programs.

(4) Inbred line and hybrid development.

(5) Developing breeding material suitable for corn improvement work in other countries.

c. Location of research activities:

(1) University of Missouri.

(a) Laboratory—in vitro screening.

(b) Field studies in summer nurseries and in winter nurseries (Florida, Puerto Rico, and Hawaii).

(2) CIMMYT.

(Central and South America.)

(3) Pioneer Hi-Bred International.

(Central and South America.)

(4) ICTA (Instituto de Ciencia y Technologia Agricolas)

Guatemala.

H. TIME-PHASED WORK PLAN

1977-1978

1. Search for resistance involving:

a. *Aspergillus flavus* and *Aspergillus parasiticus* fungal growth.

b. Inhibitors of aflatoxin due to above fungi.

2. Systematically screen exotic strains in storage:

a. ARS Plant Introduction Station, Ames, Iowa.

b. Various seed banks.

(1) CIMMYT.

(2) CIAT.

(3) ICTA.

c. Examine at least 10 accessions for each of the 130 (more or less) races of maize for response to A. flavus and A. parasiticus inoculation.

1978-1979

1. Continue screening of exotic strains not previously examined.

2. Confirm previously identified resistance.

3. Use strains with confirmed resistance in:

   a. Cyclic breeding programs for population development.

   b. Inheritance of resistance studies by:

      (1) Diallel analyses.

      (2) Generation mean analyses.

      (3) Location of genes according to chromosome arm by B-A translocations.

   c. Incorporate sources of resistance in breeding materials for use by breeders in developing countries' programs where aflatoxin is a problem.

1980-1981

1. Expand studies of confirmed resistant selections by evaluation under:

   a. Different environments, such as:

      (1) Different rainfall areas.

      (2) Tropics-coastal vs. highland.

      (3) Temperate zone.

2. Continue:

   a. Inheritance studies.

   b. Cyclic selection programs.

   c. Development of breeding material with resistance for use in commercial production or as sources of resistance.

I. AVAILABLE FACILITIES AND EQUIPMENT

Permanent equipment and facilities necessary to complete the proposed research are available at the University of Missouri. This includes laboratory space, incubators, growth chambers, greenhouses, field nurseries, and access to winter nurseries. Tentative arrangements have been made for nurseries and cooperative research at various locations in Central and South America. Recently a Waters High Pressure Liquid Chromatograph with RI, fixed wave length and multiple wave length UV has been added to the analysis laboratory. This instrument will be used for qualitative detection of aflatoxin in corn samples. Through cooperative arrangements corn samples contaminated with aflatoxin could be analyzed by the ARS Northern Central Research Laboratory at Peoria for verification of the results from the University of Missouri.

J. PROPOSED PROJECT PERSONNEL

1. M. S. Zuber - Professor of Agronomy, University of Missouri, Columbia, Principal Investigator.

   a. Estimates he will spend 50 per cent of his time on the research outlined in this proposal.

   b. Over 30 years of experience in corn breeding with special interests in host-plant resistance. Involved last 5 years in cooperative research in aflatoxin. Recently retired from ARS after 39 years of service. He is now employed by Missouri Agricultural Experiment Station as Professor of Agronomy.

   c. Other research in progress includes cooperative host-plant resistance studies on southwestern corn borer, bacterial wilt, and corn viruses. Also research on protein quality, stalk quality, and inheritance of components of grain yield. He has over 150 publications including several on aflatoxin.

   d. Received 1976 Crop Science Award, Crop Science Society of America.

2. O. H. Calvert - Professor of Plant Pathology, University of Missouri, Columbia, Co-investigator.

   a. Estimate of time commitment is 15 per cent.

   b. Almost 20 years of experience in corn - fungal pathogen associations with special interest in resistance and the last five years having been involved in cooperative research with aflatoxin.

c. Other cooperative host-pathogen research in progress includes determining the effect of cultures of Diplodia maydis in association with corn stalk quality in corn-belt hybrids, and the effect of cultures of Helminthosporium species of D. maydis on ear and leaf infections, looking for gene differences in pathogenicity in association with genes for low reaction in corn and the heritability of infection types. He has over 36 publications including several on aflatoxin.

3. A. L. Karr - Assistant Professor of Plant Pathology, University of Missouri, Columbia, Co-investigator.

   a. Estimate of time commitment is 15 per cent.

   b. Over ten years experience in enzymology, carbohydrate chemistry, gas and high pressure liquid chromatography and isolation of natural products from fungi like Helminthosporium species.

   c. Other cooperative research projects now currently engaged in other than the research described in this proposal is a project in which primary emphasis will be on the physiological genetics of the Erwinia stewartii - Zea mays complex. He has about ten publications to his credit, including some on the characterizatio of fungally produced toxins.

4. J. R. Wallin - Research Associate (USDA), Department of Plant Pathology, University of Missouri, Columbia.

   a. Estimate of time commitment is 15 per cent.

   b. Over 33 years of experience in plant pathology and epidemiology, the last seven years with fungal and bacterial pathogens of corn and within the last year has published two papers dealing with Aspergillus flavus and aflatoxin.

   c. Other cooperative research in progress other than the research described in this proposal are investigating mass selection of a maize composite for resistance and/or tolerance to two important corn viruses and response of corn strains to the bacterial pathogen Erwinia stewartii and its isolates in Missouri, monitoring the atmosphere for the incidence and distribution of A. flavus conidia and other corn pathogens and following the incidence of A. flavus on hybrids grown over the state.

K. LITERATURE CITED

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L. GENERAL APPRAISAL

This is a project of very high priority. Severity of the problem is probably even greater than presently considered, and no time should be lost in undertaking work which promises to lead to a solution.

Both the proposing institution and the proposed project leader have high professional reputations. The project design is simple and direct, and should lead to an early identification of resistant material should such be in existence. Plans for work outside the United States and for rapid dissemination of the results are excellent.

The budget is modest considering the scope and quality of the intended work; proposers have given assurance that it will be adequate. Facilities and equipment to be made available without direct charges to the project appear to be outstanding..

M. ENVIRONMENTAL CONSIDERATIONS

Since this project involves only controlled experimentation on small plots and in the laboratory, and is therefore of limited scope and conducted under strictly controlled conditions, it has been determined that it will not result in a major federal action which would have a significant environmental effect. Therefore, an Environmental Impact Statement or an Environmental Assessment will not be required.

A Negative Determination for this project is attached.

N. LIFE-OF-PROJECT BUDGET

		Salaries and Wages		
		1st year	2nd year	3rd year
1.	Project Supervisor*	7,500	7,875	8,250
2.	2 Technicians (100% time each)	24,000	27,000	29,500
3.	2 Graduate Research Assistants (50% time each)	11,000	11,400	12,000
4.	Hourly Labor	8,000	9,000	10,000
	TOTAL Salaries & Wages	50,500	55,275	59,750
5.	Staff Benefits (16% of positions 1 and 2; 5.85% of position 4)	5,500	6,100	6,625
6.	Indirect Costs	29,000	32,150	34,750
7.	Travel and Transportation	5,000	6,000	7,000
8.	Equipment	6,000	7,425	4,875
9.	Winter Nurseries	10,000	11,000	12,000
10.	Misc. Supplies	4,000	5,000	6,000
11.	Analytical Services	5,000	6,000	7,000
12.	Subcontract Services**	10,000	11,000	12,000
	TOTALS	125,000	140,000	150,000

* 25% time paid by this budget; additional 25% contributed by University.

** Expected for field and laboratory work in Central America.