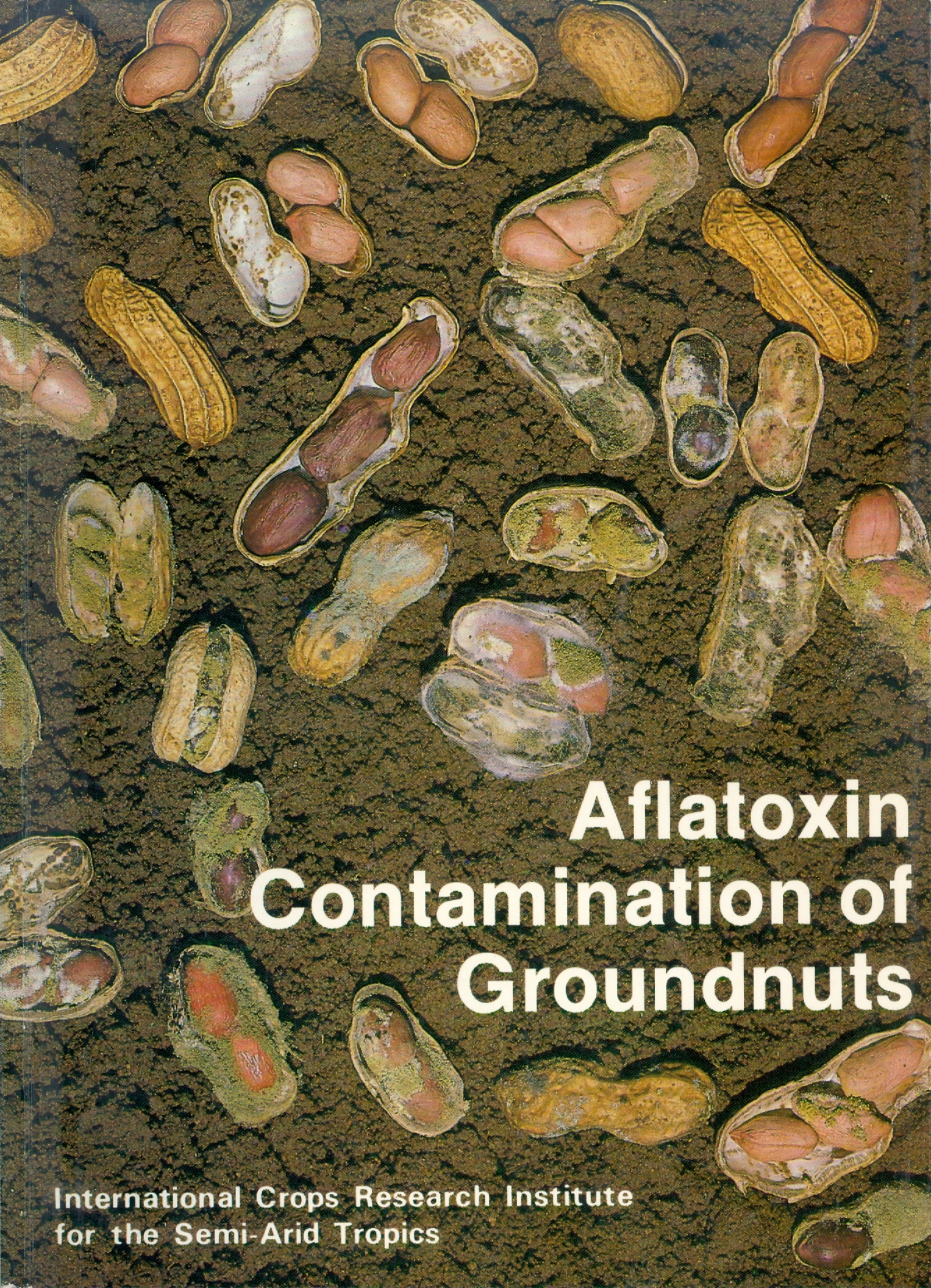

Aflatoxin Contamination of Groundnuts

International Crops Research Institute
for the Semi-Arid Tropics

Abstract

Citation: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1989. Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6–9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India: ICRISAT.

Contains a general overview of the problem of aflatoxin contamination of groundnut and over 40 papers grouped to cover the following topics: importance of aflatoxins, aflatoxins and trade, monitoring and action at national level, removal of aflatoxins, methods for aflatoxins analysis, general research on aflatoxin contamination, and genetic resistance.

Also included are group discussion reports on: evaluation and monitoring of contamination of groundnuts and groundnut products, analytical methods, research on on-farm control of aflatoxin contamination, and research on control with reference to storage, transit, processing etc. Recommendations cover information and training, strategies for control, and future research needs. Overview, summaries, group discussion reports, and recommendations, in English, French, and Spanish.

Résumé

Référence: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1989. Contamination des arachides par les aflatoxines : comptes rendus du Colloque international, 6–9 Oct 1987, Centre ICRISAT, Inde. Patancheru, A.P. 502 324, Inde : ICRISAT.

Cette publication offre une vue d'ensemble sur le problème de la contamination des arachides par les aflatoxines. Elle rassemble 40 communications portant sur les thèmes suivants : importance des aflatoxines, aflatoxines et commerce d'arachide, surveillance et lutte au niveau national, élimination des aflatoxines, méthodes d'analyse des aflatoxines, recherches générales sur la contamination et résistance génétique.

Sont présentés également les procès verbaux de discussions en groupe sur : évaluation et contrôle de la contamination de l'arachide et des produits à base d'arachides, méthodes analytiques, recherche sur les méthodes de lutte contre la contamination par les aflatoxines au niveau de la ferme ainsi qu'aux niveaux du stockage, du transport, de la transformation, etc. Les recommandations faites lors du colloque couvrent les besoins de l'information et de la formation en ce qui concerne la contamination par les aflatoxines, des stratégies de lutte et des recherches futures. Une synthèse, des procès verbaux des discussions ainsi que des recommandations sont donnés en anglais, français et espagnol.

Cover photograph: Groundnuts infected by *Aspergillus flavus*.

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Aflatoxin Contamination of Groundnut:

Proceedings of the International Workshop,

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Contents

A General Overview of the Problem of Aflatoxin Contamination of Groundnut

L.D. Swindale

The cultivated groundnut (*Arachis hypogaea* L.) is the most important oilseed in the developing world, and is a valuable source of protein for human and animal nutrition. According to the Food and Agriculture Organization of the United Nations (FAO) in 1985 nearly 19 million hectares were sown to groundnut worldwide, and 21 million tonnes of dried pods were harvested, a little better than one tonne per hectare. About 80% of the world production is from developing countries, and approximately 67% from the semi-arid tropics which is ICRISAT's mandate area. We have been charged by the Consultative Group on International Agricultural Research (CGIAR) to carry out research on groundnut, particularly aimed at small farmers, since small farmers in the semi-arid tropics are indeed the major producers of the crop. Research on groundnuts started in ICRISAT in 1976, and specific mention was made in the report that led to this program's initiation that it would be necessary to tackle the problem of aflatoxin contamination.

The problem was first recognized following outbreaks of Turkey 'X' disease in the United Kingdom in 1960. The common factor in the outbreaks was that the turkeys' diets contained groundnut meal from Brazil. Research in the United Kingdom revealed that the disease was caused by toxins produced by strains of the fungus *Aspergillus flavus* when growing on the meal, and hence these toxins were named aflatoxins.

The Tropical Products Institute, London, now part of the Overseas Development Natural Resources Institute (ODNRI), and the Central Veterinary Laboratory, Weybridge, UK, played leading parts in the extraction, purification, and identification of the toxins, and in the development of biological and physicochemical methods for identifying and quantifying toxins in groundnut and other commodities. This laid the foundation for rapidly expanding research worldwide to determine which commodities in addition to groundnut could be contaminated with aflatoxins, and which species of livestock in addition to poultry were subject to aflatoxicosis. Several important oilseeds, cereals, legumes, and spice crops were found to be naturally contaminated with aflatoxins, and a wide range of livestock were affected to a greater or lesser extent. Of the other four ICRISAT mandate crops only sorghum is likely to become contaminated with aflatoxins, and of the cereal crops the most important contamination by far occurs in maize. Maize and groundnuts are common items in the diet of many people in the tropics and are common ingredients in livestock feeds in developed countries. The most common of the aflatoxins, aflatoxin B_1, is also the most toxic.

As evidence accumulated on the actual and potential importance of aflatoxicosis in relation to farm animals, there was increasing concern expressed as to the possible hazard to human health. This concern was greatly intensified when it was shown that rats fed on aflatoxin-contaminated groundnut meal developed liver cancer. Over the past 25 years much data have been obtained on the occurrence of aflatoxins in the diet in particular regions of the world in relation to the incidence of liver cancer in those regions, and there are strong indications that the two are related.

The possible presence of such acutely toxic and carcinogenic substances in foods and in animal feeds has had a profound effect on the utilization of, and trade in groundnuts and groundnut products. Processors and importing countries have placed limits on the levels of

aflatoxins permissible in groundnuts and groundnut products. In a paper at the recent FAO/WHO/UNEP International Conference on Mycotoxins, van Egmond stated that some 50 countries have enforced or proposed aflatoxin regulations for foodstuffs. The maximum limits range from zero detectable to 50 $\mu g\ kg^{-1}$. There has been a tendency for regulations to become increasingly stringent as methods of detection have improved. It would naturally be preferred that no aflatoxins are present, but this has not proved to be practicable. It is not only groundnuts for direct human consumption that are subject to restriction. When mammals ingest aflatoxin B_1, the toxin can be passed through to the milk where it occurs in a slightly changed form called aflatoxin M_1. In August 1981 the Ministry of Agriculture in the United Kingdom banned the feeding of groundnut products to dairy cows because of the possible hazard to the health of milk-drinkers. The milk toxin is thought to be particularly important as young animals, and presumably children also, are more susceptible to aflatoxicosis and the carcinogenic effects of aflatoxins than are adults.

The human and livestock health hazards from ingestion of aflatoxin-contaminated food are much greater in the developing than in the developed world. Most developing countries lie within the tropics where temperatures and relative humidities often favor mold growth on these products. Also, conditions for storage often leave much to be desired. In many countries there are only limited or no facilities for monitoring groundnuts and groundnut products for this contamination. There are also possible synergistic interactions between aflatoxin and infectious hepatitis virus B and there is evidence that the effects of ingestion of aflatoxin are much more severe in the case of children suffering from severe protein malnutrition, an unfortunately common condition in some countries where aflatoxins occur. Where groundnut is a cash export crop there has been a tendency to concentrate efforts on ensuring the acceptability of the commoditiy to the importing country, while little attention has been paid to aflatoxin levels in produce for local consumption. It is, of course, appreciated that loss of export income can be of great importance, and it is also appreciated that it is difficult for some groundnut-producing countries to meet the stringent regulations currently imposed by importing countries. Nevertheless, local problems are important. What can be done to eliminate or reduce aflatoxin contamination of groundnuts and groundnut products? There is at present no single practice that can prevent aflatoxin contamination of the commodity. Of course, if we could breed varieties of groundnut upon which the toxigenic *A. flavus* could not grow, or on which it could grow but could not produce toxins, then the problem would be solved. Unfortunately we have not done that.

What can be done at the farm level is to grow varieties that have the highest available resistance to pod and seed invasion by *A. flavus* and also follow cultural practices that minimize damage to pods. Late-season drought stress should be avoided, and the crop should be harvested as soon as the majority of pods are mature. Postharvest drying should be rapid but not so fast as to lead to seed damage, and storage should be under clean, dry, pest-free conditions. Produce should be monitored for aflatoxin contamination as it leaves the farm or on arrival at buying stations or processing plants, and lots with aflatoxin levels above those permissible should be diverted to nonfood use, or be subjected to some detoxification process before use as food or livestock feed. Refined oil from groundnuts processed in modern solvent-extraction plants should be free from aflatoxin, but oil produced in more primitive, village-level crushing plants may contain significant levels and require additional treatment to render it safe for human consumption. Even when the groundnut product reaches the consumer, the risk of aflatoxin contamination is not over. Spores of *A. flavus* are common in the air and in water in tropical and warm temperate regions so exposed food may be colonized and aflatoxins produced if environmental conditions and the constitution of the food are suitable.

It is evident that efforts to prevent aflatoxin contamination of groundnuts must start during crop growth and continue until the product is consumed. While many of the practices recommended for prevention of contamination are simple and easy to apply, they have to be adapted to particular agroecological conditions and some may not be feasible in less-developed countries where facilities may be minimal or nonexistent. Many different groups have to be involved and must work together to tackle the problem.

This Workshop has brought together agricultural research and extension workers from 26 developing and developed countries, and from various international and regional institutions. Invited representatives from marketing and processing units and government trade interests, and medical and veterinary representatives have attended to provide a comprehensive coverage of the problem. The major objectives of the Workshop are to make an up-to-date evaluation of the problem, and to assess recent and ongoing research, with a view to providing the best possible advice to all concerned with the production of groundnuts on how to reduce, if not eliminate, contamination with aflatoxins.

ICRISAT's own approach to aflatoxin research concentrates on developing groundnuts that do not tolerate invasion by *A. flavus*, or that prohibit the development of the toxin. Related studies deal with developing in vitro methods to detect seed resistance, detecting the toxins themselves, and studying the environmental factors affecting resistance. This may seem to be a somewhat limited approach to the problem but ICRISAT is an international agricultural research and training institute that conducts most of its groundnut research in three host countries: India, Niger, and Malawi. It works, moreover, for the small farmers of all regions of the tropics where groundnut is grown. It is not appropriate for ICRISAT to undertake extensive research on postharvest aflatoxin problems since that is the clear responsibility of national scientific establishments, and it would not be appropriate for ICRISAT to undertake work that would reflect upon their ability to deal with such problems. It is, however, necessary to remember that the target group of small farmers does not, for the most part, have either the education, the information, or the means to control aflatoxin levels by sophisticated management practices. We do what it is best for us to do and we believe that our research on *A. flavus* and its toxins will enable small farmers of the tropics to obtain more profitable returns from groundnut production. Our work, however, is but a small contribution to a large problem.

It is hoped that the Workshop and the Proceedings from it will assist in presenting progress made on all fronts in dealing with the serious problem of aflatoxin contamination of groundnuts and will show how the various research achievements contribute to a greater whole.

It is also hoped that by widely distributing both the Summary and full Proceedings of this Workshop, we can bring the problem to the attention of relevant government authorities and policy makers so that they can take appropriate action to minimize the health risks to both humans and livestock from consuming contaminated groundnuts and groundnut products, and also to improve the quality of traded groundnuts, and thus increase export earnings.

Synthèse du probleme de la contamination de l'arachide par les aflatoxines

L.D. Swindale

L'arachide cultivée (*Arachis hypogaea* L.) est la culture oléagineuse la plus importante du monde en voie de développement, et une source précieuse de protéines pour la nutrition humaine et animale. Selon l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO), l'arachide occupait, en 1985, presque 19 millions d'hectares dans le monde entier, donnant une récolte de 21 millions de tonnes de gousses sèches, soit un peu plus de 1 t ha^{-1}. A peu près 80% de la production mondiale provient des pays en développement, dont 67% des tropiques semi-arides, la zone d'activité de l'ICRISAT. Le Groupe consultatif sur la recherche agricole internationale (CGIAR) nous a chargés d'effectuer des recherches sur l'arachide, portant particulièrement sur les petits paysans, ceux-ci étant les principaux producteurs des arachides dans les tropiques semi-arides. Les recherches sur l'arachide ont débuté à l'ICRISAT en 1976 et il a été souligné dans le rapport menant à l'initiation de ce programme qu'il serait nécessaire d'aborder le problème de la contamination par aflatoxines.

Le problème a été soulevé pour la première fois au Royaume-Uni en 1960 lors de l'apparition de la maladie "X" des dindes. Le facteur commun dans les manifestions de cette maladie était que la nourriture donnée aux dindes contenait de la farine d'arachide provenant du Brésil. Des recherches réalisées au Royaume-Uni ont montré que la maladie était provoquée par des toxines produites par des souches du champignon *Aspergillus flavus* présentes dans la farine, d'où le nom aflatoxine, qui leur a été donné par la suite.

Le Tropical Products Institute à Londres, intégré maintenant à l'Overseas Development Natural Resources Institute (ODNRI), et le Central Veterinary Laboratory, Weybridge, Royaume-Uni, ont joué un rôle prédominant dans l'extraction, la purification et la détermination des toxines, ainsi que dans la mise au point de méthodes biologiques et physiochimiques d'identification et de quantification des toxines présentes dans l'arachide et dans d'autres matières premières. Les fondements ainsi établis ont permis aux recherches en train de se développer rapidement partout dans le monde de déterminer quelles matières premières, outre l'arachde, risquaient d'être contaminées par les aflatoxines et quelles espèces de bétail, en plus de la volaille, étaient sujettes à l'aflatoxicose. Plusieurs oléagineux, céréales, légumes et épices importants s'avéraient être naturellement contaminés par les aflatoxines et une large gamme de bétail en était affectée à un degré plus ou moins important. Parmi les quatre autre cultures intéressant l'ICRISAT, seul le sorgho est susceptible d'être contaminé par les aflatoxines, et parmi les autres cultures céréalières, la contamination la plus importante existe surtout chez le maïs. Le maïs et l'arachide font fréquemment partie du régime alimentaire de beaucoup de gens dans les zones tropicales et sont souvent utilisés dans la préparation d'aliments pour bétail dans les pays industrialisés. L'aflatoxine la plus rencontrée, l'aflatoxine B_1, est également la plus toxique.

