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Research Report 91-01

Aflatoxin Research Review

PEANUT COLLABORATIVE RESEARCH SUPPORT PROGRAM

On the cover: Aflatoxin produced by the fungus, Aspergillus flavus, is a worldwide problem in peanut as well as other food crops. Adsorptive clay additive blocked liver accumulation of aflatoxin when chickens were fed a ration containing aflatoxin contaminated peanut (shown by the darker colored healthy liver compared to the lighter colored aflatoxin damaged liver). Photo by Dr. Timothy D. Phillips, Texas A&M University.

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For further information, contact:
Dr. David G. Cummins
Program Director
Peanut CRSP Management Entity Office
The University of Georgia
Georgia Station
Griffin, GA 30223-1797 USA

Tel: 404/228-7312
Telex: 41-4190
FAX: 404/229-3337
BITNET: CRSP-GRF
DIALCOM: 157:AGS634

AFLATOXIN RESEARCH REVIEW

Peanut CRSP Report No. 91-01

Edited by:
David G. Cummins
Program Director
Peanut CRSP

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PREFACE

Aflatoxin contamination in peanut has been a recognized problem for over 30 years. The increase in present day interest in aflatoxin developed following the death of 100,000 turkey poults on 500 farms in England in 1960. Investigation revealed a toxic fungal metabolite (aflatoxin) of Aspergillus flavus in the Brazilian peanut meal fraction of the feed. A. flavus is distributed worldwide in soil and air, and is a seed-inhabiting storage-fungus of many important food crops of man. Aflatoxins have been found not only in peanut in all major peanut-producing countries, but also in cottonseed, corn, soybean meal, fish meal, and other grains, seed, and feeds. Aflatoxin b, is the most potent, naturally occurring carcinogenic substance known and has induced cancer and caused mortality in most domesticated and experimental animals. Also, there is direct evidence from India wherein aflatoxin was incriminated as a probable cause of human infantile liver cirrhosis. The most impressive, aflatoxin-related episode is the acute poisoning in India in 1974 involving some 400 people and resulting in 106 deaths. (From Urban L. Diener, Robert E. Pettit, and Richard J. Cole, "Aflatoxins and Other Mycotoxins in Peanuts"; In Peanut Science and Technology, Edited by Harold E. Pattee and Clyde T. Young, American Peanut Research and Education Society, Inc. Yoakum, Texas 77995, 1982; pp. 482519).

The importance of aflatoxin/mycotoxin contamination in peanut was recognized in the planning of the Peanut CRSP, 1980-1982. One of the major constraints identified was "health hazards and economic losses due to mycotoxin contamination", and solving problems contributing to this constraint has been a important part of the research program of the Peanut CRSP from 1982 to the present.

Since considerable resources of time and money are expended on aflatoxin/mycotoxin research in the Peanut CRSP, it was decided to review the research underway at the Annual Meeting of the Peanut CRSP Investigators on July 10, 1992 in San Antonio, Texas. The purpose of the review was to determine if we were addressing the important problems, if there were gaps in the program, and if there was duplicative research among different projects. Two scientists representing the research in the United States Department of Agriculture/Agricultural Research Service were invited to summarize their program for a comparison in program objectives.

The IRHO-CORAF program was summarized by the French representative.

The Peanut CRSP Aflatoxin Research Program has three major areas of effort.

1. Genetic Resources - Search for genetic resistance and breeding to incorporate resistance into desirable cultivars.

2. Management - Control of pests (especially soilborne insects that damage pods and allow fungal invasion), and cultural practices to avoid stress that enhances fungal buildup.

3. Food Safety/Utilization - Mechanical, chemical, and bacterial decontamination of

aflatoxin contaminated seed; drying and storage practices to minimize contamination; and separation of contaminated seed from uncontaminated sound seed.

We concluded that there was no unnecessary duplication of effort across projects, that there were several different approaches to the aflatoxin problem that will provide solutions at different points of intervention, and that we should encourage the adoption of the technology developed. The following report summarizes presentations made at the meeting.

David G. Cummins
Program Director, Peanut CRSP

PEANUT CRSP

AFLATOXIN RESEARCH

PRINCIPAL INVESTIGATOR: Robert Pettit - Texas A&M University

PROJECT TITLE: Mycotoxin Management in Peanut by Prevention of Contamination and Monitoring

GOALS: Manage the aflatoxin problem within peanuts through prevention of contamination, monitoring, and detoxification

OBJECTIVES:

1. Characterize the life cycle of Aspergillus flavus and A. Parasiticus and determine factors which influence inoculum potential within the field.
2. Determine when and how peanut plant parts are invaded by aflatoxin-producing fungi.
3. Screen peanut cultivars and genotypes for tolerance to aflatoxin producing fungi.
4. Characterize preformed and induced fungal defense mechanisms within the peanut plant tissues.
5. Develop rapid and economical multi-mycotoxin detection systems.
6. Develop safe and efficacious methodologies for the detoxification of aflatoxin contaminated peanut products.
7. Set up training programs for students and technical staff from the developing countries.

Priorities:

1. Primary goal should be to discover technologies which are able to reduce plant stress. Aflatoxin contamination of peanut is primarily due to genetic, heat, nutritional, salt, and drought stress. Prevention of contamination through planting resistant varieties, fertilization, soil management, crop rotations, tillage practices, soil amendments, and improved harvesting procedures.
2. Screen peanut germplasm for resistance to both fungal invasions and/or colonization and aflatoxin formation within peanut seeds. Includes both field and laboratory screening.
3. Discover molecular markers which are able to characterize plant resistance. Implement by using these markers in the breeding program. Use of state of the art biotechnology procedures.
4. Construct field-practical testing devices for the analysis of binary and ternary mixtures of multi-mycotoxins.
5. Evaluate ligand specificity of the chemisorption processes associated with hydrated sodium calcium aluminosilicates.

NOTE: This is the only Peanut CRSP Project that is 100% devoted to aflatoxin/mycotoxin research. The other projects to follow have only part of their objectives on aflatoxin/mycotoxin research.