Au fur et à mesure que l'évidence d'une importance réelle ou potentielle de l'aflatoxicose chez les animaux d'élevage s'accroissait, une inquiétude croissante s'est installée quant au danger pour la santé humaine. Cette inquiétude s'est intensifiée de façon importante dès lors qu'on a démontré que les rats nourris avec des arachides contaminées par les aflatoxines contractaient des cancers du foie. Depuis 25 ans, de nombreuses données ont été collectées concernant la

présence d'aflatoxines dans les régimes alimentaires de certaines régions du monde et l'incidence des cancers du foie dans ces régions; selon ces données, il y a de fortes présomptions pour que les deux soient liées.

La présence éventuelle de produits tellement toxiques et cancérigènes dans les aliments destinés à la consommation humaine ou animale a eu des effets considérables sur l'utilisation des arachides et des produits à base d'arachides, ainsi que sur le marché de cette matière première. Les industries de transformation et les pays importateurs ont imposé des limites sur la teneur admissible des arachides et des produits à base d'arachides en aflatoxines. Dans une communication présentée à la récente Conference Internationale FAO/WHO/UNEP sur les Mycotoxines, van Egmond a fait remarquer qu'une cinquantaine de pays ont appliqué ou ont proposé des règlements relatifs à la teneur en aflatoxines des aliments. Les limites supérieures varient entre un taux de zéro détectable et 50 $\mu g\ kg^{-1}$. Au fur et à mesure que les méthodes de détection sont améliorées, ces règlements ont tendance à devenir de plus en plus sévères. La situation la meilleure serait, évidemment, une absence totale d'aflatoxines, mais ceci s'est avéré irréalisable. Les restrictions ne s'appliquent pas seulement aux arachides destinées directement à la consommation humaine. L'aflatoxine B_1, ingérée par les mammifères, peut passer dans le lait de l'animal, où elle se trouve sous une autre forme légèrement modifiée, appelée aflatoxine M_1. Au mois d'août 1981, le Ministère de l'Agriculture au Royaume-Uni a interdit l'affourragement des vaches laitières avec des produits à base d'arachides à cause du danger qui existait pour les consommateurs de lait. La toxine du lait est considérée comme particulièrement importante puisque les jeunes animaux, et vraisemblablement les enfants aussi, sont plus susceptibles à l'aflatoxicose et aux effets cancérigènes des aflatoxines que les adultes.

Le danger que représente l'ingestion d'aliments contaminés par les aflatoxines pour la santé de l'homme et du bétail est bien plus important dans les pays en voie de développement que dans les pays industrialisés. La plupart des pays en développement se trouvent dans les zones tropicales où les températures et les taux d'hygrométrie favorisent souvent le développement de moisissures sur ces produits. De plus, les conditions de stockages laissent souvent beaucoup à désirer. Dans de nombreux pays les moyens de détection de la contamination des arachides ou des produits à base d'arachides sont souvent limités ou absents. Il existe aussi d'éventuels effets synergiques entre les aflatoxines et le virus de l'hépatite B et il y a des preuves que les effets de l'ingestion des aflatoxines sont plus sévères dans le cas d'enfants souffrant d'une déficience alimentaire en protéines, situation rencontrée malheureusement très souvent dans les pays où les aflatoxines sont présentes. Les pays où l'arachide est une culture de rente destinée à l'exportation ont tendance à s'appliquer à rendre acceptable leur matière première aux pays importateurs, tandis qu'ils ne s'occupent guère des taux d'aflatoxines présentes dans les produits destinés à la consommation domestique. Bien sûr, on peut comprendre qu'une perte des revenus provenant des produits exportés puisse être d'importance majeure et que certains pays producteurs d'arachide peuvent difficilement répondre aux règlements imposés actuellement par les pays importateurs. Néanmoins, les problèmes locaux sont importants. Que faire pour éliminer ou réduire la contamination des arachides ou des produits à base d'arachide par les aflatoxines? Il n'existe, pour le moment, aucune mesure unique qui puisse éviter la contamination de cette matière première par les aflatoxines. Bien sûr, s'il était possible de sélectionner des variétés d'arachide sur lesquelles l'*A. flavus* toxinogène était incapable de s'établir, ou au moins sur lesquelles, une fois établi, il était incapable de produire les toxines, le problème serait résolu. Malheureusement, nous n'en sommes pas encore là.

Ce qui peut être fait au niveau de la ferme est de cultiver des variétés présentant la résistance la plus forte possible des gousses et des graines contre l'invasion par *A. flavus*, mettant en oeuvre en même temps des pratiques culturales qui minimisent les degâts subis par les gousses. Le stress

hydrique en fin de saison doit être évité, et la récolte doit être commencée dès que la plupart des gousses sont mûres. Le séchage post-récolte doit être rapide, mais pas trop afin de ne pas abimer les graines, et le lieu de stockage doit être propre, sec et sans ravageurs. La production doit être contrôlée au moment de quitter la ferme ou à l'arrivée aux points d'achat ou aux usines afin de détecter toute contamination par aflatoxines; les lots présentant des taux d'aflatoxines supérieurs aux niveaux admissibles doivent être dirigés vers les filières non alimentaires, ou doivent subir un procédé de détoxification avant d'être utilisés dans la préparation d'aliments destinés à la consommation humaine ou animale. L'huile d'arachide raffinée provenant d'usines modernes pratiquant l'extraction à l'aide de solvants sera normalement totalement dépourvue d'aflatoxines, mais l'huile produite dans les unités d'extraction villageoises plus rudimentaires peut avoir des teneurs en aflatoxines importantes, nécessitant un traitement supplémentaire afin de la rendre acceptable à la consommation humaine. Même au moment où le produit arrive chez le consommateur, le risque de contamination par les aflatoxines n'est pas terminé. Les spores d'*A. flavus* existant en grand nombre dans l'air et dans l'eau dans les régions à climat tropical ou chaud et temperé, les aliments non protégés peuvent être colonisés avec production d'aflatoxines, si les conditions du milieu et la composition des aliments sont favorables.

Il est evident que les efforts visant à éviter la contamination des arachides par les aflatoxines doivent démarrer pendant la période de croissance des cultures et doivent continuer jusqu'au moment où les produits sont consommés. Bien que beaucoup des pratiques recommandées pour éviter la contamination soient simples à mettre en oeuvre, il faut les adapter aux conditions agro-climatiques et certaines d'entre elles pourront s'avérer irréalisables dans les pays moins développés où les moyens de travail sont réduits ou inexistants. De nombreux groupes différents sont appelés à intervenir et doivent travailler ensemble afin de résoure le problème.

Cet Atelier a rassemblé les chercheurs et les agents de vulgarisation agricoles de 26 pays en voie de développement et industrialisés, ainsi que ceux appartenant à divers organismes internationaux et régionaux. Des invités représentant des sociétés de commercialisation ou de transformation et des organismes de commercialisation gouvernementaux, ainsi que des représentants des milieux médicaux ou véterinaires y ont participe afin de traiter tous les aspects de ce problème. L'Atelier avait pour objectifs principaux de faire une mise au point du problème et d'évaluer les recherches récentes ou en cours, en vue de fournir les meilleurs conseils possibles a tous ceux qui sont concernes par la production des arachides dans le but de réduire, voire éliminer la contamination par aflatoxines.

Les travaux réalisés par l'ICRISAT portent sur le développement de variétés d'arachide qui ne permettent pas l'invasion par *A. flavus*, ou qui empêchent le développement de la toxine. Des recherches parallèles portent sur la mise au point de méthodes *in vitro* pour déterminer la resistance des graines, sur la détection des toxines elles-mêmes et sur l'étude des facteurs du milieu ayant un effet sur la résistance. Cette façon d'aborder le problème pourrait apparaître quelque peu limitée mais l'ICRISAT est un institut international de recherche agricole et de formation qui réalise la plupart de ses travaux de recherches dans trois pays hôtes : l'Inde, le Niger et le Malawi. D'ailleurs, il travaille pour le compte de tous les petits cultivateurs de toutes les régions tropicale où l'on cultive l'arachide. Il ne serait pas souhaitable que l'ICRISAT entreprenne des recherches importantes sur les problèmes post-récolte posés par les aflatoxines, puisque ce travail est évidemment de la responsabilité des établissements scientifiques nationaux et il ne serait pas souhaitable que l'ICRISAT entreprenne un travail qui puisse remettre en cause leur aptitude à traiter un tel problème. D'ailleurs, il faut rappeler que le groupe de petits cultivateurs visé n'a, pour la plupart, ni l'éducation, ni l'information, ni les moyens nécessaires au contrôle des niveaux d'aflatoxines, ce qui exige des pratiques de gestion sophistiquées. Nous faisons ce qui est pour nous le mieux à faire et nous pensons que nos recherches sur *A. flavus* et

ses toxines permettront aux petits cultivateurs des régions tropicales de s'assurer des revenus bien plus rentables à partir de leurs productions d'arachides. Néanmoin, notre travail n'est qu'une petite contribution à la solution d'un grand problème.

Nous espérons que cet Atelier, ainsi que les Comptes rendus qui en seront faits, permettront d'avancer sur tous les fronts de la bataille contre ce grave problème qu'est la contamination des arachides par les aflatoxines et qu'ils montreront comment les diverses réalisations de la recherche contribuent à un ensemble plus important.

On espère également qu'une large diffusion des résumés et des Comptes rendus complets de cet Atelier nous permettra de porter ce problème à l'attention des autorités gouvernementales concernées et de ceux qui décident la politique à suivre, afin qu'ils puissent agir de façon à minimiser le danger que représente la consommation d'arachides ou de produits à base d'arachides contaminés pour la santé humaine ou animale, permettant également l'amélioration de la qualité des arachides commercialisées et, par conséquent, une augmentation des revenus à l'exportation.

Una revisión general del problema de la contaminación con aflatoxinas en el cacahuate

L.D. Swindale

El cacahuate (*Arachis hypogaea* L.) es la oleaginosa más importante de los países en desarrollo, y una fuente valiosa de proteínas para la nutrición humana y animal. Según la Organización de las Naciones Unidas para la Agricultura y de la Alimentación (FAO), en el año de 1985 se cultivaron casi 19 millones de hectáreas con cacahuate en todo el mundo, y se cosecharon 21 millones de toneladas de vainas secas, o sea un poco más de una tonelada por hectárea. Alrededor del 80% de la producción mundial procede de los países en desarrollo, y aproximadamente 67% de los trópicos semiáridos, que correspondan al área donde trabaja el ICRISAT. Nos ha encomendado el Grupo Consultivo sobre Investigaciones Agrícolas Internacionales (CGIAR) efectuar investigaciones sobre el cacahuate especialmente enfocadas hacia los productores en pequeños predios, dado que dentro de los trópicos semiáridos éstos son realmente los principales productores del cultivo. Las investigaciones sobre cacahuate se iniciaron en el ICRISAT en 1976, y se hizo mención específica en el informe que precedió a la iniciación de este programa, que sería necesario enfrentarse al problema de la contaminación con aflatoxinas.

El problema fue identificado por primera vez, después de ocurrir los brotes iniciales de la enfermedad "X" de los pavos en el Reino Unido, en el año de 1960. El factor común observado en dichos brotes estribaba en que la dieta de los pavos afectados contenía torta de cacahuate procedente de Brasil. Las investigaciones realizadas en el Reino Unido revelaron que la enfermedad era causada por toxinas producidas por cepas del hongo *Aspergillus flavus*, cuando se desarrollaban en la torta citada, y por tanto estas toxinas se denominaron aflatoxinas.

El Tropical Products Institute (TPI), Londres, que ahora forma parte del Overseas Development Natural Resources Institute (ODNRI), y el Central Veterinary Laboratory, en Weybridge, RU, desempeñaron papeles prominentes en la extracción, purificación e identificación de las toxinas, y en el desarrollo de métodos biológicos y fisicoquímicos para la identificación y cuantificación de toxinas en cacahuate y otros productos comerciales. Esto estableció las bases para que en las investigaciones rápidamente crecientes a escala mundial, se pudiera identificar qué productos comerciales, además de los cacahuates, podrían estar contaminados con aflatoxinas, y cuáles especies de animales, además de las aves, eran susceptibles a la aflatoxicosis. Se encontró que varias semillas oleaginosas importantes, cereales, leguminosas y cultivos de especias, estaban contaminados con aflatoxinas en forma natural, y un gran número de especies de ganado eran afectados por las aflatoxinas, en mayor o menor grado. Entre los cuatro cultivos que el ICRISAT tiene el compromiso de investigar, solamente el sorgo tiene posibilidades de llegar a contaminarse con aflatoxinas, mientras que entre los cereales, la contaminación más importante por varios órdenes de magnitud, ocurre en maíz. El maíz y los cacahuates son productos comunes en la dieta de muchos pueblos en los trópicos e ingredientes usuales en los alimentos del ganado, en los países desarrollados. La más común de las aflatoxinas, la aflatoxina B, es también la más tóxica.

A medida que se acumulaban las pruebas sobre la importancia actual y potencial de las aflatoxinas en los animales de granja, se acrecentaron las inquietudes acerca de su posible peligro para la salud humana. Estas se intensificaron marcadamente cuando se demostró que ratas alimentadas con torta de cacahuate, contaminada con aflatoxinas, eran afectadas por

cáncer del hígado. Durante los últimos 25 años se han reunido abundantes datos sobre la presencia de aflatoxinas en las dietas de determinadas regiones del mundo, en relación con la incidencia de cáncer del hígado en estas regiones, y existen fuertes indicios que están interrelacionadas.

La posible presencia de substancias tan extremadamente tóxicas y carcinogénicas en los alimentos de consumo humano y en el alimento animal, ha tenido un efecto profundo en la utilización y el comercio del cacahuate y los productos derivados de cacahuate. Los países procesadores e importadores han puesto límites máximos de niveles de aflatoxinas permisibles en los cacahuates y productos derivados de éstos. En una ponencia presentada en la reciente conferencia internacional FAO/OMS/UNEP sobre micotoxinas, van Egmond informó que alrededor de 50 países han estatuido o propuesto legislación reglamentando el contenido de aflatoxinas en los alimentos. Los límites máximos tolerables varían desde no detectables hasta 50 $\mu g\ kg^{-1}$. Ha habido la tendencia de que la reglamentación sea cada vez más restrictiva, conforme han mejorado los métodos de detección. Naturalmente sería preferible que no hubiera aflatoxinas presentes, pero esto no ha resultado factible. No sólo los cacahuates para consumo humano directo están sujetos a las restricciones. Cuando los mamíferos ingieren aflatoxina B_1, esta toxina puede pasar a la leche en donde ocurre en una forma ligeramente modificada llamada aflatoxina M_1. En agosto de 1981, el Ministerio de Agricultura del Reino Unido proscribió la alimentación de vacas lecheras con productos derivados de cacahuates, debido al posible peligro a la salud de los consumidores de leche. Se cree que la toxina en la leche es particularmente importante, debido a que los animales lactantes y presuntamente también los niños, son más susceptibles a la aflatoxicosis y a los efectos carcinogénicos de las aflatoxinas que los adultos.

Los peligros para la salud humana y de la ganadería, por ingestión de alimentos contaminados con aflatoxinas, son mucho mayores en los países en vías de desarrollo, que en los desarrollados. La mayoría de los primeros están situados dentro del trópico, donde las temperaturas y humedades relativas frecuentemente favorecen el crecimiento de los hongos en los productos citados. También las condiciones de almacenamiento frecuentemente dejan mucho que desear. En muchos países solamente hay facilidades limitadas o no existen éstas, para la vigilancia contínua contra esta contaminación en los cacahuates o sus derivados. También existen posibles interacciones sinergéticas entre las aflatoxinas y el virus B de la hepatitis infecciosa, y hay evidencia de que los efectos de la ingestión de aflatoxinas son mucho más severos en el caso de niños que padecen de una grave malnutrición proteíca, condición desafortunadamente común, en algunos de los países donde ocurren las aflatoxinas. Donde los cacahuates son un cultivo de exportacion que produce ingresos monetarios a los productores, ha habido la tendencia de concentrar los esfuerzos en asegurar la aceptabilidad del producto comercial en el país importador, mientras que poca atención se ha puesto en los niveles de aflatoxinas en los productos asignados al consumo local. Por supuesto, se reconoce que la pérdida de ingresos procedentes de la exportación puede ser de gran importancia, y también se aprecia que es difícil en algunos países productores de cacahuate, satisfacer las estrictas reglamentaciones actualmente impuestos por los países importadores. Sin embargo, los problemas locales son importantes. ¿Qué puede hacerse para eliminar o reducir las contaminaciones con aflatoxinas en los cacahuates y sus derivados? Actualmente no hay una práctica única que puede prevenir la contaminación con aflatoxina de los productos comerciales. Por supuesto, si pudieramos obtener genéticamente variedades de cacahuate sobre las cuales el hongo toxigénico *A. flavus* no pudiera desarrollarse, o sobre las cuales aun cuando se desarrollara no produjera toxinas, entonces estaría resuelto el problema. Desafortunadamente no hemos hecho eso aún. Lo que puede hacerse al nivel de predio rural, es sembrar variedades que tienen el grado más alto posible de resistencia a las invasiones de las vainas y semillas por *A. flavus* y también aplicarse prácticas de cultivo que

reducen a un mínimo las lesiones a las vainas. Deben evitarse condiciones adversas de sequía durante la última parte del ciclo de desarrollo de cultivo, y éste debe cosecharse cuando la mayoría de las vainas se encuentren maduras. El secado de post-cosecha debe ser rápido, pero no tanto que produzca daños a la semilla, y el almacenamiento deberá ser bajo condiciones limpias, secas y libre de plagas. Las cosechas deben vigilarse contínuamente contra la contaminación con aflatoxinas, al salir de los predios rurales o al arribar en los centros de acopio o plantas procesadoras, y aquellos lotes de materiales con niveles de aflatoxinas mayores que los permisibles, deberán separarse para uso no-alimentario, o someterse a algún proceso que elimine las toxinas, antes de usarse como alimento humano o animal. El aceite refinado obtenido de los cacahuates procesados en las plantas modernas de extracción con solventes está libre de aflatoxinas; pero el aceite producido en las plantas extractoras de aceite por presión, que son las más primitivas y de uso frecuente en los pueblos pequeños, puede contener altos niveles de aflatoxinas y requerir un tratamiento adicional para que sea apta para el consumo humano. Cuando el producto derivado de los cacahuates llega a manos del consumidor, el riesgo de contaminación con aflatoxinas aún persiste. Las esporas de *A. flavus* generalmente están presentes en el aire y el agua de las regiones con climas tropicales o cálido-templados, de manera que los alimentos expuestos pueden llegar a ser inoculados y producir aflatoxinas, si las condiciones ambientales y la composición de los alimentos en cuestión son favorables.