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PRINCIPAL INVESTIGATOR: Olin Smith - Texas A&M University

PROJECT TITLE: Disease-Resistant Peanut Varieties for Semi-Arid Environments

Mycotoxin Related Research

GOAL: Increase safety of peanut consumption through development, release, and dissemination of productive, agronomically adapted peanut varieties with acceptable quality and low mycotoxin production potential.

OBJECTIVES:

1. Ascertain the relative tendency for mycotoxin contamination of new breeding lines compared to checks and current varieties.
2. Identify germplasm with low mycotoxin production.
3. Incorporate germplasm and breeding lines with reduced mycotoxin production potential into testing & breeding programs.
4. Avoid extended testing and release, as varieties, lines with enhanced tendency for high mycotoxin production.

Action:

1. Texas. Collaboration with the Texas A&M mycotoxin project includes the joint selection and leasing of a naturally infested field site (non-irrigated) near Waller TX, and planting and harvesting are joint efforts. Germplasm and advanced lines are provided to the Texas A&M mycotoxin project for mycotoxin related studies.

2. Texas. Support grant funds have supplemented Peanut CRSP funds in the establishment of a site for use in screening for aflatoxin resistance at the Texas A&M Agricultural Research Station at Yoakum beginning in 1990. Purposes for the new test site are two-fold: researcher access and control of field operations; and availability of irrigation facilities to enhance management of test to better ensure adequate seed production and A.— flavus production. Inoculum was introduced to the test site and the tests are being repeated in 1991. Concurrent tests at a naturally infested, nonirrigated site are being conducted for comparison of results with those at the new test site. Neogen test kits are being used for the mycotoxin analysis. Twenty-five each of early and late maturing lines are being tested. Additional lines in preliminary yield tests at the non-irrigated site will also be analyzed for aflatoxin in 1991. A small, equipment grant by a commercial firm has facilitated this effort.

3. Senegal. Screening of selected advanced lines in the breeding program for aflatoxin contamination is being conducted in conjunction with the Texas A&M mycotoxin project. Some of the lines under test derive from crosses used in studies initiated by a HC peanut breeder and a French pathologist.

4. Burkina Faso. Entries in multiple location advanced line yield tests are being analyzed for relative mycotoxin contamination in collaboration with the Alabama A&M Food Technology project. Mycotoxin analysis are being conducted on selected entries only at test sites where fungal association with plants is observed in order to contain the number of samples for analysis at a manageable number.

PRINCIPAL INVESTIGATOR: T. G. Isleib - North Carolina State University

PROJECT TITLE: Peanut Varietal Improvement for Thailand and the Philippines

A Summary of Aflatoxin-Related Research

There are three mechanisms of host-plant resistance currently under study for the reduction of aflatoxin in peanut: resistance of the plant to preharvest infection (PIR), resistance of rehydrated dry seed to invasion (DSR), and inability of the infected seed to support production of aflatoxin (APR). DSR is easily measured as the frequency of rehydrated seeds that develop mycelium after inoculation and incubation. APR requires quantitation of the amount of aflatoxin produced by infected seeds and is substantially more costly than DSR measurement. PIR is measured by promoting infection of plants in a medium with natural or augmented populations of *Aspergillus*. High temperature and drought stress imposed at mid-flower stage of growth can weaken the plant sufficiently to allow this ubiquitous but weak pathogen to invade the pods and seeds.

At NCSU, 14 lines reported to carry resistance to Aspergillus species were crossed with NC7 to initiate study of the inheritance of resistance (see appended table). Most of the lines used were reported to exhibit DSR; some were reported to carry PIR; and none were reported to carry APR. Mr. Setyo Dwi Utomo, a masters student from Indonesia, estimated broad-sense heritability in two of the crosses, AR-4/NC7 and GFA-2/NC7, finding fairly high values for DSR (H=0.55-0.63) and intermediate values for APR (H=0.20-0.40). While estimates of heritability for PIR were also in the intermediate range, the precision of measurement was quite poor as obtained in the greenhouse. The data on PIR are suspect. There was transgressive segregation for DSR and APR in the two populations. A few lines combining the two forms of resistance have been identified. Utomo also performed a generations means analysis of the four crosses from a factorial mating of NC7 and the Indonesian cultivar 'Gajah' with **'J-11'** and U4-75, a line reported to carry APR. Additive genetic effects and additive-by-additive epistatic interactions predominated in the four crosses for DSR and APR, indicating that selection for those traits should be effective. PIR exhibited nonadditive gene action and was again subject to high error of measurement.

Dr. H. T. Stalker screened 114 accessions of wild peanut in section Arachis for DSR. None of the accessions screened exhibited a level of resistance superior to those observed in cultivated peanut. While there may be genes for resistance in species of other sections of the genus, those other sections are reproductively isolated from A. hypogaea to a much greater degree than are the species of section Arachis.

In North Carolina, aflatoxin contamination is a problem that develops in storage rather than in the field. The proportion of infected seeds at harvest is usually quite low. In storage, pods absorb water from "driplines" where respired water vapor condenses on walls or ceiling and drips back into the stored crop. Therefore, our breeding strategy for reduction of aflatoxin in the Virginia-Carolina area focuses on the incorporation of DSR and APR into lines with economic potential in the production area. Ten selections from Utomo's populations are being

inter-mated in a program of recurrent selection for the two traits as well as being backcrossed to NC7 to eliminate the small seed size and fastigiate growth habit exhibited by most of the lines. To obtain field measurements of PIR, we have installed 200 microplots at the Sandhills Research Station where temperatures are typically 3-4 C warmer than at Raleigh and the predominating soil type is a deep sand. Twenty lines reported to exhibit a range of infection rates have been planted in a ten-rep test of the system. Both infection rate and aflatoxin production will be measured.

in Southeast Asia, the aflatoxin problem may be exacerbated by the farmers' practice of harvesting freshly dug pods into tight-mesh plastic bags for transport to the market or shelling plant. Even when the buyer demands dried pods, the farmer may not dry the crop immediately after digging but only immediately before sale. The combination of high temperature and high moisture in peanut handled in this way may increase aflatoxin levels even though diseased pods and seeds are removed by hand. The Thai collaborators are evaluating methods of field screening for host-plant resistance with infection rate rather than aflatoxin production being the primary measure of resistance or susceptibility.