Es obvio que los esfuerzos para evitar la contaminación de los cacahuates con aflatoxinas deben iniciarse durante el desarrollo del cultivo y continuarse hasta que la cosecha sea consumida. Aun cuando muchas prácticas recomendadas para evitar la contaminación son sencillas y fáciles de aplicar, éstas deben adaptarse a las condiciones agroecológicas específicas y algunas no pueden aplicarse en los países menos desarrollados, donde las facilidades necesarias son mínimas o inexistentes. Muchos grupos diferentes de productores tendrán que participar y trabajar juntos para resolver el problema común.

Este taller ha reunido a los investigadores agrícolas y extensionistas procedentes de 26 países en vías de desarrollo y desarrollados, y de varias instituciones internacionales y regionales. Han asistido representantes invitados de unidades comerciales, de procesamiento, e intereses comerciales gubernamentales, así como representantes de los sectores médicos y veterinarios, para lograr una cobertura comprensiva del problema. Los principales objetivos del taller son: lograr una evaluación actualizada del problema y desglosar las investigaciones recientes, así como las investigaciones actualmente en marcha, con el propósito de proporcionar los mejores consejos posibles a todas las personas interesadas en la producción de cacahuates, sobre cómo reducir o eliminar las contaminaciones con aflatoxinas.

El enfoque de las acciones del ICRISAT en las investigaciones sobre aflatoxinas se concentra sobre el desarrollo de cacahuates resistentes a la invasión de *A. flavus*, o que impiden el desarrollo de las toxinas. Estudios colaterales se realizan para desarrollar métodos *in vitro*, para detectar la resistencia de la semilla, la detección de las toxinas, y la evaluación de los factores ambientales que afectan la resistencia del cultivo. Esto pudiera parecer un enfoque un tanto restringido al problema, pero el ICRISAT es un instituto internacional de investigación y capacitación agrícola, que realiza la mayoría de sus investigaciones sobre cacahuate dentro de tres países anfitriones: India, Niger y Malawi. Además, trabaja en beneficio de los pequeños agricultores de todas las regiones del trópico donde se siembran cacahuates. No se considera conveniente que el ICRISAT emprenda investigaciones amplias sobre problemas de aflatoxinas en el período post-cosecha, dado que esto claramente es responsabilidad de las instituciones científicas nacionales, y no sería apropiado que el ICRISAT emprendiera estos trabajos, si existe la capacidad nacional para la resolución de problemas de esta índole. Sin embargo, es necesario recordar que el importante grupo de pequeños agricultores carece, en su mayor parte,

de la educación, la información, y los medios necesarios para mantener bajos los niveles de aflatoxinas si es necesario el uso de prácticas sofisticadas de manejo en cultivos. Hacemos aquello que consideramos prioritario, y creemos que nuestras investigaciones sobre *A. flavus* y sus toxinas permitirán a los pequeños agricultores en los trópicos derivar mayores ingresos de la producción de cacahuates. Nuestro trabajo, sin embargo, es solamente una pequeña contribución a la solución de un problema mayor.

Se espera que este taller y las memorias derivadas del mismo, ayudarán en la presentación del progreso logrado en todos los frentes, en la lucha contra el serio problema de la contaminación con aflatoxinas en los cacahuates, y mostrarán como cada uno de los diversos logros de las investigaciones contribuyen a alcanzar un objetivo general de mucho mayor magnitud.

También se espera que mediante la amplia distribución tanto del resumen como las memorias completas de este taller, podremos poner el problema ante la atención de las autoridades gubernamentales y los formuladores de políticas apropiados, a fin de que puedan tomar las acciones necesarias para minimizar los riesgos para la salud, tanto de humanos como para la ganadería, derivados del consumo de cacahuates o productos derivados, contaminados con aflatoxinas, así como mejorar la calidad de los cacahuates que entran al comercio y consecuentemente incrementar los ingresos por concepto de exportación de los mismos.

Objectives and Structure of the Workshop

Objectives

The main objectives of the Workshop were to:

- Bring together research workers and others concerned with the many different aspects of the groundnut aflatoxin problem to exchange the latest information,
- Evaluate the status of research on aflatoxins in different countries/regions,
- Identify areas for collaborative research,
- Discuss ways of evaluating and managing the aflatoxin contamination problem in groundnuts around the world,
- Identify specific training needs and organizations that can offer training, and
- Develop plans for disseminating information useful to groundnut growers, processors, users, advisory services, and policy makers.

Structure

In order to cover the wide range of topics and disciplines represented at the Workshop, papers were arranged in sessions that were ordered to move from general aspects of the problem to specific research topics. With over 40 papers presented, discussions at the end of each were of necessity brief, but the program allowed for the participants to break into groups and hold in-depth discussions within these groups. At a final plenary session each group chairman presented a report and recommendations that are included in this Proceedings.

The recommendations of each group were considered during the plenary session by all participants and the final recommendations of the Workshop have been formulated from these deliberations.

A major recommendation, and a continuing theme throughout presentations and discussions was the need to increase awareness of the problem of aflatoxin contamination at all levels, from the general public and farmers, to food policy makers and representatives of trade and industry. In recognition of this a decision was made to prepare a Summary Proceedings in English, French and Spanish and to distribute it as widely as possible. This was done in 1988.

Throughout this document the term groundnut is used for *Arachis hypogea* L. except in proper names of organizations e.g., Peanut CRSP or in widely recognized names for groundnut products e.g., peanut butter.

In order to achieve uniformity in reporting all aflatoxin contents are expressed in micrograms per gram ($\mu g\ g^{-1}$) or per kilogram ($\mu g\ kg^{-1}$).

Objectifs et organisation de l'atelier

Objectifs

L'Atelier avait pour objectifs principaux de :

- Rassembler les chercheurs et tous ceux qui sont intéressés par les nombreux aspects divers du problème des aflatoxines chez l'arachide pour échanger les dernières informations;
- Evaluer la situation des recherches sur les aflatoxines dans les différents pays et régions;
- Definir les possibilités de collaboration dans le domaine des recherches;
- Etudier les moyens d'évaluation et de contrôle du problème de la contamination des arachides par les aflatoxines dans le monde entier;
- Identifier les besoins spécifiques en formation, ainsi que les organismes susceptibles de fournir cette formation;
- Etablir des plans de diffusion d'informations utiles auprès des cultivateurs, des industries de transformation, des utilisateurs, des services de conseils et des autorités.

Organisation

Afin de pouvoir traiter toute la gamme de thèmes présentés à l'Atelier, les communications ont été regroupées en sessions, organisées de façon à passer des aspects généraux du problème aux thèmes de recherches spécifiques. Une quarantaine de communications ayant été présentées, les discussions à la fin de chacune d'elles étaient forcément brèves, mais l'organisation du programme permettait aux participants de se diviser en groupes afin de discuter plus en détail au sein de ces groupes. Au cours d'une session plénière de clôture, le président de chaque groupe à présenté un procès verbal et des recommandations qui sont inclus dans ce présent compte rendu.

Les recommandations de chaque groupe ont été examinées par tous les participants au cours de cette session plénière et les recommandations finales de l'Atelier ont été formulées à partir de ces délibérations.

Une recommandation principale, et un thème qui est revenu tout au long des présentations et des discussions, a été la nécessité de rendre les gens plus conscients, a tous les niveaux, de ce problème de contamination des arachides par les aflatoxines, depuis le grand public et les cultivateurs, jusqu'aux responsables des politiques alimentaires et aux représentants du commerce et de l'industrie. Afin de tenir compte de ces préoccupations, il a été décidé de préparer un résumé des travaux en anglais, en français,et en espagnol et de le diffuser aussi largement que possible. Cette démarche a été complétée en 1988.

Tout au long de ce document, le terme "arachide" est utilisé pour désigner *Arachis hypogaea* L., sauf dans le cas de noms propres d'organismes, par exemple le Peanut CRSP.

Dans un souci d'homogénéité, toutes les teneurs en aflatoxines sont exprimées en microgrammes par gramme ($\mu g\ g^{-1}$) ou par kilogramme ($\mu g\ kg^{-1}$).

Objetivos y estructura del taller

Objetivos:

Los principales objetivos del taller fueron:

- Reunir a investigadores y otras personas interesados en los múltiples y diversos aspectos del problema de aflatoxinas en el cacahuate, para intercambiar la información más reciente.
- Evaluar el estado de las investigaciones sobre aflatoxinas en diferentes países y regiones.
- Identificar áreas de investigaciones conjuntas.
- Discutir maneras de evaluar y manejar el problema de la contaminación con aflatoxinas en los cacahuates, en todos los países del mundo.
- Identificar las necesidades específicas de capacitación y las organizaciones que puedan ofrecer capacitación.
- Desarrollar planes para la diseminación de información útil para los productores de cacahuates, procesadores, usuarios, servicios de asistencia técnica y los formuladores de políticas apropiadas.

Estructura

Para cubrir el amplio rango de temas y disciplinas representadas en el taller, las ponencias se agruparon en sesiones de trabajo, que se ordenaron para su desarrollo, desde el aspecto general del problema a los temas de investigación específica. Con la presentación de más de 40 ponencias, las discusiones al final de cada una de éstas fueron inevitablemente breves, pero el programa desarrollado permitió a los participantes separarse en grupos y efectuar discusiones más profundas dentro de los mismos. En la sesión plenaria final, cada presidente de grupo presentó su informe y recomendaciones, que se incluyen en estas memorias. Las recomendaciones de cada grupo fueron analizadas durante la sesión plenaria por todos los participantes, y en base a las deliberaciones respectivas, se formularon las recomendaciones finales del taller. Una recomendación importante del taller, y tema que se reiteró a través de las intervenciones y discusiones, es la necesidad de sensibilizar a la gente acerca del problema de contaminación con aflatoxinas, a todos los niveles, desde el público general y los agricultores, hasta los formuladores de políticas de producción de alimentos y representantes del comercio y la industria. Con fin de reconocer tales recomendaciones, se decidió preparar un resúmen de las memorias en inglés, francés, y español, y para distribuirlas con la mayor divulgación posible. Esto se logró en 1988.

En este documento el término cacahuate se utiliza para designar a *Arachis hypogea* L.

Con la finalidad de lograr uniformidad, todos los contenidos de aflatoxinas se expresan en microgramos por gramo ($\mu g\ g^{-1}$) o microgramos por kilogramo ($\mu g\ kg^{-1}$).

Session II

Importance of Aflatoxins

Risk to Human Health Associated with Consumption of Groundnuts Contaminated with Aflatoxins

R.V. Bhat[1]

Abstract

Acute and chronic effects of aflatoxins in man are well documented. The reported outbreaks of aflatoxicosis in man were due to the consumption of staple foods such as maize and not to the consumption of groundnut. Circumstantial evidence has implicated groundnut meal containing aflatoxin as causing Indian Childhood Cirrhosis. Dietary intake of aflatoxin through groundnut has been implicated in the development of liver cancer in certain parts of the developing world. The incidence of liver cancer associated with the ingestion of aflatoxin from groundnuts is low in developed countries such as the USA.

Food consumption surveys in India have indicated that the consumption of nuts, (mostly groundnuts) varies from 2 to 35 g per consumption unit per person per day depending on the region and season. Data from the Indian Multicentric Food Contamination Monitoring Program have indicated that though aflatoxins could be detected in 13% of the groundnut samples they tested, the toxins exceeded the official permissible limit of 30 $\mu g\ kg^{-1}$ in only 2.6% of the samples. Studies carried out in Thailand, the Philippines, and the USA have indicated that the dietary intake of aflatoxins from groundnut is lower than that from maize.

The aflatoxin regulatory actions taken by the European Economic Community (EEC), Japan, and other developed countries on importing groundnut and its derivatives have resulted in safeguarding exports rather than minimizing health hazards in the developing countries.

Résumé

Risques pour la santé humaine dus à la consommation des arachides contaminées par les aflatoxines : *Les effets aigus et chroniques des aflatoxines chez l'homme sont bien documentés. Les cas d'aflatoxicoses signalés chez l'homme sont dus plutôt à la consommation des aliments de base tels que le maïs, et non pas des arachides. En Inde, la cirrhose infantile est souvent attribuée à la farine des arachides contaminées par les aflatoxines. L'évolution du cancer du foie dans certains pays en voie de développement est également imputée à ces mycotoxines. Cependant, dans les pays développés tels que les Etats-Unis, la corrélation entre les aflatoxines d'arachide et le cancer du foie est faible.*

D'après les enquêtes menées en Inde sur l'alimentation, le taux de consommation journalière des fruits secs, en particulier des arachides, varie de 2 à 35 g par unité de consommation et par personne, en fonction de la région et de la saison. D'après les données collectées par le Indian Multicentric Food Contamination Monitoring Program, les aflatoxines ont été détectées dans 13% des échantillons d'arachides analysés; le taux de toxines n'a dépassé la limite autorisée de 30 $\mu g\ kg^{-1}$ que dans 2,6% des échantillons. Des études conduites en Thaïlande, aux Philippines et

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aux Etats-Unis ont révélé que le niveau d'aflatoxines ingérées suite à la consommation des arachides est inférieur à celui dû au maïs.

Les législations sanitaires mises en vigueur dans les pays de la CEE, au Japon et dans d'autres pays développés, concernant la présence des aflatoxines dans les arachides importées et leurs produits, ont permis de sauvegarder les exportations plutôt que de réduire les risques pour la santé dans les pays en développement.

Resúmene

Peligro para la salud humana asociado con el consumo de cacahuate contaminado con aflatoxinas: *Los efectos agudos y crónicos de las aflatoxinas sobre el hombre están bien documentados. Los brotes reportados de aflatoxicosis en el hombre fueron atribuídos al consumo de alimentos básicos, tales como el maíz, y no al consumo de cacahuates. Pruebas circunstanciales han implicado a la torta de cacahuate que contiene aflatoxinas, como causa de la cirrosis infantil en la India. La absorción dietética de aflatoxinas a través de cacahuates ha sido implicada en el desarrollo del cáncer del hígado, en ciertas partes del mundo en desarrollo. La incidencia de cáncer del hígado asociado con la ingestión de aflatoxinas presentes en cacahuates es baja en países desarrollados, como Estados Unidos de América.*

Los estudios de consumo de alimentos en la India han indicado que el consumo de nueces de diferentes clases (predominantemente cacahuates) varía de 2 a 35 g por unidad de consumo, por persona, por día, dependiendo de la región y la estación del año. Los datos del Programa Multicéntrico para la Vigilancia de la Contaminación Alimentaria de la India han indicado que aun cuando se pudo detectar aflatoxinas en el 13% de las muestras de cacahuate analizados, las toxinas excedieron el límite oficial permisible de 30 $\mu g\ kg^{-1}$ en solamente 2.6% de las muestras. Estudios efectuados en Tailandia, Filipinas y Estados Unidos han indicado que la absorción dietética de las aflatoxinas contenidas en cacahuates es menor que la absorción de aflatoxinas contenidas en el maíz.

Las disposiciones legales que reglamentan el contenido máximo de aflatoxinas, adoptadas por la Comunidad Económica Europea (CEE), Japón y otros países desarrollados, en relación con la importación de cacahuates y productos derivados, han logrado como resultado la protección de los consumidores de las exportaciones, más bien que la reducción de los peligros a la salud en los países en desarrollo.

Introduction

Aspergillus flavus and *A. parasiticus* often infect groundnuts and develop secondary metabolites popularly called aflatoxins. Aflatoxins are known to be hepatotoxic, carcinogenic, and teratogenic. For historical reasons, the problem of aflatoxins has been commonly associated with groundnuts although it is much more of a problem in maize. A Global Significance Survey carried out in different parts of the world revealed that maize and groundnut are the commodities most affected by the mycotoxin (Hesseltine 1986). In a developed country like the USA, the frequency distribution survey of the total aflatoxin level in raw shelled groundnuts and shelled maize clearly indicated that the problem is at least five times more serious in maize than in groundnuts (Stoloff 1985). Outbreaks of aflatoxic hepatitis in India in 1975 and Kenya in 1982 have demonstrated that the health hazard from aflatoxins in developing countries is more from maize than groundnuts (Krishnamachari et al. 1975, Nagindu et al. 1982). However, the

economic significance of aflatoxin contamination in terms of reduced export earnings and loss at different levels of the trade chain such as; shelled groundnuts, edible oils, and oil cakes is more in groundnuts than in maize.

Aflatoxins in Groundnut and their Association with Various Diseases

During the last two decades, efforts have been made in different parts of the world to correlate the consumption of groundnuts contaminated with aflatoxins or aflatoxin-producing fungi with several diseases in man. In most cases, only circumstantial evidence based on epidemiological observations occasionally supported by analytical data on groundnut aflatoxin levels have been provided, and direct evidence implicating aflatoxins and the cause of the disease are lacking.

Aflatoxins and occupational diseases among groundnut workers

Workers engaged in postharvest activities related to shelling, bagging, storage, transport, etc., may be exposed to aflatoxin through the respiratory route. There is evidence from epidemiological studies to indicate that aflatoxins in respirable particles pose a potential occupational hazard. A chemical engineer involved in sterilizing Brazilian groundnut meal contaminated by *Aspergillus flavus* developed alveolar cell carcinoma and died within a year. Aflatoxin B_1 was detected in his lung tissue. His colleague had also developed alveolar cell carcinoma (pulmonary adenomatosis); (Dvorackova 1976). Three cases of pulmonary interstitial fibrosis were recently reported from Czechoslovakia, of whom two were agricultural workers. Aflatoxin B_1 (10 - 54 μg kg^{-1}) was detected in lung samples of all the workers, (Dvorackova and Pichova 1986).