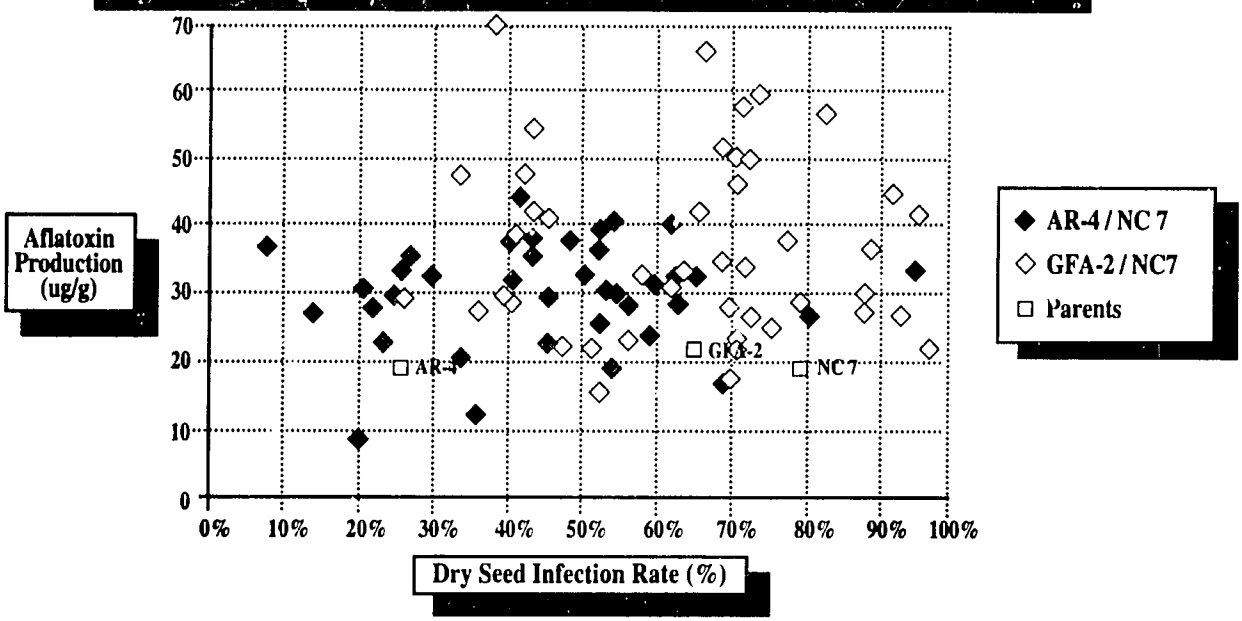
Other aflatoxin-oriented research conducted at NCSU but not directly supported by the CRSP includes the transformation work conducted by Dr. Arthur K. Weissinger. With the development of a system for the routine transformation of peanut tissue and subsequent selection and regeneration of transformed plants, it will be possible to explore the efficacy of genetic resistances from organisms other than peanut. These include genes for enzymes that might degrade structural components of the fungal mycelium that do not occur in the host, such as chitin.

In other work, Dr. Gary Payne has isolated a gene from A. flavus that mediates synthesis of a precursor to aflatoxin. By transferring the promoter region from this gene to the GUS (glucuronidase synthase) gene, Dr. Payne hopes to develop an assay for host plant constituents that alter the level of expression of this particular gene. It may be possible to identify plants that either fail to produce the chemical signal that promotes synthesis of aflatoxin or produce a substance that suppresses the gene (see page 29).

Table of parents used in crosses with NC 7 to investigate resistance to A. flavus.

Line	Source	Pedigree	Type of Resistance		
			Seed Coat	Pre-harvest	Toxin Production
Ah 7223	Nigeria		✓	✓	-
AR - 1 (GP35)	USDA-ARS/ Univ. of Georgia	Florrunner / PI 337.409 (GP4, var. fastigiata)	✓	✓	-
AR - 2 (GP 36)	USDA-ARS/ Univ. of Georgia	UF 392-12-B-28 (Florigiant sib) / Dixie Spanish, A5-5 // PI 337.409	✓	✓	-
AR - 3 (GP37)	USDA-ARS/ Univ. of Georgia	PI 337.432 (var. fastigiata) / Tifspan (var. vulgaris)	✓	✓	-
AR - 4 (GP38)	USDA-ARS/ Univ. of Georgia	PI 337.394F (GP3, var. fastigiata)/ Florunner	✓	✓	-
C55-437	Senegal		✓	-	-
Faizpur	India		✓	-	-
GFA-1	Univ. of Georgia	UF 439-16-10 (Florunner sib) / PI 246.388 ('Koboka Tjina' from South Africa), A137 // a5-5, UF 392-12-B-28 (Florigiant sib) / Dixie Spanish	✓	✓	-
GFA-2 (GP34)	Univ. of Georgia	UF 439-16-10 (Florunner sib) / PI 246.388 ('Koboka Tjina' from South Africa), A137 // Florunner	✓	✓	-
J11	India		✓	✓	-
Monir 240-30			✓	-	-
NC Ac. 841	North Carolina State Univ.		✓	-	-
PI 337.394F	Argentina		✓	✓	-
PI 337.409	Argentina		✓	✓	-
Sunbelt Runner			-	-	
U4-47-7	Uganda		✓	✓	-
UF 71513	Univ. of Florida		✓	✓	-
Var.	Cuba		✓	-	-

Distribution of means for APR and DSR among F4-derived families from two populations.



PRINCIPAL INVESTIGATOR: Robert Lynch - The University of Georgia

PROJECT TITLE: IPM Strategies for Peanut Insects in SAT Africa

Peanut CRSP Aflatoxin Research

GOAL: Determine the interrelation between insect damage to peanut pods and the incidence of aflatoxin contamination of seed.

OBJECTIVES:

1. Determine the influence of termite damage to peanut pods on the level of aflatoxin contamination of seed in Burkina Faso.

2. Determine the effect of peanut harvest date on the extent of termite damage to peanut pods and level of aflatoxin in seeds in Burkina Faso.

3. Evaluate peanut lines from ICRISAT with reported resistance to termites for termite damage and aflatoxin contamination in Burkina Faso.

4. Determine the influence of lesser cornstalk borer damage to peanut pods and the incidence of aflatoxin contamination of seed in Georgia.

5. Determine the interrelation between extent of pod damage by termites and lesser cornstalk borer on the occurrence of aflatoxin in peanut seed.

Research in Burkina Faso has shown that termite injury to peanut pods increases substantially with a delay in harvest. Research is currently underway to determine if this increased termite injury increases aflatoxin contamination in seed. Twelve peanut lines from ICRISAT-India with resistance to pod injury due to termite feeding are currently being evaluated for termite injury in Burkina Faso and for aflatoxin contamination in relation to level of termite damage. Research in the U.S. has shown that pod injury by the lesser cornstalk borer increases the percentage of pods and seed contaminated with Aspergillus flavus and aflatoxin. Furthermore, only external pod scarification by the lesser cornstalk borer without pod penetration is required to significantly increase aflatoxin content of seed. Research is currently being conducted in the U.S. to determine the mechanism of increased A. flavus and aflatoxin contamination as a result of lesser cornstalk borer injury to peanut pods.

PRINCIPAL INVESTIGATOR: Bharat Singh - Alabama A&M University

PROJECT TITLE: An Interdisciplinary Approach to Optimum Food Utility of Peanut in SAT Africa

Summary of Aflatoxin Research

OBJECTIVES: To determine amount of aflatoxin contamination in peanut and peanut products for human consumption during storage, processing and handling of the products in market channels.

Earlier Work: Earlier work under this project included (1) survey of aflatoxin contamination of peanut in Sudan and Caribbean countries, (2) development of methods of decontamination using various methods (University of Florida).

Currently: As a part of objectives an evaluation of quality of peanut and peanut products, surveys have been conducted for last three years in various regions of Burkina Faso. The main objectives of this research is to avoid contamination by proper handling of the products, such as manual sorting and grading, coupled with appropriate storage and packaging.

Aflatoxin analysis on a cooperative basis is provided to the Texas A&M Breeding and The University of Georgia Insect Management projects.

PRINCIPAL INVESTIGATOR: Larry Beuchat - The University of Georgia

PROJECT TITLE: Appropriate Technology for Storage/Utilization of Peanut

Summary of Current Aflatoxin Research Activities

1. Aflatoxin in Ground Roasted Peanut (Kasetsart University)

   Generally, ground roasted peanut products commercially available in Thailand are made from low quality peanut. The Department of Medical Science (Ministry of Public Health) reported that 95 % of ground roasted peanut in Thai markets contained more than 20 ppb aflatoxin. Manufacturers use misshaped and dirty peanut to prepare this product since identity of these peanut is not evident in the finished product. Inspection of this product is not enforced because it is not sold in certified packages. Because of these problems, ground roasted peanut has been given top research priority among products which might be contaminated with aflatoxin in Thailand. Problems associated with rancidity, nonuniform grit size, color and high microbial populations also characterize this product. There is a need to improve the qualities of ground roasted peanut products in order to decrease risk of public health and increase the utilization of peanut products. Research is underway to determine safety and sensory qualities of ground roasted peanut in Thailand, the most suitable grade of peanut for making ground roasted product, the best process for manufacture and consumer acceptance and market potential for modified products.

2. Aflatoxin in Commercial Peanut Products (University of the Philippines at Los Banos)

   Surveys of commercial peanut products in the Philippines have revealed that a high percentage are contaminated with aflatoxin. Products will be continually monitored for the presence and levels of aflatoxin as influenced by changes in handling and processing procedures as advised by the UPLB Peanut CRSP team.

3. Biological Control of Aflatoxigenic Aspergilli (University of the Philippines at Los Banos)

   Work at UPLB has resulted in the isolation of a mold which is antagonistic against aflatoxin production by Aspergillus flavus and A. parasiticus. Investigations to determine the culture conditions under which maximum antagonism occurs are in progress.

4. Separation of Contaminated and Sound Seed (The University of Georgia)

   The use of chemical detoxification methods to reduce aflatoxin content in contaminated peanut has not been very successful for reasons of safety and wholesomeness of resulting products. Efforts toward finding a solution to this growing dilemma therefore have to be redirected. A method that holds promise is the use of a hydrogen peroxide blanching

process in which the extent of action of catalase results in separation of contaminated peanut seed from uncontaminated seed. Catalase, an enzyme present in the skin and endosperm of the peanut, will react with H_2O_2 to yield water and oxygen gas. The oxygen generated is theorized to form an oxygen jacket between the skin and the cotyledon, allowing easier skin removal during the blanching process. Preliminary studies in our laboratory revealed that this phenomenon occurred with sound peanut seed but not with seed on which Aspergillus parasiticus had grown. It is hypothesized that the mold may contain constitutive catalase which reacts upon contact with H_2O_2 in the blanching solution, thereby reducing the amount of H202which would come in contact and be able to react with peanut catalase. If this occurred, skins would not separate from cotyledons as readily during the blanching process. The objective of experiments carried out in the past year was to investigate the relationship between catalase activity and growth of A. parasiticus, using an aqueous extract of peanut (peanut milk) as a model substrate.

Results show that catalase content in uninoculated peanut milk was reduced by approximately half within 3 days. No significant changes occurred after 6 and 10 days of incubation. A dramatic increase in catalase content, however, was observed in peanut milk inoculated with A. parasiticus. A 3-to 10-fold increase was observed after 6 days of incubation followed by an additional 3- to 5-fold increase after 10 days. This observation supports the hypothesis that A. parasiticus produces catalase. The observed difficulty in removing skins from infected peanut seed may therefore be due, in part, to insufficient peroxide concentration in the blanching solution rather than absence of peanut catalase at the interface of the cotyledon and the skin. This supports our hypothesis that A. parasiticus produces catalase that reacts with H_2O_2 in the blanching solution, thereby reducing the amount of H_2O_2 which would come in contact and be able to react with peanut catalase to otherwise separate the skin from the cotyledon.