In a Dutch groundnut oil factory the workers were exposed to an estimated 0.039 - 2.5 μg aflatoxin per working week (45 h). An epidemiological study of 55 workers indicated that 11 developed various forms of cancer, mainly of the respiratory tract (van NieuWenhuize et al. 1973).

However, a study conducted 18 years after initial exposure did not give a definite indication of cancer risk associated with respiratory exposure to aflatoxin, although it did suggest certain associations warranting further study (Hayes et al. 1984).

Baxter et al. (1981) were also of the view that while there could be considerable risk associated with exposure to grain dust from groundnut, cottonseed, and maize contaminated with aflatoxin, the exact magnitude of the risk cannot be adequately estimated.

Farmers who clean out moldy grains from storehouses suffer from burning of the eyes, nose, and throat, chills, fever, and dry irritating coughs (Emanuel et al. 1975). The number of fungal spores present in grain dust ranges from 3.5 to 200 million m^{-3} of air and predominantly includes species of *Aspergillus* (Palmgren 1985).

Association between aflatoxin and protein energy malnutrition

Aflatoxins have been recognized to be more harmful to malnourished than well-nourished animals and humans. Thus, malnourished children in developing countries are more susceptible to aflatoxin toxicity. In a series of studies in the Sudan, Nigeria, Ghana, and South Africa, in both autopsied as well as biopsied livers from children with protein energy malnutrition, aflatoxins were detected in levels ranging from 62 to 33 206 μg kg^{-1} in kwashiorkor and marasmic kwashiorkor, but not marasmus (Coulter et al. 1986, Hendrickse 1984). Groundnuts and peanut butter were the major source of aflatoxins among these populations (Hendrickse et

al. 1982). Aflatoxins M_1 and M_2 were frequently detected in breast milk of Sudanese women (Coulter et al. 1984). Based on these studies it is postulated that kwashiorkor might result from chronic aflatoxin poisoning (Anon. 1984). Recent evidence indicates that children with kwashiorkor have a reduced ability to metabolize and excrete aflatoxins compared to marasmic children (Coulter et al. 1986). Thus, aflatoxins are unlikely to be primarily responsible for kwashiorkor development, although malnourished children would be more susceptible to the toxic effects of aflatoxin.

Aflatoxin in groundnuts and Indian Childhood Cirrhosis (ICC)

Indian childhood cirrhosis is a liver disorder found only among children in the Indian subcontinent. Toxins (including aflatoxin), virus, and hereditary factors have been put forward as possible etiologic agents. According to Amla et al. (1969), there is sufficient circumstantial evidence to indicate that children exposed to aflatoxin through breast milk and dietary items such as unrefined groundnut oil and parboiled rice may develop ICC. In each 24-h collection of urine from cirrhotic children aflatoxin B_1 ranging from 0.02 to 0.05 μg was detected in 7% of the urine samples. This was substantiated when malnourished children in a pediatric ward of a hospital developed liver lesions similar to than seen in ICC cases after accidentally consuming 30-50 g of groundnut protein flour contaminated with 300 $\mu g\ kg^{-1}$ of aflatoxin for periods ranging from 5 days to 1 month, (Amla et al. 1971).

Aflatoxin and liver cancer

Studies carried out in Kenya, Swaziland, the Transkei region of South Africa, Mozambique, and Thailand have found a positive correlation between hepatocellular carcinoma (HCC) and aflatoxin ingestion by man. The incidence of HCC in Taiwan, Japan, the People's Republic of China, the Republic of Korea, Greece, and large areas of subSaharan Africa is also associated with the prevalence of the hepatitis-B surface antigen (HBS Ag) carrier state (Okuda and Mackay 1982). Studies carried out in Swaziland to correlate aflatoxin and hepatitis-B with liver cancer indicated that 4.6% of the diet was positive for aflatoxins. The major source of contamination was sauce containing groundnuts which was used on maize meal. The mean level of aflatoxins was $302.5 \pm 108.5\ \mu g\ kg^{-1}$ (IARC 1984). In these studies, aflatoxin exposure and HBS Ag prevalence were measured across 10 geographical areas. Aflatoxin exposure was a more important factor in liver cancer incidence than the prevalence of hepatitis infection (Peers et al. In press). These studies also indicate that, over a decade, as a result of carrying out a rural development program, the aflatoxin incidence has lowered. These data, coupled with what is known about the carcinogenic effect of aflatoxins, in animals as well as the secular trends in HCC incidence rates as evidenced by the correlation of reduction of mycotoxin incidence in foods and the decreased HCC incidence in South Africa (Bradshaw et al. 1982), and in Mozambique (van Rensburg et al. 1985) should be considered as supporting evidence for the role of aflatoxins in the causation of HCC (Friesen et al. in press, Hsieh 1986).

Association between occurrence of endemic osteoarthrosis and consumption of contaminated groundnuts

Several chronic, progressively disabling, polyarthritic diseases with fairly similar features have been described from different countries during the last decade. These include the Kashin-beck or

Urov diseases from USSR, People's Republic of China, and North Vietnam (Sokoloff 1985), Mseleni Joint Disease (MJD) from South Africa (Fellingham et al. 1973), and Endemic Familial Arthritis of Malnad, in Karnataka, India (Bhat and Krishnamachari 1977). Mycotoxicological etiology has been suggested for this clearly familial disease (Nesterov 1964, Walser et al. 1982). When samples of home-grown maize and groundnuts from the endemic areas were examined, several *Fusarium* spp; *F. poae, F. oxysporum, F. moniliforme, F. equiseti*, and *F. compactum* besides *Penicillium* spp and *Lasiodiplodia theobromae* were found to be more predominantly present in households affected by MJD than in non-affected ones. The incidence of *Aspergillus flavus* was low in all the samples (Marasas and van Rensburg 1986). A diet containing home-grown maize and groundnuts from affected households in the endemic area of MJD caused fibrous osteodystrophy and nephrolithiasis accompanied by certain biochemical changes in serum such as plasma alkaline phosphatase, hypocalcaemia, or hyperphosphataemia (Fincham et al. 1985). However, there is as yet no conclusive evidence to show that these oestoarthritic diseases are associated with consumption of contaminated maize and groundnuts. A recent meeting held in Beijing by the World Health Organization (WHO) concluded that "Several hypotheses indicate involvement of certain chemicals in food and water (manganese, phosphorus, mycotoxins, microbial toxins, humic acid, etc.) in combination with nutritional disbalance, in particular selenium deficiency, as the causation or contributing factors to the etiology of this disease" (WHO 1985).

Cost effectiveness of lowering aflatoxin tolerance levels in groundnuts

A case study on the cost effectiveness of alternative aflatoxin tolerance for groundnut and groundnut products in the USA was conducted by Dichter (In press). The number of cases of liver cancer which could potentially be prevented at lower tolerance levels (5 $\mu g\ kg^{-1}$) was estimated using both experiments and an epidemiological data base. Since well-documented scientific evidence is available to indicate that the harmful effects of aflatoxins are aggravated in malnourished conditions such as protein and vitamin A deficiencies, the effect of lower tolerances on nutritional status was also assessed in order to determine if there would be adverse health consequences. The consumption patterns of US consumers and the protein adequacy of their diets was assessed quantitatively using individual food intake records obtained in a national survey, with special attention to the importance of groundnut products in the diets of nutritionally vulnerable groups. The analysis revealed that even at the 5 $\mu g\ kg^{-1}$ level the predicted protein deficit would be less than 1% of the recommended daily allowance for children as well as for all consumers living below the poverty level. This finding indicated adverse health effects would be unlikely if more stringent standards for aflatoxins were adopted.

By applying cost-effectiveness analysis techniques to evalute health- improvement measures, taking lowering deaths from liver cancer due to aflatoxins in groundnuts in USA, Dichter (In press) concluded that as far as reducing cancer mortality is concerned, a tolerance level of 15 $\mu g\ kg^{-1}$ is the most effective measure.

Assessment of risk through consumption of groundnut and its products

Data collected over the last quarter century indicates that the risk from consumption of aflatoxin-contaminated groundnuts and groundnut products is likely to be more to animals than to humans.

Groundnut consumption pattern

Groundnut consumption varies in different countries, and even within a country. It depends on the region, season, and food habits. Food consumption surveys in India have indicated the consumption of nuts (mostly groundnuts) varies from 2 to 35 g per day per person. In countries like Senegal and the Gambia, where more than 70% of the population is engaged in some aspect of the groundnut trade, the consumption could be as high as 85 g per day per person. When the groundnuts are consumed roasted, boiled, canned, or in other forms; the moldy, shrivelled, and damaged kernels that are likely to contain more aflatoxins are unlikely to be consumed. Moreover, roasting or frying in oil is known to partially (up to 70%) destroy the toxin. Groundnut-based snack foods are often prepared in the home-based, nonorganized sector, where it is difficult to ensure quality control.

In the developed countries, peanut butter is quite popular. In the USA, the maximum annual consumption of peanut butter by children has been assumed to be 225 g (Stoloff 1985). The marketing and consumption pattern of groundnut in developed countries creates a relatively uniform exposure. The quality control in these countries is organized and methodical. However, in developing countries up to 80% of the agricultural produce is consumed at home or at the village level without entering the organized market chain. This would mean there would be no dilution effect i.e., the contaminated commodity being mixed with noncontaminated lots reducing aflatoxin levels. Moreover, there is no possibility of the aflatoxin being detected by official regulation-enforcement agencies.

Aflatoxins in edible groundnuts

Aflatoxin contamination in groundnuts was prevalent in 22 countries participating in the Food Contamination Monitoring Project (FCMP) of the FAO/WHO/UNEP under their Food Contamination Monitoring Project Global Environmental Monitoring System. Among 185 000 samples analyzed between 1980 and 1983, most median and 90th percentile levels were below 20$\mu g\ kg^{-1}$ in raw groundnuts and 15 $\mu g\ kg^{-1}$ in consumer products. In 2350 samples, aflatoxins were above the 20 $\mu g\ kg^{-1}$ limit (Jelinek In press).

In the USA, based on annual crop reports of the Peanut Administrative Committee, data expressed as a frequency distribution curve, for 1973 to 1984 on aflatoxin contamination incidence and level, has been drawn by Stoloff (1985). These studies indicate that fixing a tolerance level of 35 $\mu g\ kg^{-1}$ would allow the use of virtually all the groundnut crop in a year with least amount of contamination, 98% of the crop in an average year, and 96% of the crop in a year with most aflatoxin contamination. These studies also indicate that under existing agroecological conditions it would not be possible to eliminate the natural occurrence of aflatoxins in shelled groundnuts even in the developed countries where there is considerable awareness of the aflatoxin problem.

A multicentric study carried out by the FAO under the Food Contamination Monitoring Project (FCMP) in India, Nepal, Pakistan, and Sri Lanka in 1984 indicated that groundnut is the commodity most frequently contaminated with aflatoxin. The data from India indicated that though aflatoxins could be detected in 13% of the groundnuts sampled, it exceeded the official permissible limit of 30 $\mu g\ kg^{-1}$ in only 2.6% of the samples. Various surveys carried out in different parts of India also point to groundnut as a high-risk commodity for aflatoxins (Tulpule et al. 1982).

Based on the extent of the aflatoxin contamination in groundnuts and the per capita

consumption rate of 7.6 g day^{-1} for groundnut and its products, the exposure to aflatoxins has been calculated to be 3 $\mu g\ kg^{-1}$ body weight (Stoloff 1985).

Aflatoxins in edible grade flour

The greatest danger to human health from aflatoxins in groundnuts could arise from the use of edible groundnut cake in the supplementary feeding program. Normally, the best kernels are selected either for export or table consumption purposes. The moldy, damaged, and shrivelled kernels are mixed with sound kernels to obtain oil with little chance of the level of contamination being detected. When oil is extracted from aflatoxin contaminated groundnuts, 15% of the aflatoxins go into the oil, while 85% remain in the oil cake. The level of contamination in edible-grade groundnut flour supplied to a manufacturer of children's food in Hyderabad varied from traces to 200 $\mu g\ kg^{-1}$.

Aflatoxins in edible oils

Groundnut oil is one of the major edible oils produced in south India. The higher income groups consume up to 100 g of oil per head per day while the poorer segments consume about 15 g a day. The level of aflatoxin in unrefined oil in India varies, the highest recorded level being 5000 $\mu g\ kg^{-1}$ (Tulpule et al. 1982). Assuming a realistic average toxin level of 80 $\mu g\ kg^{-1}$, use of 50 g of unrefined groundnut oil day^{-1} has been calculated to contribute 4 μg of aflatoxin to the diet day^{-1} (Achaya 1986). Aflatoxin is removed from oil during the process of refining, and by the use of certain filters in conventional plate and frame presses.

Aflatoxins in groundnut cake and extractions

Oil is extracted either by the traditional bullock or hand-drawn 'Ghanis', or through mechanical expellers in rural areas in the unorganized sector, or by the solvent extraction method in the organized sector. In India the solvent-extracted oil cake is called 'extractions'. Considerable quantities of groundnut cake produced in developing countries were exported to EEC and eastern European countries until legislation on aflatoxins in secondary sources like milk were introduced in EEC countries. The groundnut cake consumption in EEC countries fell from 9.88 million t in 1979 to 3.42 million t in 1981 and was estimated to be around 3 million t in 1986. India exported 0.53 million t of groundnut cake in 1978/79 while in 1985/86 it was 0.16 million t. The mean level of aflatoxins found by French official services (Briantais in Barre) in 1984 for imported groundnut meal were as follows: Argentina 0.08 $\mu g\ kg^{-1}$; Brazil 2.24 $\mu g\ kg^{-1}$; India 0.68 $\mu g\ kg^{-1}$; Senegal 0.97 $\mu g\ kg^{-1}$; and Sudan 0.88 $\mu g\ kg^{-1}$. The level of aflatoxins in exportable Indian groundnut 'extractions' ranged from 200-700 $\mu g\ kg^{-1}$ while a reputed survey organization in the UK reported a 915-1500 range of aflatoxins averaging 424 $\mu g\ kg^{-1}$ for 344 Indian samples imported into the UK (Achaya 1986). A detailed survey on the trend of aflatoxins in groundnut cake exported from India and the groundnuts that have been imported to countries like Japan indicated that the levels of aflatoxins in export commodities are generally low (Bhat In press).

Due to the enforcement of stringent quality measures by the importing countries, the best of the commodities are exported from the developing countries and the remainder, often contaminated, is used within the country as animal feed. The bulk of the oil cake (about 60%) is used as direct livestock feed and a small percentage (about 15%) is used for compound livestock feed.

Risk to human health from mycotoxins in secondary sources

It is recognized that when animals consume feed contaminated with aflatoxins, part of the toxin is metabolized in the body and may get into the milk as aflatoxin M_1, or may be found in animal products such as meat, eggs, etc., which are consumed by man. The levels of toxin that are present in these secondary sources are lower than those in agricultural commodities, and evidence of chronic or acute diseases in man/animals attributable to these secondary sources is lacking. However, to minimize the health risk, legislation has been passed in several countries restricting the level of aflatoxins in the feed. Worldwide data from the Global Environmental Monitoring System (GEMS) on levels of aflatoxin M_1 in 3700 samples of whole milk, dried skimmed milk, and cheese indicates that the aflatoxin levels were below 0.4 $\mu g\ kg^{-1}$ calculated on a whole-milk basis (Jelinek 1987). These results agree with those reported elsewhere that the levels of aflatoxin M_1 in milk and milk products are generally low. On the other hand, in developing countries where milk is drawn from cattle at the home level, where pooling of samples from different animals does not take place, the levels of aflatoxin M_1 can be as high as 4.8 $\mu g\ kg^{-1}$ (Tulpule et al. 1982).

Residues of mycotoxins or their metabolites may be found in milk and various tissues, particularly in the livers and kidneys of livestock that have ingested mycotoxin-contaminated feed. The factor of dilution from feed ingestion to tissue varies greatly, e.g., 1:6250 for muscle, 1:5000 for liver, while that in milk varies from 1:34 to 1:1600 (Rodricks and Stoloff 1976). The levels of aflatoxin found in animal tissues consumed by man are unlikely to cause serious health hazards.

Risk to animal health from aflatoxins

The historic discovery of aflatoxins was due to the death of turkeys in the UK as a result of consuming contaminated groundnut meal imported from Brazil. The harmful effects of consuming contaminated groundnut cake have been mainly observed in poultry and milch cattle (Bhat et al. 1978). The effects in poultry include mortality, feed refusal, slowed growth, fertility and reproduction problems, and decreased resistance to disease. The major reason for not giving aflatoxin-contaminated feeds to cattle is the problem of metabolites of aflatoxin being secreted in the milk. A 3% live weight loss of poultry in USA representing a total weight loss of over 100 million kg of meat, worth US $ 143 million, has been estimated to be due to consumption of feed contaminated with mycotoxins (Hesseltine 1986).

Detoxification of aflatoxins in food and animal feed has been gaining importance in recent years. Commercial facilities have been established in Senegal to detoxify groundnut cake by the ammonia formol and sodium hypochlorite processes. The additional cost is claimed to be 7 CFA (2.69 US$) kg^{-1} for ammonia formal, and 5 CFA (1.92 US$) kg^{-1} for sodium hypochlorite. In India, Hindustan Lever Ltd, have established a facility to detoxify groundnut meal by the ammonia process. They have demonstrated the ability to bring down the aflatoxin level from 500 $\mu g\ kg^{-1}$ to 50 $\mu g\ kg^{-1}$ in a closed unit at 90° C with 7.5% moisture and 2.5% gaseous ammonia. The cost is estimated at Rs.180 (14.4 US$) t^{-1}. An added advantage claimed is a 1% increase in the nitrogen content of the meal.