No significant change in pH was observed in peanut milk inoculated with A. parasiticus. The pH of uninoculated milk increased from 6.7 to 9.2 within the 10-day incubation period, irrespective of the initial amount of catalase present. This indicates that catalase may cause chemical changes in peanut milk other than H202decomposition. Catalase can be intimately associated with cell respiration. Growth of A. parasiticus may have resulted in a buffering effect to minimize change in pH.

Biomass production by A. parasiticus was essentially unaffected by initial catalase content of the medium. The mycelial dry weight ranged from 0.83 to 1.21 g per 50 ml of various peanut milk substrates within the 3- to 10-day incubation time.

Production of catalase by aflatoxigenic aspergilli, and perhaps other molds, could serve as a basis for developing a method for separating infected and sound peanut seed on a commercial scale. Separation would be achieved by seed flotation characteristics. The higher the catalase activity on the surface of the seed skin, i.e., the more advanced the deterioration due to catalase-positive molds, the more rapid would be the decomposition

of H_2O_2 in the blanching solution. Hence, more oxygen would be evolved, causing contaminated seed to float more rapidly than the uncontaminated ones. A continuous separator could be designed to remove contaminated seed from bulk lots. We are in the process of investigating the efficacy of this system.

5. Detoxification Studies (The University of Georgia)

A bacterium, originally classified as Flavobacterium aurantiacum, was observed in the 1960's to degrade and/or bind aflatoxin. The mechanism(s) by which this occurs has never been defined. Investigations designed to determine the mechanism of aflatoxin removal were recently initiated in our laboratory. We plan to determine whether aflatoxin is bound to bacterial cell components or if it is degraded by bacterial enzymes or perhaps some other mechanism. Work in this area will continue in FY 92 and FY 93.

PRINCIPAL INVESTIGATOR: Manjeet Chinnan - The University of Georgia

PROJECT TITLE: Postharvest Handling Systems for the Small Peanut Producer

Summary of Aflatoxin Research

GOAL: The major goal of this project has been to develop improved peanut postharvest handling technologies including improved drying, handling, shelling, and storage techniques.

There have been two thrusts related to aflatoxin research in this project.

Aflatoxin Monitoring during- Storage, Handling and Marketing

Work was conducted in four countries in the Caribbean - Antigua, Belize. Jamaica and St. Vincent. Work in Jamaica involved examination of aflatoxin development as a result of drying levels and storage of peanut in improved storage conditions. The tests were done as part of on farm trials. Belize studies involved monitoring of aflatoxin during storage. Both Jamaica and Belize experiments employed mixing the test peanut with peanut flour inoculated with Aspergillus flavus spores to ensure presence of mold spores. These experiments were designed in collaboration with Dr. Larry Beuchat, PI, The University of Georgia Food Technology Project.

Density Characterization of Aflatoxin Contaminated Peanuts

This study was done by Mr. Vivek Gnanasekharan as part of his Master's thesis. The purpose of this study was to determine density distributions of naturally contaminated peanut and peanut inoculated with Aspergillus parasiticus. Methods were developed to determine density and aflatoxin level of individual seed. The study conclusively demonstrated the depression of peanut seed density in the presence of high levels of aflatoxin. However, additional data is needed to model and design appropriate separation schemes.

Vivek received two prestigious awards based on his research mentioned above. First, E. Broadus Browne given to a best M.S. student by the College of Agriculture, University of Georgia ($500 cash award). Second, first prize Phi Tau Sigma Graduate Paper Competition Award given by Institute of Food Technologists ($1500 cash award).

ASSOCIATED AFLATOXIN RESEARCH

USDA/ARS

NORTH CAROLINA STATE UNIVERSITY

CORAF

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PRINCIPAL INVESTIGATOR: Corley Holbrook - United States Department of Agriculture/Agricultural Research Service, Tifton, GA

PROJECT TITLE: Breeding Peanut for Resistance to Preharvest Aflatoxin Contamination

COOPERATORS: D. M. Wilson, M. E. Matheron, W. F. Anderson and M. E. Will. USDA-ARS, Tifton, GA; Univ. of Georgia, Tifton, GA; Univ. of Arizona, Somerton, AZ.

Preharvest aflatoxin contamination (PAC) is one of the most significant challenges facing the U.S. peanut industry. The development of peanut cultivars with resistance to PAC would be a valuable tool in reducing the problem. There are two requirements for developing PAC resistant cultivars First, there must be genes for resistance to PAC. Second, there must be a reliable and efficient screening technique which can be used to identify material which contains these genes. The objectives for 1990 were developed to address these requirements. The first objective was to select a core collection for peanut which can be used to efficiently identify genes for resistance in peanut. Multivariate statistical analysis was used to select a core collection of 831 accessions from the U.S. germplasm collection (7,432 accessions) of peanut. Examination of data for six phenotypic traits indicated that the core collection is representative of the entire collection and that the genetic variance which exists for each trait in the entire collection has been maintained in the core collection. The use of this core collection should greatly speed the process of identifying genes for resistance to PAC. The second objective for 1990 was to begin research on development of a reliable and efficient screening technique. Ten methods of inoculation were examined to determine which one would be best for insuring Aspergillus as reflected by higher colonization of pods and seeds. However, aflatoxin contamination was high in seed from all treatments, probably due to an adequate background population of Aspergillus. Three locations were also studied to determine which would provide the appropriate environmental conditions for PAC. Aflatoxin contamination was consistently high in rain protected field plots at Tifton, Georgia in 1990. Aflatoxin was high, but inconsistent in field plots at Yuma, Arizona. Aflatoxin levels up to 2,000 ppb were observed in peanut from Yuma. Aflatoxin was more prevalent in peanut subjected to summer drought than in peanut subjected to a fall stress period. The use of shade cloth to minimize the extreme summer temperature at this location did not result in greater aflatoxin contamination. These results demonstrate that soil temperatures at Yuma do not consistently exceed the range necessary for PAC. The inconsistency in PAC at Yuma may be due to the difficulty in imposing an extended drought on the pods without killing the plants. Aflatoxin was low and inconsistent from greenhouse grown peanut at Tifton. Studies being conducted in 1991 are aimed at refining the screening techniques for field screening at Tifton and Yuma and for greenhouse screening at Tifton. In addition, approximately one-half of the accession from the core collection are being evaluated for resistance to PAC.

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PRINCIPAL INVESTIGATOR: Richard J. Cole - United States Department of Agriculture/Agricultural Research Service, National Peanut Research Laboratory, Dawson, GA

PROJECT TITLE: National Peanut Research Laboratory Research Program on Aflatoxin Management in Peanut

COOPERATORS: Joe W. Dorner, Paul D. Blankenship and Bruce W. Horn

Prior to 1985, the aflatoxin research program at the National Peanut Research Laboratory (NPRL), Dawson, Georgia, was primarily oriented toward studies on analytical methodology development and evaluation, removal technology, and elucidation of the environmental factors responsible for preharvest aflatoxin contamination. Subsequently, the program has evolved primarily toward the development of strategies for prevention of preharvest aflatoxin contamination and other aflatoxin management strategies.

At this point in time, we have identified and are vigorously pursuing several prevention strategies that we feel could provide relief in the shortest period of time.

These strategies are (1) the use of non-aflatoxin-producing strains of Aspergillus parasiticus as biocompetitive agents that are capable of replacing the wild toxic strains in the soil environment, (2) the use of chemical additives such as aluminosilicates (on GRAS List) to peanut butter and peanut products that irreversibly bind the aflatoxins, making them both chemically and biologically unavailable, (3) the enhancement of the natural resistance to A. flavus of commercially acceptable varieties such as the Florunner variety, and (4) development of aflatoxin early warning system.

Biocompetitive Agents

We initiated these studies in 1987 when we introduced a non-aflatoxin-producing strain of A. parasiticus into the soil of some of our environmental control plots. The plot facility enables us to test any resistance strategy under conditions ideal for preharvest aflatoxin contamination. The first year results were not conclusive but showed a degree of promise. However, results from the second year showed the non-aflatoxin-producing agent dominated the soil environment and resulted in a tremendous reduction in aflatoxin in the edible peanut compared to expected levels. The presence of high levels of a chemical marker compound (OMS) in the edible and non-edible peanut showed that the added biocompetitive agent had replaced the wild strains in the contamination process. The results of the third year studies were equally promising with the added agent still maintaining a high level of dominance in the soil environment. We currently have two other agents under test that could be ultimately utilized commercially. We are now able to accelerate this area of research with the new funds. We have hired a Ph.D. microbiologist (mycologist), and will hire one technician. We are entering into a specific cooperative agreement with Gustafson Chemical Company to help bring this research approach to fruition in the shortest time possible.

Sequestering Agents

Recent animal studies have shown that certain aluminosilicates added to feed are capable of binding the aflatoxins contained in the feed making them unavailable for absorption in the gut. The binding is irreversible which protects animals consuming aflatoxin contaminated feed. These compounds are currently on the GRAS List and are routinely used in the food and feed industry for a number of different uses.

We have initiated studies to evaluate the feasibility of using these agents in peanut butter as a last defense against low levels of aflatoxin that have evaded all other management strategies.

A detailed research project has been undertaken in this area that would (1) look for effective sequestering agents acceptable for food purposes and (2) test these agents in peanut butter and other peanut products for biological effectiveness and consumer acceptance (organoleptically). These studies will also be accelerated with the availability of new funding.

Enhancing Natural Resistance in Florunner Cultivar

Our previous studies have shown that Florunner peanut possess natural resistance to A. flavus and aflatoxin contamination that breaks down during late-season drought stress. This resistance is most probably based on several factors. One is the capacity of peanut seed to synthesize fungitoxic compounds (phytoalexins) in response to fungal invasion. This capacity is controlled primarily by the moisture content of the peanut, and as this moisture decreases during drought, the capacity for phytoalexin production is lost. It is only after this happens that peanut become contaminated with aflatoxin. Our current research strategy is to identify peanut varieties or genotypes that are capable of producing these phytoalexins at reduced moistures or are capable of maintaining high seed moisture under drought conditions and incorporating the traits into the Florunner cultivar or other commercial varieties.

Florunner resistance is based on other factors as well since studies of single seed and seed from single plants showed >90% of the contamination is confined to < 10% of peanut plants, even though all plants were subject to ideal conditions for contamination. Studies are underway to identify the basis for this apparent resistance. Once identified, genetic selection toward these factors and away from susceptibility should result in increased resistance in an already commercially acceptable variety. We are seriously considering hiring a post-doctoral candidate to accelerate these on-going studies.

Aflatoxin Early Warning Systems

Another technology we are currently researching is the development of "aflatoxin early warning systems. " These devices, when placed in a farmer's field, will predict how much drought stress the peanut can endure and still be relatively free of aflatoxin. It will provide the farmer with the management option of digging early and compromising, to some extent, other quality factors, such as maturity, but maintaining his crop relatively free of aflatoxin. With our input, one

device is currently commercially available and we have another device under development.

As stated previously, these approaches could provide relief from the aflatoxin problem in the shortest period of time. The rate-limiting factor prior to last year was available resources. We feel that the recent additional funding made available to us will accelerate our total program to identify and develop effective prevention and management strategies against the aflatoxin problem. We feel that prevention is the effective and economical management strategy for the aflatoxin problem.