Recently, there have been doubts on the safety of the ammonia process. It is claimed that the ammonia-detoxified meal when ingested by the animal can undergo transformation in the stomach and there are chances of aflatoxin being formed again because of the activity of gastric juices. If these findings are confirmed, there is real danger of aflatoxin intoxication under such circumstances.

Conclusion

Based on the available data it can be concluded that evidence to directly implicate aflatoxins through consumption of groundnuts and its products in various diseases in man is inconclusive, although such possibilities have been clearly shown in various farm animals. The major reason for such a scenario is the consumption of comparatively small amounts of groundnuts and groundnut products by man as compared to such staple foods as maize.

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Discussion

I.A. Rana. Is there a definite causal relationship between aflatoxin ingestion and kwashiorkor? If the relationship between aflatoxin and liver cancer is not established, this would not be surprising as this is the case for other dietary factors in relation to other cancers. The Pakistan Medical Research Council has shown that incidence of liver cancer is higher in Karachi than in the North. Urinary aflatoxin levels in low income groups were double in high income groups in Karachi. These differences were not sex- or age-related.

R.V. Bhat. A definite causal relationship between aflatoxin ingestion and kwashiorkor has not been established. The presence of aflatoxins in livers of children suffering from kwashiorkor is primarily due to the fact that food contaminated with aflatoxin has been consumed by those children. Epidemiological evidences are available from several countries linking aflatoxins with liver cancer.

Hazards to Livestock of Consuming Aflatoxin-contaminated Groundnut Meal in Africa

J.D. Reed and O.B. Kasali[1]

Abstract

The outbreak of Turkey 'X' disease in England led to the discovery of aflatoxicosis, caused by feeding groundnut meal contaminated by Aspergillus flavus *to livestock. The high content of aflatoxins in groundnut meal in African countries has serious implications for livestock feeding. The risks depend on the level and type of aflatoxin in the diet, the strain of animal, and its nutritional status. Subclinical aflatoxicosis is characterized by reduced feed intake and poor productivity, but may not be associated with overt clinical symptoms. Chronic problems occur when aflatoxins are present in the diet at less than 1000 $\mu g\ kg^{-1}$ but the lower limits for effects on productivity are not certain.*

The principal lesions of aflatoxicosis occur in the liver and may be classified as toxic hepatitis. Natural cases usually result from repeated ingestion of the toxin. One of the most constant responses to aflatoxin B_1 is bile ductule hyperplasia at the periphery of hepatic lobules. Changes in hepatocytes (vacuolization, fatty change) leading to necrosis, are usually localized in one part of the hepatic lobule, depending on the species. Hepatic veno-occlusive lesions are also common.

The immunosuppresive effect of aflatoxin, coupled with high exposure to diseases and poor nutrition, are detrimental to increased livestock production in Africa.

Résumé

Risques entraînés pour les animaux d'élevage par la consommation de la farine d'arachides contaminées en Afrique : *L'apparition de la maladie X du dindon en Angleterre a permis la découverte de l'aflatoxicose, due à la consommation par les animaux de la farine d'arachides contaminées par* Aspergillus flavus. *En Afrique, la forte teneur en aflatoxines de la farine d'arachides a une conséquence grave sur l'alimentation animale. Les risques dépendent du niveau et du type d'aflatoxines dans le régime, de l'espèce animale et de son état nutritionnel. L'aflatoxicose subclinique est caractérisée par une perte d'appétit et une faible productivité; il n'y a pas de symptômes cliniques notables. Un taux d'aflatoxine inférieur à 1000 $\mu g\ kg^{-1}$ dans le régime peut également entraîner des problèmes chroniques, cependant les limites inférieures liées à une baisse de productivité ne sont pas connues.*

Les lésions dues à l'aflatoxicose se produisent surtout au niveau du foie, et sont considérées comme une hépatite toxique. Les causes naturelles résultent d'une ingestion répétée de la toxine. La réaction la plus fréquente à l'aflatoxine B_1 est une hyperplasie du canal biliaire à la périphérie des lobules hépatiques. En fonction de l'espèce animale, les modifications des hépatocytes (vacuolisation, modification des lipides) aboutissent à une nécrose d'une partie du lobule hépatique. Les lésions véno-occlusives hépatiques sont également fréquentes.

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L'effet immunosuppressif des aflatoxines, ainsi que la forte incidence des maladies et une alimentation inadéquate, limitent l'augmentation de la production animale en Afrique.

Resúmene

Peligro para el ganado originado por el consumo de torta de cacahuate contaminado con aflatoxinas en Africa: *La aparición de la enfermedad "X" de los pavos en Inglaterra condujo al descubrimiento de la aflatoxicosis, causada por la alimentación del ganado con torta molida de cacahuate contaminada por* Aspergillus flavus. *El alto contenido de aflatoxinas en la torta molida de cacahuate en los países africanos tiene serias consecuencias en la alimentación del ganado. Los riesgos dependen del nivel y tipo de aflatoxinas presentes en la dieta, el tipo de animal y su estado nutricional. La aflatoxicosis subclínica se caracteriza por un reducido consumo de alimentos y una baja productividad en los animales, pero a veces no está asociada con síntomas clínicos claros. Problemas crónicos ocurren cuando las aflatoxinas presentes en la dieta son menores que 1,000 $\mu g\ kg^{-1}$, pero los límites inferiores para efectos sobre la productividad no han sido definidos.*

Las principales lesiones de la aflatoxicosis ocurren en el hígado y pueden clasificarse como hepatitis tóxica. Los casos típicos generalmente son el resultado de la ingestión repetida de las toxinas. Una de las respuestas más constantes a la aflatoxina B_1 es la hiperplasia del ducto biliar en la periferia de los lóbulos hepáticos. Los cambios en los hepatocitos (vacuolización y cambios en la grasa) que llevan a la necrosis, están localizados en alguna parte del lóbulo hepático, dependiendo de la especie animal. Las lesiones hepáticas veno-oclusivas, también son comúnes.

El efecto inmunosupresivo de las aflatoxinas, conjuntamente con altas exposiciones a las enfermedades y regímenes alimenticios deficientes, perjudican el mejoramiento de la producción ganadera en Africa.

History and Occurrence of Aflatoxins in Groundnut Meal

The outbreak of aflatoxicosis in the United Kingdom in 1960 caused the death of large numbers of livestock (Blount 1961) and led to the discovery of aflatoxins in groundnut meal contaminated by *Aspergillus flavus* (Hesseltine 1979). Subsequently, aflatoxins have been found in other feeds, especially maize and cottonseed meal (Lillehoj 1979). The potential for death and declining productivity in livestock consuming aflatoxin-contaminated feed has led to the introduction of regulations in European Economic Community (EEC) countries restricting the content of aflatoxin B_1 to 10–50 $\mu g\ kg^{-1}$ depending on the feed and class of livestock concerned (Patterson 1983). However, it is interesting to note that the level of aflatoxin B_1 in imported groundnut meal in non-EEC countries in Europe can be quite high ($>1000\ \mu g\ kg^{-1}$ according to Chelkowski et al. 1978).

Increased restrictions on the importation of aflatoxin-contaminated groundnut meal into developed countries may force countries in Africa to utilize this feed resource internally. For instance, The Gambia exports over 90% of its groundnut meal in order to obtain foreign exchange, although there is a shortage of protein for feeding livestock. However, increased use of groundnut meal for feeding livestock will necessitate an understanding of the aflatoxin problem in the African context.

Effects of Aflatoxins on Livestock

Clinico-pathology

Early signs of aflatoxicosis in livestock are reduction in feed intake and weight loss, often followed by rapid death. The susceptibility of animals to aflatoxin depends on the species (duckling, rabbit, turkey, chicken, swine, cattle, and sheep in decreasing order), the form of toxin (aflatoxin B_1 is the most toxic), and on the animals' nutritional status. Aflatoxin is both teratogenic and carcinogenic. The liver is the primary target organ in most cases (Jones and Hunt 1983).

The clinico-pathologic features of aflatoxins depend on hepatic injury that is related to the dose administered. Doses adequate to produce death within 24 h will result in detectable liver damage within 3 h, and several alterations in liver function within 6 h. The serum alanine transferase (SAT), ornithine carbamyl transferase, and isocitric dehydrogenase (IDH) are markedly elevated. The serum levels of albumin, albumin-globulin ratio, non-protein nitrogen (NPN), and urea nitrogen are reduced (Edds 1979).

The principal lesions of aflatoxin occur in the liver and may be classified as toxic hepatitis (Kaneko 1980). One of the most constant responses to aflatoxin B_1 is the proliferation of small bile ductules. This is usually accompanied by loss of hepatic glycogen, fatty inflitration, fibroblastic proliferation, and perivascular edema. These degenerative changes lead to necrosis that is usually localized in one part of the lobule (centrolobular or midzonal). Edema of the gallbladder has been frequently noted in the pig (Nelson 1979).

One of the common effects of carcinogens at the cellular level is to cause degranulation of the endoplasmic membranes. Hepatic changes at the cellular level in aflatoxin-treated calves were loss of ribosomes from the endoplasmic reticulum, loss of nuclear chromatin material, and altered nuclear shapes (Lynch et al. 1971).

Aflatoxin B_1 is metabolized by hepatic microsomal mixed function oxidase. The hydroxylation of aflatoxin B_1 to aflatoxin M_1 in the lactating cow is of particular concern. Aflatoxin M_1 is also found in the liver, kidney, and urine of sheep (Masri et al. 1974).

The strong accumulative effects of aflatoxin B_1 on lipid metabolism may be manifested by an early decrease in feed conversion (Lynch et al. 1979). Aflatoxicosis in farm animals produces growth depression, fatty livers, and a decrease in the lipid content of extra hepatic tissue. The accumulation of fat in livers has been suggested to be due to a blockage of fat removal from the liver. The aflatoxin effect on lipid metabolism is also marked by reduction in such fat-soluble vitamins as vitamin A (Lynch et al. 1979).

Several mycotoxins exert biological actions that impair the effectiveness of native defense mechanisms and immunogenesis (Pier et al. 1979). Most of the experimental information concerns aflatoxin and its effect on poultry, swine, and cattle.

The major effect of aflatoxin is on the cell-mediated immune system. This failure has been demonstrated in poultry with fowl cholera and in swine erysipelas. Aflatoxin consumption has also been reported to increase susceptibility of poultry to salmonellosis, candidosis, and coccidiosis, and of calves to fascioliasis (Pier et al. 1979). The diminished resistance and immunogenesis are thus a problem of substantial economic importance and deserve further investigation.

Ducks and Poultry

Ducklings are the most susceptible class of livestock to aflatoxicosis and are preferentially used in bioassays for aflatoxins in feeds (Ostrowski-Meissner 1984). Ducklings showed a depressed utilization of dietary protein on diets containing only 70 μg aflatoxin B_1 kg^{-1}, whereas chickens did not show a depression until they consumed diets containing 280 μg kg^{-1} (Ostrowski-Meissner 1984).

Generally chickens will show depressed levels of performance on diets containing more than 250 μg kg^{-1} aflatoxin B_1 and, at higher levels (>500 μg kg^{-1}), liver lesions become severe (Davli 1986).

Swine

Relatively low concentrations of aflatoxin B_1 (182 μg kg^{-1}) reduced average daily gain and feed efficiency in piglets fed on contaminated maize, but this effect was reversed by increasing the concentration of crude protein in the diet, and by the addition of fat (Coffey 1986). Levels of 100 to 300 μg kg^{-1} in swine rations usually do not produce toxin effects from weaning to market, but levels greater than 400 μg kg^{-1} may produce aflatoxicosis (Edds 1979). Residues of aflatoxin B_1 and M_1 may be present in liver, kidney, and muscle tissues in swine fed on diets containing only 100 μg kg^{-1} aflatoxin B_1 and these residues may be hazardous for human consumption (Edds 1979). Although it is suspected that aflatoxin B_1 is a liver carcinogen in man, evidence to support this is largely circumstantial (Roe 1987).

Ruminants

The rumen fermentation does not appear to detoxify aflatoxins, but aflatoxins may inhibit rumen microorganisms (Mertens 1979). Aflatoxin B_1 in diets for feedlot steers did not affect growth rate or feed intake at concentrations of 60 and 300 μg kg^{-1}, but at 600 μg kg^{-1} both growth rate and feed intake were reduced. Afltoxins B_1 and M_1 were not detected in muscle, fat, or liver 7 days after withdrawal from aflatoxin-contaminated diets. Liver lesions were only apparent in steers fed the diets containing 600 μg kg^{-1} (Helferich et al. 1986). Only 0.7 to 1.4% of an oral dose of aflatoxin B_1 was excreted in milk from lactating goats, 25% of the aflatoxin in this milk was M_1. About 50% of the oral dose was detected in the faeces (Helferich et al. 1986).

Implications for Feeding Groundnut Meal in Africa

Most African countries have feed-mixing enterprises that rely heavily on oilseed cakes for the manufacture of concentrate feeds for poultry, swine, and dairy cattle. The dependency of livestock industries on these by-products will increase because restrictions on the import of concentrates will become greater, especially in West Africa. However, little information is available on the aflatoxin contamination of groundnut meals and other oilseed cakes in Africa, and its potential effects on livestock production. Tropical environments favor the growth of *Aspergillus flavus* (Ostrowski-Meissner 1984). All of the samples of groundnut meal from an oil extraction plant in Nigeria were contaminated by aflatoxins (Abalaka and Elegbede 1982). The overall effect of aflatoxins on livestock production in Africa is unknown, but is probably large

because of their immunosuppresive effects coupled with high exposure to diseases and the poor nutritional status of livestock in that continent.

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Discussion

R.V. Bhat. Regarding the international harmonization of aflatoxin levels in feeds, the Codex Alimentarius Commission of the FAO/WHO has just sent a circular to all the national Codex points suggesting certain limits, and has invited comments from the respective governments by November 1987. We hope that after the comments are received, internationally agreed limits for aflatoxins in animal feeds will be set.

J.D. Reed. The levels of aflatoxin allowed in livestock materials vary considerably throughout the world. Some countries go as high as 300 $\mu g\ kg^{-1}$ in materials for livestock feeding. So an internationally agreed level would be very useful.

R.V. Bhat. The proposed limit in groundnut cake and other animal feed ingredients suggested by the Codex is 200 $\mu g\ kg^{-1}$.

Session III

Aflatoxins and Trade in Groundnuts

Groundnut Trade in India and the World: Implications of Aflatoxin Contamination

G. Chandrashekhar[1]

Abstract

Although India is the largest producer of groundnuts, her share in the world edible groundnut trade has declined sharply in the last 10 years. A persistent deficit in oilseeds production resulting in high groundnut prices in India compared to those from other producing countries has made Indian groundnuts more expensive and less attractive to world trade. Apprehensions of aflatoxin contamination of groundnuts have done much less damage to the Indian groundnut trade than have vacillating government policies. In India selection of edible groundnuts is still done manually because of the high cost of mechanization and investment risk. Yet, Indian graders are capable of supplying groundnuts of internationally acceptable quality. In the present setting, the impact of aflatoxin incidence in groundnuts is at best marginal for India. Government support to ensure larger exports of edible groundnuts from India at competitive prices is bound to bring greater awareness and motivation amongst graders to prepare aflatoxin-free high quality groundnuts for the world market.

Résumé

Commerce d'arachide en Inde et dans le monde-conséquences de la contamination par les aflatoxines : *L'Inde est le plus important producteur d'arachides dans le monde. Cependant, sa part dans le commerce mondial d'arachides de bouche a fortement baissé pendant ces dix dernières années. La production déficitaire d'oléagineux a augmenté les prix d'arachides en Inde par rapport à d'autres pays producteurs et les a ainsi rendues moins intéressantes au niveau du commerce mondial. L'instabilité des politiques gouvernementales s'est avérée plus défavorable au commerce indien d'arachide que les appréhensions concernant la contamination par les aflatoxines. Le triage des arachides de bouche est encore manuel, à cause des coûts élevés de la mécanisation et des risques liés à l'investissement. Pourtant, les trieurs indiens sont à même de garantir des arachides de qualité acceptable pour l'exportation. L'effet de l'incidence des aflatoxines est actuellement encore marginal. L'appui de l'Etat en vue de favoriser l'exportation des arachides de bouche à des prix compétitifs permettra de sensibiliser et de motiver les trieurs indiens à préparer des arachides exemptes d'aflatoxines et de bonne qualité destinées à l'exportation.*

Resúmene

El comercio del cacahuate en la India y con el mundo : Repercusiones de la contaminación con aflatoxinas : *Aunque la India es el mayor productor del cacahuate, su participación en el*

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comercio mundial de cacahuate comestible ha declinado marcadamente en los últimos 10 años. El déficit persistente en la producción de oleaginosas ha originado los altos precios de los cacahuates en la India, en comparación con otros países productores, y ha hecho que los cacahuates de la India sean menos atractivos para el comercio mundial. Los temores a la contaminación con aflatoxinas en los cacahuates han hecho mucho menos daño al comercio de cacahuates de la India que las políticas gubernamentales vacilantes. En la India, la selección de los cacahuates comestibles aún se realiza en forma manual, debido al alto costo de la selección mecanizada y el riesgo de las inversiones en este rubro. Sin embargo, los clasificadores hindúes pueden seleccionar cacahuates de calidad aceptable internacionalmente. En el escenario actual, el impacto de la incidencia de aflatoxinas en los cacahuates cuando mucho es marginal dentro de la India. El apoyo gubernamental para asegurar mayores exportaciones de cacahuates comestibles de la India a precios competitivos obligatoriamente producirá una mayor sensibilización y motivación entre los clasificadores, para que se seleccionen cacahuates de alta calidad, libres de aflatoxinas, para el mercado mundial.