PRINCIPAL INVESTIGATOR: Gary A. Payne - North Carolina State University

PROJECT TITLE: Development of a Screen for Resistance to Aflatoxin Accumulation Based on Gene Expression

Cooperators: M. K. Beute and T. G. Isleib, North Carolina State University, Raleigh, NC

INTRODUCTION

We have isolated a gene for aflatoxin biosynthesis from Aspergillus flavus (2). The gene (afl-2) appears to be involved in the regulation of the aflatoxin pathway before the biosynthesis of the first stable intermediate. Strains with a defective alf-2 gene do not produce aflatoxin, nor any of the pathway intermediates, nor any of the enzymes in the pathway. Because this gene appears to be a key regulator in the aflatoxin pathway, any naturally occurring peanut compound that blocks the expression of this gene has the potential to be important in resistance to aflatoxin accumulation.

We propose to develop an assay to identify compounds present in plants that affect the expression of this gene and thus the biosynthesis of aflatoxin. Such an assay would expedite the development of resistant lines by allowing plant breeders to either select for plants that contain inhibitory compounds or select against plants that contain inducing compounds.

The assay system we propose is based on a gene fusion construct. The promoter region of the afl-2 gene (the region that responds to environmental stimuli and turns on the gene) will be fused to the gene for B-galacturonidase (GUS). The product of the GUS gene catalyzes the cleavage of a number of B-glucuronides to produce a colored product that can be quantified by fluorescence. Thus, by using this construct we will be able to identify, by the production of a blue compound, those metabolites that either turn on or turn off aflatoxin biosynthesis. Because this gene affects regulation of aflatoxin at the beginning of the pathway, this assay will identify those compounds that block biosynthesis early before the accumulation of toxic intermediates. A further advantage of this system is that it will not require aflatoxin analysis.

The probability of finding a compound that inhibits or stimulates aflatoxin formation is high. A number of compounds have been identified from various plant sources that inhibit aflatoxin formation in culture (6). Also, peanut genotypes have been identified that suppress aflatoxin B1 production (1).

OBJECTIVE

To develop a rapid and inexpensive assay for naturally-occurring plant compounds that influence the regulation of aflatoxin biosynthesis.

RESEARCH PLAN

We have isolated a gene involved in the regulation of aflatoxin biosynthesis. This gene, afill-2, has been cloned and sequenced. The promoter region of the afl-2 gene will be characterized, removed from the afl-2 gene, and fused to the GUS gene to replace the GUS promoter. In this construct, production of GUS will be under the regulation of the afl-2 promoter and no GUS will be formed unless the afl-2 promoter is activated. The induction or inhibition of the afl-2 promoter by plant metabolites can be evaluated by the expression of the GUS. GUS activity can be quantified by the production of a blue pigment. GUS fusion constructs have been used to measure gene expression in A. nidulans and in the plant pathogen Cladosporium fulva. (4).

The gene construct will be prepared according to the methods of Roberts et al (4). The afl2/GUS construct will be transformed into strain 650-33 which has a defective afl-2 allele and does not produce aflatoxin (3,5,). A transformed strain of 650-33 containing the afl-2/GUS fusion will be grown in media amended with extracts from differing lines of peanut, and the activity of the afl-2 promoter will be evaluated by the cleavage of the substrate MUG (4-methyl umbelliferyl glucuronide) which results in a colored product that can be easily quantified by its fluorescence.

The assay will be tested by comparing lines of peanut that have shown some resistance to aflatoxin accumulation to those lines that are known to be susceptible. If this assay is successful, it may be possible to isolate and characterize the metabolite that is either inhibitory or stimulatory to aflatoxin formation. The identification of the product will allow for a screening program based on the presence or absence of this compound or lead to the genetic engineering of a plant containing the inhibitory compound.

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REPRESENTATIVE: Robert Schilling - Deputy Director, IRHO (Institute for oilseeds Research - France) and CORAF correspondent.

TITLE: The CORAF Program

Conference des Responsables Africains et Francois de la Recherche Agronomique (CORAF) in French is the "Conference for Representatives of African Agricultural Research." It was founded in 1987 by national research institutions of 14 African countries, partners of French cooperation in the field of tropical agriculture research. CORAF was entirely africanized in 1990, France becoming an "associate member. " Membership is opened to other African institutions and association is opened to any national or international organization.

CORAF is a networking structure with a steering committee (opened to associate members), an African president, a permanent secretary located in Senegal and a number of specialized networks: groundnut/cotton/rice/maize/cassava/drought resistance.

Each network has an African coordinator, a non-African correspondent, a scientific directorate of at least two members and a local delegate in each of the member-institutions. It publishes a newsletter and holds a general assembly or technical workshop once a year. A base-center for each network is created in a location where equipment can be concentrated and where a critical mass of scientists can be reached, and where strategic research with a regional impact can be conducted in the form of associative projects.

The Groundnut network comprises National Agricultural Research Systems of Benin, Burkina Faso, Cameroon, Congo, Madagascar, Mali, Niger, Ivory Coast, Central African Republic, Senegal, Chad, Guinea, Togo. Zaire and Guinea-Bissau have joined recently. Nigeria has made offers of collaboration.

The network coordinator is Dr. Amadou Ba in Senegal, the correspondent is myself (Dr. Robert Schilling) in France. The base-center is located in Bambey, Senegal, and 1.5 million ff (250,000 $U.S.) have been granted by France for equipment. Two sub-centers are to be created, one in Farafio-Ba, Burkina Faso for foliar disease problems and the other is Louddrime, Congo for problems specific to equatorial areas.

Areas of priority have been defined:

Breeding and physiology for drought tolerance,
Foliar diseases,
Postharvest and seed technology,
Aflatoxin,
Irrigation,
Sustainable production through mineral fertilization,
Crop improvement in equatorial areas.

Federative projects have been set up to cover more or less each of these fields of priority. Four projects have been funded and are under way. Main donors are the European Economic Community (3 projects) and France (1 project).

As regards funding, France provides a small budget for an annual workshop, travel, two secretaries and office facilities for Amadou Ba and myself, plus a special grant for the basecenter. All the rest comes through our projects.

Problems and projects:

1. First, the concentration of activities is in two countries, Senegal and Burkina Faso, where groundnut research has reached a minimum level of efficiency. All other countries are below this minimum, and take-off of the program is difficult.

2. Second, there is the prevailing francophone image of the organization. It is the will of CORAF, and still more of France, to draw other non-francophone african members and other associates and donors.

3. A meeting of the ministers of research of the member countries will be held in November 1991 in Togo in order to give CORAF an official political support and a formal juridical status.

4. SPAAR (Support Programs for African Agricultural Research) has identified 65 networks operating in Africa, among which 14 are considered eligible for aid through SPAAR. The CORAF groundnut network is one of them, which gives hope for more financial help.

5. The Peanut CRSP could be a member of the Network, if you are interested.

This is the last issue of our network bulletin*. We would be glad to include a presentation of the Peanut CRSP organization and activities in the next issue, if you provide us a page before the end of the year.

Thank you for your attention.

*Arachide Infos, B. P. 5035, 34032 Montpellier Cedex, France.

PARTICIPANTS

San Antonio, TX
July 10, 1991

1991 Peanut CRSP Investigator's Meeting and Aflatoxin Research Review

The meeting was held from 10:30-12:00 at the Hilton Palacio Del Rio Hotel. David Cummins and Olin Smith presided. Those attending were:

1.	John C. Anderson	Alabama A&M University, Dept. of Food Science, Normal, Al 35762
2.	Larry Beuchat	Dept. of Food Science, Georgia Station, Griffin, Ga.30223
3.	Marian Beremand	Texas A&M University, Plant Sciences Department. College Station, TX 77843
4.	Marvin Beute	North Carolina State University, Dept. of Plant Pathology, Box 7616, Raleigh, NC 27695
5.	Louis J. Boyd	The University of Georgia, Office of Int'l Agricultural Sponsored Programs, 201 Conner Hall, Athens, GA 30602
6.	Manjeet Chinnan	Dept. of Food Science, Georgia Station, Griffin, GA 30223
7.	Rick Brandenburg	North Carolina State University, Dept. of Entomology, Box 7613, Raleigh, NC 27695-7613
8.	Richard J. Cole	National Peanut Research Laboratory, USDA-ARS, Dawson, Ga. 31742
9.	David G. Cummins	Peanut CRSP, Georgia Station, Griffin, GA 30223
10.	Jim Demski	University of Georgia, Dept. of Plant Pathology, Georgia Station, Griffin, GA 30223
11.	Idrissa 0. Dicko	University of Ouagadougou, Ouagadougou, Burkina Faso
12.	Julius F. Fajardo	Texas A&M University, Plant Pathology and Microbiology, College Station, TX 77843
13.	Don Faris	ICRISAT, Patancheru, Andhra Pradesh 502 324, India
14.	Ron Gibbons	ICRISAT Sahelian Center, Niamey, (via Paris) Niger, West Africa
15.	W. James Grichar	Texas A&M University, Plant Disease Research Station, Box 755, Yoakum, TX 77995
16.	Ray Hammons	1203 Lake Drive, Tifton, GA 31794-3834 (EEP)
17.	Geoff Hildebrand	SADCC/ICRISAT, Chitedge Agricultural Research Station, P.O. Box 1096, Lilongwe, Malawi
18.	Margaret Hinds	University of the West Indies, St. Augustine, Trinidad
19.	C. Corley Holbrook	USDA-ARS, Coastal Plain Station, Tifton, GA 31793
20.	T. G. Isleib	North Carolina State University, Dept. of Crop Science, Box 7629, Raleigh, NC 27695-7629

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21.	Wm. Fred Johnson	Agency for International Development, University Center/BIFADEC, Rm 900 SA-38, Washington, DC 20523-3805
22.	Nancy Keller	Texas A&M University, Dept. of Plant Pathology & Microbiology, College Station, TX 77843
23.	Robert E. Lynch	USDA-ARS, Coastal Plain Station, Tifton, GA 31793
24.	Ousmane NDoye	ISRA, Secteur Centre-Sud, B.P. 199, Kaolack, Senegal, West Africa
25.	A. J. Norden	Rt. 2 Box 1651, High Springs, FL 32643 (Peanut CRSP EEP)
26.	B. Onuma Okezie	Alabama A&M University, Office of International Programs, Normal, Al 35762
27.	Ted Oplinger	Texas A&M Univ., Dept. of Plant Pathology & Microbiology, College Station, TX 77843
28.	Johnny Pendleton	1722 Robert Drive, Champaign, IL 61821
29.	Robert E. Pettit	Texas A&M University, Plant Sciences Dept. College Station, TX 77843
30.	Robert Schaffert	Agency for International Development, R&D/AGR/AP, RM 420 SA-18, Washington, DC 20523-1809
31.	Robert A. Schilling	Annual Oil Crop Division, IRHO, B. P. 5035, Montpellier Cedex 01, France
32.	Mike Schubert	Texas A&M University, Plant Disease Research Station, Yoakum, TX 77995
33.	C. E. Simpson	Texas A&M University, Box 292, Stephenville, TX 76401
34.	Bharat Singh	Alabama A&M University, Dept. of Food Science, Normal, AL 35762
35.	Donald A. Smith	ICRISAT, Patancheru, Andhra Pradesh 502 324, India
36.	Dudley Smith	Texas A&M University, Agricultural Experiment Stations, College Station, TX 77843
37.	Olin Smith	Texas A&M University, Soil & Crop Sciences, College Station, TX 77843
38.	Tom Stalker	North Carolina State University, Dept. of Crop Science, Box 7629, Raleigh, NC 27695-7629
39.	Farid Waliyar	ICRISAT Sahelian Center, Niamey, Niger (via Paris) West Africa
40.	Arthur Weissinger	North Carolina State University, Dept. of Crop Science, Box 7620, Raleigh, NC 27695-7620
41.	Monique Wells	Texas A&M University, Plant Disease Research Station, P.O. Box 755, Yoakum, TX 77995
42.	Johnny Wynne	North Carolina State University, North Carolina Agricultural Research Service, P.O. Box 7643, Raleigh, NC 27695-7643