Introduction

India has been the largest producer of groundnut in the world for the last several years, with an annual production of between 6 and 7 million metric tonnes (unshelled). Groundnut is the most important of the wide variety of oilseed crops grown in India and accounts for 50% of the total annual oilseed output of approximately 12 million tonnes.

Almost all the groundnuts produced in India are used for oil extraction. Its use as a table nut is not as widespread here as it is in some West European countries. However, India has traditionally been an exporter of handpicked selected (HPS) groundnuts, also known as edible groundnuts. Indian groundnuts are known for their nutty flavor, natural taste, and crunchy texture.

Recent Events in HPS Groundnut Trade

The year 1977 marked a watershed in the history of Indian edible groundnut exports. In April 1977 the Government banned export of HPS groundnuts because of domestic shortages and the high price of edible oil (Table 1). The ban closely followed an earlier ban imposed in July 1976,

Table 1. Export of handpicked selected groundnuts: changes in Indian Government policy, 1974 to date.

Year	Agency	Remarks
Before Oct 1974	None	No official agency
Nov 1974 - Jun 1976	IOPEA[1]	Through IOPEA
Jul 1976 - Oct 1976	-	Export banned
Nov 1976 - Mar 1977	IOPEA	Ban partially lifted
Apr 1977 - Nov 1978	-	Export banned
Dec 1978 - Oct 1982	NAFED[2]	Through NAFED and private trade barred
Oct 1982 onwards		Private trade allowed along with NAFED
May 1984 onwards		IOPEA designated as monitoring agency

1. Indian Oil and Produce Exporters Association.
2. National Agricultural Cooperative Marketing Federation.

which was partially lifted between November 1976 and March 1977 to permit the export of 50 000 t.

The export ban imposed in April 1977 continued until almost the end of December 1978, when, to the dismay of private exporters, business was channelled solely through a government-supported cooperative organization, which had little expertise or infrastructure. Private exporters, who had so assiduously cultivated lucrative overseas markets, were totally excluded from the export business.

During this period of monopoly of exports through the National Agricultural Cooperative Marketing Federation (NAFED), as the cooperative organization is known, performance on the export front plummetted. As a result, private trade was reinducted into the export business in October 1982.

During the period of export channelling through NAFED from 1978 to 1982, India lost touch with most of her buyers. The switch-on-switch-off export policy as a result of frequent embargoes on export and the change in exporting agency had a disastrous effect (Table 2). The confidence of traditional Indian groundnut buyers like the United Kingdom, the Netherlands, the Federal Republic of Germany, and Japan was thoroughly shaken. Naturally, buyers started cultivating more reliable sources of supply.

Even as India's export policy was undergoing frequent changes, major importing countries in Western Europe such as the United Kingdom and the Federal Republic of Germany tightened their aflatoxin regulations and imposed more stringent standards. As a result of a uniform policy for European Economic Community (EEC) members, the acceptable aflatoxin B_1 level was pegged at a maximum of 5 $\mu g\ kg^{-1}$.

Although the export control was lifted in 1983, there has not been any significant improvement in India's export performance. Currently, exports average 20 000 t per annum which is less than 4% of the total world trade.

Moreover the production of groundnut has itself become unstable owing to persistent drought in the principal growing areas. Over the last 6 crop years from 1981/82 to 1986/87 production has oscillated between 5.0 and 7.3 million t.

The Saurashtra region of Gujarat, known as the "groundnut bowl" of the country, where export-quality groundnuts are produced, has been severely affected by drought. Inadequate rainfall during the last 3 years has also led to quality problems, with an increased proportion of shrivelled and discolored nuts. For export purposes, it would have been possible to select edible grade groundnuts, but only at a high cost because of the need for multiple grading.

Table 2. Handpicked selected groundnut exports from India, 1974-1987.

Year	Quantity (t)	Agency	Year	Quantity (t)	Agency
1974/75	53 200	IOPEA[1]	1981/82	24 000	NAFED
1975/76	111 100	IOPEA	1982/83	32 600	NAFED + Trade
1976/77	132 400	IOPEA	1983/84	24 700	NAFED + Trade
1977/78	-	Banned	1984/85	27 800	IOPEA
1978/79	4 500	NAFED[2]	1985/86	8 980	IOPEA
1979/80	23 100	NAFED	1986/87	29 500	IOPEA
1980/81	58 300	NAFED			

1. IOPEA = Indian Oil and Produce Exporters Association.
2. NAFED = National Agricultural Cooperative Marketing Federation.

The domestic and international scene has undergone a sea change for India during the last few years. Because of internal shortages, Indian groundnut prices are generally high and effectively prevent large-scale export of edible groundnuts. Since 1984, domestic prices have exceeded international prices by 10–20%. Overseas sales now are largely confined to the USSR.

Market Competition and Contractual Terms

An important term of the export contract for edible groundnuts relates to quality, including aflatoxin level. At the height of their popularity, Indian groundnuts were traded on "shipped weight and quality final" condition, i.e., the quality of export goods obtained at the time of export shipment was final and the shipper/sheller was contractually not responsible for quality deterioration, if any, occurring during transportation. Such a condition effectively shifted the risk arising out of possible postharvest contamination to the buyers. So long as India was at the forefront of groundnut exporters, buyers were forced to accede to this condition.

Since the beginning of this decade, the world groundnut market has been swamped by USA, the People's Republic of China, and Argentina, in that order. At present it is not uncommon to find major suppliers willing to offer edible groundnuts on "delivered weight and quality final" terms. This change reflects an increase in market competition amongst exporters who are under pressure from importers in a buyers' market, in spite of the fact that the importers are free to appoint their own surveyors/superintendents (which invariably they do) to verify and be satisfied about the quality of goods shipped.

It would be possible to cite high prices as the single most important reason for the decline in Indian edible groundnut exports, but it is more likely that Indian exports have suffered because of a combination of factors - high prices, vacillating government policy, and aflatoxin, with "high prices" being the predominant factor. Moreover, the switch-on-switch-off export policy of the government resulting in frequent embargoes or restrictions on export has shaken the confidence of traditional buyers in the continuity of supplies from India.

Export Plans

The Indian Oil and Produce Exporters Association (IOPEA), is striving to break this vicious circle of sluggish exports, and lack of quality assurance, which again leads to low volumes of exports. The Association is engaged in an ongoing dialog with the Ministry of Commerce in the Government of India to improve export of Indian HPS groundnuts.

The target countries identified for mounting a marketing thrust are the United Kingdom, the Netherlands, and the Federal Republic of Germany in the West, and Japan in the East. These markets had once shown preference for Indian nuts.

Market studies conducted by IOPEA in western Europe and Southeast Asia have revealed that buyers are not so apprehensive about aflatoxin contamination of Indian groundnuts, as concerned about continuity of supplies from India at competitive prices. Recommendations have been made by the IOPEA to Indian policy makers to guarantee a fairly long-term (5 to 8 years) fixed export policy, and through fiscal support, make Indian edible groundnuts as competitive as those of say Chinese or American origin.

Let's turn our attention to roasted groundnuts. It is common knowledge that roasting reduces the level of aflatoxin in groundnuts. Efforts are afoot in India to develop export markets for roasted groundnut kernels. Demand for roasted groundnut in-shell is also on the increase. One

or two major markets have already been identified and it should be possible to service these markets with appropriate guarantees regarding aflatoxin.

According to the planned projection of the government of India, by the year 1990 which happens to be the terminal year of India's Seventh Five Year Plan, the aggregate production of oilseeds will be 18 million t of which groundnut will account for 52% or 9.3 million t.

At that level of production, India should be in a position to offer for export at least 200 000 t of edible groundnuts at competitive prices. It is at that stage, in my opinion, that the question of aflatoxin will assume significance for the Indian groundnut trade.

It has been observed in the past that buyers tended to discount Indian groundnuts because of absence of guarantees regarding aflatoxin. If India hopes to capture a respectable share of the world edible groundnut market, it will indeed be necessary to introduce right away amongst farmers and HPS graders such systems and practices as will minimize, and if possible eliminate, the incidence of aflatoxin. We have identified the following factors as influencing aflatoxin formation:

- drought during pod maturation;
- damage caused by insects, pests, and pathogens;
- delayed harvesting; and
- soil temperature between 25° and 27°C during the latter part of growing season.

The Association has already taken steps to educate farmers in Gujarat. We have recommended the following:

- proper sun drying of pods;
- avoidance of dampness caused by rain;
- removal of wet seeds;
- proper storage to ensure moisture level of not more than 8%; and
- avoidance of high humidity during storage.

Many Gujarat farmers are already aware of the problem of aflatoxins and hence take preventive steps as far as possible. We also plan to introduce the Gujarat practices in Andhra Pradesh, another major groundnut-producing state.

During the last two seasons, the IOPEA has sponsored aflatoxin sample surveys during the harvest of the winter groundnut crop. Scores of samples are drawn from major groundnut-growing districts and analyzed for aflatoxin. The survey gives a broad indication of the levels of aflatoxin found in farmers' stock groundnuts drawn from different regions, but it is too early to discuss the results, or to draw any valid conclusions from the survey findings.

Over the next 3 years, the Association hopes to identify specific areas which are less aflatoxin-prone and can produce groundnuts with relatively low aflatoxin contents.

The package of measures including price competitiveness, long-term export policy, farmers' education in aflatoxin control, and field surveys, evolved by IOPEA as a part of its overall strategy should soon start to yield results. Historically, the aflatoxin problem has been only a minor contributor to the failure of Indian exports. Improved export prospects are bound to result in more serious aflatoxin control efforts including the mechanization of sorting and grading.

Discussion

R.V. Bhat. I still strongly feel that the loss in Indian exports of both groundnuts and groundnut extractions is mainly due to aflatoxins. The groundnut extract export has fallen from 550 000 t valued at Rs 850 billion in 1977/78, to 161 000 t valued at Rs 270 billion in 1985/86; and the HPS from 130 000 t in 1977/78 to 20 000 t in 1985/86. Most buying countries will not accept a product containing more than 15 μg kg^{-1} aflatoxin whatever the price. Another factor is transport. If you want to ship one tonne of groundnuts from Bombay to Rotterdam it costs US $60 whereas the shipping price is only US $3 from New York to Rotterdam. Because of this high price, generally ships of convenience registered in Greece, Malta, or Liberia are used, and these ships are in a bad shape. So although the Association will ensure excellent aflatoxin-free groundnut meal or HPS groundnuts at the dockside, there is a problem of aflatoxin developing during shipment.

G. Chandrashekhar. I agree. Because of the aflatoxin problem groundnut extractions exports have dwindled, but that is not the case with HPS groundnut exports that have declined mainly because of price disparity. Over recent years our costs have been about US $700–750 t^{-1} whereas the US is selling at $ 600, China at $ 550, and Argentina quotes at $ 500. All the buyers we have approached stated categorically that India must compete on price. India is in a position to supply groundnuts with less than 5 μg aflatoxin kg^{-1}. We have the technology, we have the know-how, we have the manpower, and we have specialized skilled labor in Gujarat and Saurashtra. The problem is to sell the groundnuts at a competitive price. If the Government were to subsidize exports, I am sure we could have a successful export trade. We have given a 5-year plan to the Government and we visualise that we should easily be able to export 200 000 t in the next 5 years, if our proposal is accepted.

I.A. Rana. You have said that your organization can supply groundnuts with aflatoxin levels within the permissible limit. If this has been done by detoxification, the aflatoxin may again be formed in the stomach of the animal, and the same problem will arise. What are your views about that?

G. Chandrashekhar. The question of detoxification arises only the in case of groundnut cake. My organization promotes the use and export of groundnut kernels, not cake. We are in a position to select and grade these kernels in such a way as to ensure that the aflatoxin level is limited to a maximum of 5 μg kg^{-1}. Basically, as an exporter, I am prepared to guarantee to any buyer a supply of edible-grade groundnuts, with 5 μg aflatoxin kg^{-1} or less. And I think as an exporter my responsibility should cease the moment I deliver the goods to him.

P. Subrahmanyam. You mentioned that there is a move to export roasted groundnuts to European countries. What is the shelf life of roasted groundnuts?

G. Chandrashekhar. As a matter of fact, we have recommended to the Government that we should have a warehouse in Rotterdam or Hamburg, send raw groundnuts from here, get them roasted there on a contractual basis, and then supply to the buyers. To get the goods roasted here and then ship them would be more expensive and difficult. It will be much easier for us to get Indian groundnuts blanched and roasted at the overseas ports on a contractual basis. The question of shelf life does not therefore arise.

R.D. Coker. Concerning the increasing interest in roasted nuts in shells, it occurs to me that everybody is concerned with trying to control the quality of roasted nuts. If the nuts remain in shell this represents quite a problem because it is very difficult to sample a nut while it is still in the shell. I am not convinced that roasting groundnuts reduces aflatoxin levels by any appreciable amount. The consistency figure seems to be about 25–30%.

G. Chandrashekhar. We visited Japan in June 1987. The limit for aflatoxin in Japan is 10 μg

kg^{-1}. They are importing $ 13 million worth of roasted groundnuts in shell every year, most of them from the People's Republic of China and Indonesia, and they claim that they have had no problem so far in importing roasted nuts in shell.

R.D. Coker. The problem with quality control of groundnuts in shell is that of sampling.

G. Chandrashekhar. There are no generally accepted quality specifications for in-shell groundnuts. Business is conducted on the basis of samples. If the buyer is satisfied with the appearance of the trade sample, he decides to buy. Some buyers shell the in-shell samples to ascertain their aflatoxin level. The buyer does take some risk in purchasing in-shell nuts. Perhaps the ODNRI can help evolve a suitable sampling procedure for in-shell nuts.

R.J. Cole. If you enter the export market, the aflatoxin problem is a major consideration. With regard to the price and quality factors, when you send a consignment to Europe or wherever, they will not accept your analysis. If your consignment is unacceptable on the basis of their tests you will get a telex as to what you will have to do with your groundnuts. So it is one of the most important aspects in the export market. You will not convince the importers, or the FAO for that matter, that aflatoxin is not a problem, as it has been recognized that it produces liver cancer in selected animals. You may feel that you have been treated unjustly, but if you want to compete in the export market you will have to accept the conditions operating there.

G. Chandrashekhar. We certainly do realize that aflatoxin is carcinogenic. We are in regular touch with buyers and end-users in major importing countries. It would be a mistake to think that in my paper I have run down the importance of aflatoxin in groundnut exports. No; far from it. What I have said is that as far as India is concerned, her exports have been affected not because of high incidence of aflatoxin, but because of high price. Indian shellers are capable of preparing edible groundnuts to conform to international standards and we are under no illustion about the importance of aflatoxin in groundnut exports, because we hope to get back into business shortly.

The Problem of Aflatoxin Contamination of Groundnut and Groundnut Products as seen by the African Groundnut Council

B. Coulibaly[1]

Abstract

In member states of the African Groundnut Council (AGC) groundnuts are an important traditional and economic crop. For the past 25 years, the problem of aflatoxin has confronted the groundnut industry and AGC. The economic and commercial problems worsen each year. Aflatoxin is a serious constraint to exports particularly in groundnut cake and meal destined for the traditional western European markets. The nature of the aflatoxin problem is indisputable, but its solution involves matters of trade and politics as well as scientific research.

On the basis of scientific information on Aspergillus flavus *and other mycotoxin-producing fungi, and despite the controversies, the AGC launched an aflatoxin control program in 1975. Phases I and II have been completed with financial aid from the European Economic Community (EEC) and the United Nations Development Programme (UNDP) and with technical assistance from the Food and Agriculture Organization of the United Nations (FAO). The following results have been achieved: (1) staff have been trained to survey and monitor aflatoxin in fields and laboratories; (2) laboratories for aflatoxin analysis have been established and equipped; (3) control measures have been identified; and (4) two pilot detoxification plants have been constructed to supplement cultural control measures.*

The AGC monitors contacts with EEC representatives and exporters of groundnut products on relevant legislation and standards and their application.

A scientific solution is not sufficient in itself, it can only be implemented by the combined efforts, goodwill, and initiatives of trade, industry, and politicians.

Résumé

Problème de la contamination par l'aflatoxine des arachides et de leurs produits, vu par le Conseil africain de l'arachide : *Dans les Etats membres du Conseil africain de l'arachide (CAA), l'arachide représente une production traditionnelle d'importance économique. Depuis 25 ans, l'industrie d'arachide et le CAA se heurtent au problème des aflatoxines dont les conséquences économiques et commerciales tendent à s'aggraver. La présence des aflatoxines freine les exportations, en particulier celles des tourteaux et de la farine d'arachides destinés aux marchés ouest-européens. L'aflatoxine pose donc un problème important dont la solution touche non seulement à la recherche scientifique mais aussi aux questions commerciales et politiques.*

Malgré les polémiques soulevées autour du problème, en 1975, le CAA a lancé un programme fondé sur les connaissances scientifiques sur Aspergillus flavus *et d'autres champignons*

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producteurs de mycotoxines, pour lutter contre l'incidence des aflatoxines. Les Phases I et II ont été menées à terme grâce à l'appui financier de la Communauté économique européenne (CEE) et du Programme des Nations Unies pour le développement (PNUD), avec le concours technique de l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO). On peut déjà noter les acquis suivants : formation du personnel au contrôle d'aflatoxine aux champs et en laboratoire; établissement et équipement des laboratoires pour l'analyse des aflatoxines; identification des mesures de lutte; et construction de deux unités pilotes pour la détoxification afin de compléter les mesures culturales de lutte.

Le CAA entretient des contacts avec les représentants de la CEE et les exportateurs des produits arachidiers, en ce qui concerne la réglementation et les normes ainsi que leur application.

Une solution scientifique seule ne serait pas suffisante; elle doit être complétée par des efforts conjugués des industriels, des responsables du commerce et des politiciens.

Resúmene

El problema de la contaminación con aflatoxinas del cacahuate y sus derivados, visto por el Consejo Africano del Cacahuate : *En los estados miembros del Consejo Africano del Cacahuate (AGC), el cacahuate es un cultivo tradicional y económicamente importante. Durante los últimos 25 años, el problema de las aflatoxinas ha preocupado seriamente a la industria cacahuatera y al AGC. Los problemas económicos y comerciales resultantes han empeorado cada año. Las aflatoxinas son un serio impedimento para las exportaciones, especialmente de la torta y la torta molida de cacahuate destinadas a los mercados tradicionales de Europa. La naturaleza del problema de las aflatoxinas es inconvertible, pero su solución incluye tanto aspectos comerciales y políticos, como de investigación científica.*

En base a la información científica disponible sobre Aspergillus flavus *y otros hongos que producen micotoxinas, y a pesar de las controversias existentes, el AGC inició un programa de control de las aflatoxinas en 1975. Las fases I y II del mismo se han completado con el apoyo financiero de la Comunidad Económica Europea (CEE) y el Programa de las Naciones Unidas para el Desarrollo (PNUD), y con el apoyo técnico de la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO).*

Se han logrado los siguientes resultados: (1) se ha capacitado personal para el muestreo, estudio y vigilancia continua de las aflatoxinas, en los campos y laboratorios; (2) se han establecido y equipado laboratorios para el análisis de aflatoxinas; (3) se han identificado medidas de control; y (4) se han construido dos plantas experimentales para eliminar toxinas de productos alimenticios, como complemento de las medidas basadas en el manejo de los cultivos.

El AGC mantiene en forma continua contacto con los representantes de la CEE y los exportadores de productos derivados de cacahuates, en relación con medidas legislativas relevantes y sobre el estándar de calidad y su aplicación.

Una solución científica no es suficiente en sí; solamente puede ser ejecutada eficazmente mediante los esfuerzos conjuntos, la buena voluntad y las iniciativas de los comerciantes, los industriales y los políticos preocupados por este problema.

Introduction

Groundnut is considered to be an indispensable speculative crop in any socioeconomic development program undertaken by the member states of the African Groundnut Council (AGC). This is due to its importance both as a food crop and an export crop. The problem of aflatoxin contamination has been posing a grave economic threat to AGC member states for more than a

decade. The situation is becoming increasingly complicated, and aflatoxin contamination also has serious implications for the export of such groundnut products as oilseed cake and meal to western European markets, the major traditional outlets for AGC products.

In its attempts to find a solution to aflatoxin contamination, the AGC has faced the problem of accurately identifying and quantifying the damage caused by aflatoxin contamination of groundnuts in different areas.

In 1975, the AGC began a permanent control program based on existing scientific knowledge of *Aspergillus flavus* and other related toxigenic fungi. The first and second phases of this program were carried out with financial assistance from the European Economic Community (EEC), and the United Nations Development Programme (UNDP) and the Food and Agriculture Organization of the United Nations (FAO), and resulted in:

- Training of laboratory assistants and supervisory personnel.
- Construction of laboratories with equipment for analysis and determination of mycotoxins in groundnuts and groundnut products.
- Evolution of measures to prevent aflatoxin contamination.
- Establishment of two pilot detoxification plants.

The AGC, in coordination with the EEC, is attempting to organize a series of consultations between the EEC and exporting countries before further restrictive measures are taken against groundnut products. There are many indications that aflatoxin contamination is causing a serious problem, necessitating the involvement of both political and diplomatic authorities.

Aflatoxin Contamination in Groundnut and Groundnut Products and its Economic Implications for AGC countries

The income generated by groundnut is of great importance to the economy of most member nations of the AGC. Groundnuts are important as food, for use in industries, and as exports to obtain foreign exchange. The crop is very important to the small farmer.

In some member states of the AGC groundnut is the most strategically important product in the economy and is an indispensable element for equilibrium and viability in any socioeconomic develpment program, involving considerable human, financial, and technical resources.

Aflatoxin can occur in organic substrates other than groundnut if they are exposed to the causal fungi and conditions are favorable for colonization. There is no specific internationally accepted norm or limit for an acceptable aflatoxin content, as each country establishes its own norm. Groundnut is unnecessarily discriminated against in favor of other products. The AGC annually mobilises about 50% of its human, technical, and financial resources in addressing this problem.

In its search to find a durable solution the AGC needs to accurately identify and quantify in different but related areas, the damage to groundnuts incurred by aflatoxin contamination.

A concerted international effort utilizing the most advanced technology is required to find a solution. It is in this context that the present workshop is particularly important to the AGC.

Aflatoxins and their Toxicity

Nature of aflatoxin

The term "aflatoxins" is used to designate a group of organic metabolites, more or less toxic to

animals, of certain strains of fungi belonging to the species *Aspergillus flavus* and *A. parasiticus*. Six aflatoxins have been identified:

aflatoxin $B_1 = C_{17} H_{12} O_6$
aflatoxin $B_2 = C_{17} H_{14} O_6$
aflatoxin $G_1 = C_{17} H_{12} O_7$
aflatoxin $G_2 = C_{17} H_{14} O_7$
aflatoxin M_1 = 4-hydroxyaflatoxin B_1
aflatoxin M_2 = 4-hydroxyaflatoxin B_2

Detailed studies show that aflatoxin B_1 is the most commonly occurring form. All the aflatoxins can be toxic at certain concentrations and may cause primary liver cancer in animals and humans. They can also cause lack of appetite, loss of weight, haemmorrage, ascites, and abortion. Young animals are more sensitive and vulnerable to aflatoxicosis than older ones. There are two types of aflatoxin toxicity: direct toxicity, and relay toxicity; both can be acute or chronic.

Direct toxicity

Caused by direct intake of high doses of aflatoxins B_1, B_2, G_1, or G_2.

Relay toxicity

Occurs when aflatoxins B_1 or B_2 are ingested by a mammal which secretes them in the converted form as trysin-aflatoxin M_1 or M_2 in its milk.

Controversial issues

The determination of a precise, quantified maximum aflatoxin content in foodstuffs has always led to differences in opinion between sellers and buyers; the former do not want to suffer unduly, and the latter want to avoid taking any risk.

Representative samples

The representative nature of samples is a disputed topic since the distribution of alfatoxin in lots of kernels is generally uneven. Usually only a few contaminated kernels contribute to the toxicity of the sample. The sampling methods developed to date do not guarantee that samples are completely representative. Thus, there is a risk of rejecting a healthy lot or retaining a contaminated one. Further research is necessary to develop a method that is easily applicable, and acceptable to both sellers and buyers.

Determination of toxicity limits/levels

Toxicity levels determined using certain sensitive species (ducklings and turkey poults) bred under laboratory conditions may not be directly applicable to older animals of more resistant species living under normal conditions. It is even more difficult to determine dangerous levels of aflatoxin in food destined for consumption by humans.

It has been proved that the toxicity of a sample depends on such parameters as the nature of the aflatoxin(s) present, the nature and properties of other metabolites present in the sample, the species, age, and sex of the animal consuming the sample, the duration of food intake, etc. It

follows that there are many areas to be investigated before it is possible to precisely evaluate the toxicity limits of mycotoxins, especially those which undergo metabolic transformation of their molecules to release biologically active compounds.

Possibility of dilution

The aflatoxin level in a given raw material can be lowered to an acceptable level in the end product by dilution with other ingredients, and this may render efforts towards fixation of maximum aflatoxin levels futile.

EEC Directive 74/63 and its Modifications

Following the EEC Directive 74/63 that is presently in force, the AGC was compelled to invest heavily in human and financial resources in its fight against aflatoxin contamination, spending $4 500 000 from the EEC, $2 000 000 from UNDP, and $10 000 000 in kind including property, personnel, and services as part of the contribution from all AGC member states. Just when all these efforts were yielding results, the EEC set new non-tariff obstacles that affect groundnut and groundnut products. The existing regulation relating to the maximum level of undesirable substances in cattle feed that includes groundnut products was modified.

The Directive was enforced with effect from 11 Feb 1974 (EEC's official Journal no. L-38, page 31) and the modifications were introduced in 1986 (Official Journal of the European Community no. L-212, dated 2 Aug 1986).

EEC Directive 74/63

The maximum authorized levels of aflatoxin B_1 indicated for the final product by the Directive are:

- Simple cattle feed = 50 $\mu g\ kg^{-1}$ of aflatoxin B_1.
- Composite cattle feed (for oxen, sheep, and goats) = 50 $\mu g\ kg^{-1}$ of aflatoxin B_1.
- Complementary cattle feed for milch cows = 20 $\mu g\ kg^{-1}$ of aflatoxin B_1.
- Other composite cattle feed = 10 $\mu g\ kg^{-1}$ of aflatoxin B_1.

Modifications to EEC Directive 74/63

These include:

a. Extension of EEC Directive 74/63 to include all raw materials coming under the category of cattle feed.
b. A change in the name of the Directive to: Directive of the Council of Undesirable Substances and Animal Nutritional Products.
c. Introduction of a new annexe comprising three columns, which are:
   Column I: undesirable substances (including aflatoxin B_1)
   Column II: plant/vegetable raw materials
   Column III: indicates the maximum permissible aflatoxin level at 12% relative humidity (the limit is 0.2 ppm or 200 $\mu g\ kg^{-1}$ for aflatoxin B_1)
d. A new article, 3 (a) was included that stipulates:
   That the member states can introduce into the market any commodity listed in Annexe (2) only when the undesirable substances content does not exceed the level fixed in Column III.

If, for a given raw material, the undesirable substances content as stipulated in Annexe (2) exceeds the limit prescribed in the Directive, then it can be brought into the market only if it is accompanied by a document stating:

- that the raw material is reserved solely for recognized cattle feed dealers;
- that the raw material should be given to cattle only after prior processing/treatment; and
- the nature and the effective content of the undesirable substances.

e. All countries are at liberty to unilaterally fix provisory regulations based on safeguard clauses regarding the maximum content of undesirable substances in commodities not covered by the Directive; if however the maximum level is fixed, then they can reduce it at the community level. They must notify the measures taken and justify them to the Commission, and these measures can be applicable only so long as the Commission does not enforce its own statute.

AGC and the Aflatoxin Problem

In order to improve the quality of its groundnut products, the AGC has taken up a two-pronged action: (1) direct action to control aflatoxin contamination, (2) participation in dialogue and negotiations seeking a consensus to fix a tolerable aflatoxin level in groundnut products.

Direct Action to Control Aflatoxin Contamination

In 1975, in order to combat aflatoxin contamination in groundnut and its products, AGC members formulated a Regional Research Program to Control Aflatoxin, which comprises eight projects. This program was partly financed by the EEC (US $4.5 million) and UNDP (US $2 million), while member states contributed US $10 million. A major part of this finance was used to: (1) update laboratories and to produce an aflatoxin map for each member nation; (2) train personnel in the methodology of sampling and analysis for aflatoxins, and in the detection and supervision of infested areas; (3) establish two pilot factories for detoxification, and provide training, and (4) pay consultants to undertake field studies in order to improve the preventative measures taken against aflatoxin.

At the national level, efforts are still being made to improve the quality of groundnut and its products. The well-established techniques of pneumatic, electronic, and manual sorting facilitate the separation of seeds visibly damaged by *Aspergillus flavus* or other fungi. Industrial detoxification of oilseed cakes with ammonia and formaldehyde is thought to be 98% effective. The detoxified cake is acceptable in the European market. Apart from the two detoxification plants already established in Senegal and Sudan, a third, set up by Senegal with nationally allocated funds, became operational in 1983.

Consultations, dialogues, and negotiations to fix a tolerable level of aflatoxin in groundnut products

The AGC held consultations, dialogues, and negotiations with appropriate international authorities to fix a level of aflatoxin acceptable to sellers, buyers, and consumers.

Scientific authorities approach. Two groups belonging to this category, i.e., the IUPAC mycotoxin group and the R. Truhant, R. Ferrand, A. Lamine N'Diaye group, both groups are particularly experienced in the study of mycotoxins and toxicology and have regularly collaborated with the AGC. Professors M. Jemmali and A. Lamine N'Diaye are consultants and

advisors to the AGC, and regularly supply up-to-date information on the methods of determining levels of direct and relay toxicity of aflatoxin.

It must also be mentioned here that the Wolff laboratories in France, and the Research Centers of the Departments of Agriculture in Tifton and New Orleans, USA, have often conducted training programs and provided consultancy services on aflatoxins at the request of the AGC. The Tifton scientists have also freely donated to the AGC, germplasm resistant to invasion by *A. flavus*.

Professional authorities approach. In this category, the Federation of Oils, Seeds, and Fats Associations (FOSFA) and the Grains and Feed Trade Association (GAFTA), with their headquarters in London, have always helped the AGC in its efforts to study the development and practical application of methods of analysis, the evolution of regulations and standards, and their interpretation and implication in the field of animal and human nutrition with special reference to mycotoxins.

International organizations' approach. In its attempts to find other solutions to the aflatoxin problem, following the EEC Directive 74/63 that introduced more restrictions, the AGC has come to an agreement with the Ambassadors' Committee President (ACP), the EEC, and FAO.

Since 23 May 1983 (the date on which the modifications to the EEC Directive were announced), the AGC has continually striven towards making the EEC withdraw all constraining modifications from that Directive. Thus, the Ambassadors' Committee President, the African Ambassadors' Group President (both in Brussels), and all the Ambassadors of the AGC member nations met in Belgium to review the aflatoxin situation; its sanitary, socioeconomic, commercial, and legislative implications. This review concentrated on the protectionist and discriminatory character of the EEC modifications. An appeal was made to the diplomatic authorities mentioned above to consider the objections made by the AGC to the modifications envisaged by the EEC in Directive 74/63.

Instigated by the AGC, meetings were organized with the Technical Bodies of the ACP and the EEC to consider certain pertinent elements before preparing a detailed memorandum of aflatoxin problems. During these meetings, the EEC put forward its justification for the amendments in the Directive 74/63. It has however, been admitted that there are dissensions and differences within the EEC as to the nature of the product, related to groundnut, which is directly affected by the extension of the Directive to all raw materials.

Following these dialogues, a memorandum entitled: "Determination of the Groundnut Situation in the Common Market", was presented to the President of the EEC Commission, with the approval of the ACP. This document detailed the ACP stand concerning the propositions of the new EEC Directive to lower the tolerable level of aflatoxin B_1 in cattle feed.

Following strong protests from different interested parties, the EEC sought to give additional explanations to its partners in the ACP on the new proposals, before submitting them for approval by the Council of Ministers.

A meeting of the AGC/ACP/EEC with scientific experts from different concerned parties, was held at Brussels on 1 Feb 1984. During the discussions, it was proved that the aflatoxin B_1 level as laid down by the EEC is too extreme and far from scientific. The AGC/ACP group took this opportunity to:

1. propose a level of 400 $\mu g\ kg^{-1}$ instead of 200 $\mu g\ kg^{-1}$ (proposed by EEC) for raw materials;
2. ask for the approval of a well-defined list of raw materials;
3. demand that all accompanying measures included in article 3(a) in the new proposal/Directive be declared null and void, and

4. see that the following points made by the ACP are taken into account before any amendment is introduced in the EEC Directive 74/63:
   - Standardization of all the measures in each country belonging to the EEC.
   - Exclusion of oilseeds from the control.
   - Relieve exporters and transporters from expensive obligations and storage problems incurred by bank insurance to cover goods during shipment.
   - Revision and standardization of methods of sampling and analysis.
   - Common definition of the term "Recognised dealer" for all the EEC members.

Agreement with the FAO. At the 18th Session of the FAO Intergovernmental Group on Oilseeds, Oils, and Fats, 20–24 Feb 1984, in Rome the AGC was part of the delegation which discussed the proposed modifications to the EEC Directive regarding tolerance limits of undesirable substances (especially aflatoxin B_1) in cattle feed. After detailed discussions, the Group finally declared that "all legislative regulatory measures in this field should necessarily be based on data which can be verified through reliable means, in order to prevent any unjust harm to the concerned parties and also to the liberty of international oilseeds commerce".

The Group also asked FAO to closely follow the international standardization of norms so the Joint Committee FAO/OMS of the Codex Alimentarius could fix aflatoxin limits which are internationally recognized, uniform, and reasonable.

Present Requirements of the AGC

The main objective of the AGC in its effort to protect groundnut and its products in the world market in general, and in the EEC market in particular, is to make the European Parliament delete any modification in the EEC Directive 74/63 if it is not related to sanitary protection. Any other amendment should be discussed in advance with the concerned parties; it should not be decided unilaterally and arbitrarily by a single party. The following aspects should be considered for any acceptable amendment, if it concerns the AGC:

- Standardization of the tolerance levels across EEC member states.
- Legislation should essentially concern the finished feed products for different categories of animals (monogastric, polygastric, young and milch animals); the specific tolerance levels for these categories presently in force vide EEC Directive 74/63 should be maintained.
- A precise and exhaustive list of raw materials should be made available.
- Prevention of any departure from the standardization of levels on the pretext of resorting to safeguards and withdrawal of all accompanying measures.
- Use of standard methods of sampling and analysis accepted by all concerned parties.

Recommendations

In order to find a solution to the socioeconomic, scientific, and technical problems posed by aflatoxin contamination of groundnut and its products, action should be embarked upon and/or improved upon in the following areas:

Research

- The establishment of groundnut varieties resistant to the fungi causing aflatoxin contamination.

- The search for techniques aimed at identifying in a precise manner the damage attributable solely to aflatoxin contamination.
- A study of the links that may exist between groundnut contamination and the incidence of liver cancer in man.
- A search for a precise, acute, and accumulative toxicity rate for aflatoxin in the metabolism of different kinds of animals (monogastric and polygastric).
- Examination of aflatoxin relay toxicity in man.
- Careful assessment of the financial losses attributable solely to aflatoxin contamination of groundnut and its products.
- The standardization of methods used for sampling, analysis, and determination of the mycotoxin content in a manner acceptable to both the seller and the buyer.
- The improvement of methods used for the direct extraction of protein from groundnut for the nutrition of young animals and man.

Implementation of preventive and curative measures

- Informing the authorities, extension workers, and educating the masses on the danger aflatoxin poses to public health and to the economy (involving schools and the mass media).
- Training and equipping groundnut producers with adapted, resistant genetic materials, technical assistance and logistics for harvesting, drying, transportation, and storage operations under conditions aimed at controlling aflatoxin.

Regulations

The standardizing of regulations and the establishment of international standards for aflatoxins and other important mycotoxins.

Session IV

Aflatoxins in Groundnut: Monitoring and Action at National Level

Aflatoxin Contamination of Stored Groundnuts in Zimbabwe

A.H. Siwela[1] and A.D. Caley[2]

Abstract

Aflatoxins were analyzed in groundnuts stored for either local or export sales in the period 1982/83 to 1986/87. Four hundred and forty-one samples of seven groundnut varieties were collected for analysis. Sixty-eight percent of the samples had total concentrations of aflatoxin B_1 and G_1 of up to 25 $\mu g\ kg^{-1}$. In the 1986/87 groundnut crop, the Flamingo and Makulu Red varieties, which constitute the bulk of export sales, had up to 25 $\mu g\ kg^{-1}$ aflatoxin B_1 in 79% of the samples, plus aflatoxin G_1 in 67% of the samples. Overall, the Egret variety was the most susceptible to aflatoxin contamination during this period.

Résumé

Contamination des arachides stockées par les aflatoxines au Zimbabwe : *Les analyses des aflatoxines ont été effectuées sur les arachides stockées, destinées soit à la vente locale, soit à l'exportation, au cours de la période allant de 1982/83 à 1986/87. Elles ont porté sur 441 échantillons de 7 variétés d'arachide. Dans 68% des échantillons, la concentration totale des aflatoxines B_1 et G_1 s'est élevée à 25 $\mu g\ kg^{-1}$. Dans la récolte de 1986/87, les variétés Flamingo et Makulu Red, qui constituent la majeure partie des ventes à l'exportation, ont présenté une concentration d'aflatoxine B_1 allant jusqu'à 25 $\mu g\ kg^{-1}$ dans 79% des échantillons et de l'aflatoxine G_1 dans 67% des échantillons. En général, la variété Egret s'est montrée la plus sensible à la contamination par les aflatoxines au cours de cette période.*

Resúmene

La contaminación con aflatoxinas en el cacahuate almacenado en Zimbabwe : *Se cuantificaron aflatoxinas en cacahuates almacenados para ventas locales o de exportación, durante el período 1982/83 a 1986/87. Se recolectaron 441 muestras de siete variedades de cacahuate, para su análisis. Sesenta y ocho por ciento de las muestras tuvieron concentraciones de aflatoxina B_1 y G_1 hasta 25 $\mu g\ kg^{-1}$. En 1986/87, las variedades Flamingo y Makulu Red, que constituian la mayor parte de las ventas de exportación, tuvieron un contenido hasta de 25 $\mu g\ kg^{-1}$ de aflatoxina B_1 en 79% de las muestras, y además aflatoxina G_1 en 67% de las muestras. En general, la variedad Egret fue la más susceptible a las contaminaciones con aflatoxinas durante este período.*

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Introduction

Groundnut is the third most important oilseed in Zimbabwe, after cotton and soybean. It is grown throughout the country in both the commercial and peasant sectors. The peasant sector predominantly produces short-season varieties, i.e., spanish and valencia, which constitute about 25% of the confectionery nuts while the commercial sector produces the longer-season varieties (Hildebrand 1980).

The problem of aflatoxin contamination in groundnuts and other commodities is not new in Zimbabwe. Investigations of aflatoxin contamination of groundnuts were started in 1962 following the discovery by the Veterinary Research Laboratories of two cases of aflatoxicosis in poultry.

Aflatoxin contamination did occur in an appreciable proportion of the crop (Bushnell 1965). Since then, results of a 10-year survey have been published (du Toit 1977). Several interesting points were raised enabling the prediction of aflatoxin contamination in the Zimbabwean groundnut crop resulting from differing climatic, seasonal, and agronomic practices. It was shown that the levels of aflatoxins in groundnuts varied from season to season. When the end-of-season rainfall was low, an increase in the levels of aflatoxin contamination of the groundnut crop was observed. Similarly, an increase in the aflatoxin levels was observed when irrigation of the groundnut crop was stopped prematurely. It was also shown that contamination of groundnuts with aflatoxin increased when lifting was delayed beyond pod maturity. Variation of aflatoxin levels in groundnuts from individual Grain Marketing Board (GMB) depots were observed. GMB depots drawing their groundnuts from the cooler, high-rainfall areas had groundnuts with less aflatoxin contamination than those serving the hotter and drier areas.

To protect the health of the population and livestock, a code of conduct was introduced (Table 1), and surveys to monitor aflatoxin levels have continued. No groundnuts are released by the GMB for use without being analyzed for aflatoxin B_1 and aflatoxin G_1.

In this paper, we report the analytical data from 1982/83 to 1986/87.

Table 1. Aflatoxin level classifications and code of conduct in Zimbabwe.

		Aflatoxin concentration ($\mu g\ kg^{-1}$)	
Category[1]		B_1	G_1
Negative	(N)	<5	<4
Low_1	(L_1)	5- 10	4- 8
Low_2	(L_2)	10- 25	8- 20
Medium	(M)	25- 50	20- 40
High	(H)	50-100	40- 80
Very High	(VH)	100-250	80-200

1. The negative category is used for export, human consumption, and poultry feed. All other categories are used for stockfeed, as determined by the maximum permissible percentage inclusion of contaminated cake (du Toit 1971).

Material and Methods

A 10-kg random sample representing 20 t was reduced to a 500-g working sample for submission to our laboratory by the GMB. The sample was further reduced by the quartering technique until a 100-g subsample was obtained. This sub-sample was ground to a fine meal of which 50 g were used for analysis.

Extraction

A 50-g ground sample was extracted with a 250 mL methanol - water (55:45) solution containing 3 g sodium chloride. The slurry was vigorously stirred and left to stand for 30 mins after which it was blended at high speed for 2 mins in the presence of 100 mL of hexane. The slurry was finally filtered, a 25-mL aliquot was extracted with 25 mL chloroform and evaporated on a steam bath to near dryness. A gentle nitrogen stream was used to dry the extract.

Analysis by Thin-Layer Chromatography (TLC)

The residue was redissolved in 0.5 mL chloroform, and 0.02 mL spotted on G_{254} thin-layer chromatographic plates alongside authentic aflatoxin standards. The plate was developed first in ether and then in chloroform + acetone (9+1) solvent systems. Quantification was achieved by visual comparison of fluorescence of standards against samples when viewed under UV light. Confirmation of aflatoxin identity was achieved by the sulphuric acid spray method.

Results and Discussion

Table 2 shows the incidence of aflatoxin in confectionery groundnuts. In both Tables 2 and 3, aflatoxin B_1 and aflatoxin G_1 were totalled and the category defined by the aflatoxin B_1 scale.

The maximum permissible level in Zimbabwe has been set up at 25μg kg^{-1}. From 1982/83 to 1986/87, intertemporal variability in the level of groundnut contamination up to 25μg kg^{-1} was not significant at the 5% level of probability (chi-square test). However, the incidence of contamination at or below 5μg kg^{-1} fluctuated from season to season, with the 1982/83 and

Table 2. Incidence of aflatoxin in confectionery groundnuts in Zimbabwe from 1982/83 to 1986/87.

Storage year Apr-Mar	Total samples	Samples in each category(%)[1]					
		N	L_1	L_2	M	H	VH
1982/83	157	29	7	22	17	15	10
1983/84	80	56	-	8	8	18	10
1984/85	19	21	-	42	5	16	16
1985/86	61	66	-	13	5	10	6
1986/87	124	69	2	6	5	11	7

1. Defined in Table 1.

1984/85 crops being the worst affected. The seasons preceeding 1982/83 and 1983/84 were relatively dry. It is conceivable that the higher levels of contamination occurred before storage. Du Toit (1977) showed that groundnut invasion by *A. flavus* and subsequent aflatoxin contamination occurred in the crop before lifting. He found no evidence to indicate that the normal methods of storage used by either the GMB or the farming community predispose groundnuts to aflatoxin contamination. However, no rational explanation can be advanced as to why no difference is observed when 25μg kg^{-1} is taken as the upper limit.

Table 3 shows the incidence of aflatoxins in different varieties of groundnuts for the storage year 1986/87. Of the samples tested, contamination was most frequent in the Egret variety with only 46% of the samples at either 25μg kg^{-1} level or below 5μg kg^{-1} compared to other varieties which varied from 67–100% in the 25μg kg^{-1} category and 53–100% in the negative category. No detectable levels of aflatoxins were found in over 50% of samples of Makulu Red and Flamingo varieties which constitute the bulk of the groundnut exports.

These data represent preliminary results of an on-going study on the susceptibility of a particular variety to aflatoxin contamination. Such a study might help persuade producers of that variety to switch to other varieties which are not at risk. We also hope to sample at various GMB depots and eventually go to individual suppliers, thus tracing the source of aflatoxin contamination.

Table 3. Aflatoxin incidence in different varieties of groundnuts in Zimbabwe, preliminary survey, storage year Apr-Mar 1986/87.

Variety	Total samples	Samples in each category (%)[1]					
		N	L_1	L_2	M	H	VH
Egret	13	46			8	38	8
Valencia	6	100					
Makulu Red	15	53	7	7	13	7	13
Spanish	22	59	5	18	5	9	4
Flamingo	58	76		3	3	9	9
Plover	2	100					
Swallow	1	100					
Mixed/Ration	7	72	14			14	
All varieties	124	69	2	6	5	11	7

1. Defined in Table 1.

Acknowledgments

The authors wish to thank the GMB for collecting the samples; Special Analysis Laboratory, Chemistry and Soils Research Institute for aflatoxin analysis; Mr R.J. Fenner, Director of the Department of Research and Specialist Services for granting permission to publish the results; Mr T.J.T. Madziva, Head, Chemistry and Soil Research Institute for his constructive criticism of the script, and Messrs M. Reeves, N. Kutukwa, and J. Mashavane of the GMB for helping in the compilation and data analysis.

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Discussion

G. Chandrashekhar. Dr Siwela said that he found high aflatoxin contamination in groundnut samples when rainfall towards the end of the season was low. The Indian Oil Producers and Exporters Association (IOPEA) conducted a study in November 1986 when late season rainfall was low. In Gujarat over 160 samples were drawn and analyzed for aflatoxin and over 70% of the samples showed aflatoxin levels of less than 5 $\mu g\ kg^{-1}$.

A.H. Siwela. Our finding is in line with other workers' results. It is believed that when end-of-season rainfall is low, pods may crack due to drought stress or water deficit, and expose kernels to direct contamination by *A. flavus* or *A. parasiticus* and subsequent aflatoxin contamination.

F. Waliyar. In Senegal we made similar observations.

V.K. Mehan. I think that the seed invasion problem becomes more severe as drought intensity increases. The length of the drought period also affects aflatoxin contamination; if drought stress occurs for less than 20 days it may not lead to significant fungal invasion and subsequent aflatoxin contamination, but longer periods result in contamination.

J.H. Williams. I think one needs to also take into account genetic variation in the drought response. Some varieties will have 30 to 40% of seeds infected, others will have only 3% infected. So there is very large variation in response to drought.

N.E. Ahmed. You have shown us several methods of drying, I would like to know if you have any preference?

A.H. Siwela. The four methods shown are used routinely in the country and all the methods have been found adequate - they do not predispose groundnuts to aflatoxin contamination at all.

R.W. Gibbons. When I was in Malawi, we did quite a lot of work on different drying methods. We found that some of the methods were very effective, particularly the making of hollow stacks.

Aflatoxin and *Aspergillus flavus* Contamination Problems of Groundnuts in Zambia

J. Kannaiyan[1], R.S. Sandhu[2], and A.L. Phiri[3]

Abstract

In Zambia, groundnut kernels meant for export are routinely monitored for aflatoxin contamination. Since 1979, 6.3% of the 28 410 samples analyzed had contamination levels of more than 5 μg aflatoxin kg^{-1}. A 2-year study with promising varieties revealed the variability of A. flavus *seed infection. Seed treatment with Benlate®/Labilite® at 3 g kg^{-1} seed was found to control* A. flavus *in groundnut seed and can improve crop stand.*

Résumé

Problèmes liés à l'aflatoxine et à *Aspergillus flavus* chez l'arachide en Zambie : *En Zambie, les graines d'arachide destinées à l'exportation subissent un contrôle de routine pour la contamination par les aflatoxines. Depuis 1979, 6,3% des 28 410 échantillons analysés ont présenté des taux de contamination dépassant 5 μg kg^{-1}. Les résultats d'une étude s'étendant sur deux ans sur les variétés prometteuses ont révélé une contamination variable des grains par* A. flavus. *Le traitement des semences avec Benlate® ou Labilite® à raison de 3 g kg^{-1} a permis de maîtriser* A. flavus *dans les semences tout en améliorant la levée des plantes.*

Resúmene

Problemas de contaminación con aflatoxinas y *Aspergillus flavus* en el cacahuate en Zambia: *En Zambia, los granos de cacahuate asignados a la exportación, son analizados regularmente para determinar su contenido de aflatoxinas. Desde 1979, el 6.3% de 28,410 muestras analizadas tenían niveles de contaminación de más de 5 μg kg^{-1} de aflatoxina. Un estudio de dos años con variedades prometedoras reveló la gran variabilidad existente en el grado de infección de la semilla con* Aspergillus flavus. *El tratamiento de la semilla con Benlate® o Labilite®, a razón de 3 g kg^{-1} de semilla, fue eficaz en el control de A. flavus* presente en la semilla de cacahuate y puede mejorar el establecimiento del cultivo.

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important food and cash crop in Zambia. Information on the climate, cropping areas, production, and cultivation practices of groundnuts in Zambia has been reported by Sandhu et al. (1985). Most of the groundnuts produced in Zambia are locally consumed, while the quantity exported has been fluctuating. Very little of the crop is used for edible oil. Monitoring kernels for aflatoxin contamination has been limited to export quality control. Recently, research has also been initiated on infection of groundnut kernels by *Aspergillus flavus* . Aflatoxin contamination of kernels is a result of such infection and crops grown from infected seed suffer from aflaroot disease.

Monitoring for Aflatoxin in Export Samples

Quality control of export materials for aflatoxin contamination is handled by the Eastern Co-operative Union (ECU) Limited, Chipata, a parastatal organization handling all Zambian groundnut kernel exports. From each 100- kg batch of export groundnut kernels, a representative sample of 1 kg is analyzed for aflatoxin content using the Tropical Products Institute's methods (Jones 1972). From 1977/78 to 1985/86, a total of 28 410 samples were analyzed. A mean of 6.3% of samples had aflatoxin contents of more than 5 $\mu g\ kg^{-1}$ (Table 1), and these were rejected for export. Locally consumed groundnuts, which are not directly monitored, may well have had aflatoxin contamination problems. In the Eastern Province, where most of the groundnuts are grown, a number of cases of suspected aflatoxin-induced health problems have been observed. However, these cases remain unconfirmed for want of suitable clinical facilities and lack of awareness of the importance of the aflatoxin problem.

Table 1. Frequency of aflatoxin contamination detected in export samples of groundnut kernels in Zambia monitored by the Eastern Co-operative Union Limited (ECU), Chipata, Zambia, from 1977/78 to 1985/86.

Harvest	No. samples tested	Samples containing >5 $\mu g\ kg^{-1}$ aflatoxin
1977/78	9200	3.3
1978/79	1050	9.5
1979/80	1060	9.4
1980/81	3300	3.0
1981/82	2400	8.3
1982/83	4900	8.2
1983/84[1]	-	-
1984/85	3900	5.1
1985/86	2600	3.9
Overall	28410	6.3

1. No export in this year.

Prevalence of *A. flavus* in Market Samples

Several samples were obtained from groundnut kernels sold locally in Chipata market, a common and informal channel for domestic consumption. Eight random samples of the predominant Chalimbana cultivars were collected from the market in July 1987; these had been harvested in May 1987. Each sample consisted of a few replicates each of 100 seeds, and was subjected to the standard 'blotter test' (ISTA 1976). Seeds of Chalimbana from Msekera Research Station, were used as controls. After incubation for 1 week at room temperature (25°C) the seeds were examined for percentage germination and *A. flavus* colonization.

The results (Table 2) showed that *A. flavus* was present in all the market samples as well as in the Chalimbana control. Seed infection of samples ranged from 0.8 to 8.0% among samples while the Chalimbana control had 4.5% of seeds infected. The market samples differed widely in seed germination (44–86%) but these differences did not seem to be related to *A. flavus* colonization. Since *A. flavus* was viable in seed of market samples 2 months after harvest, we speculate that aflatoxin contamination could further increase if the storage period were extended to 6–8 months and storage conditions were sub-optimal (Mehan and McDonald 1983).

Table 2. Results of laboratory screening[1] market samples of groundnut for germination (%) and *Aspergillus flavus* infection (%), Msekera Research Station, Zambia, Jul 1987.

Sample	Germination (%)	*A. flavus* infection (%)
MS 4	86	6.8
MS 7	84	2.3
MS 1	59	8.0
MS 3	58	2.0
MS 5	56	0.8
MS 6	53	3.0
MS 2	50	0.8
MS 8	44	6.0
Control		
Chalimbana	56	4.5
SE	±3.8	±1.5
Mean	60	3.8

1. Results are means of four replications, each of 100 seeds.

To study if *A. flavus* infection differed among common cultivars of groundnut, eight cultivars grown during the 1985/86 season at Msekera Research Station were sampled. Of these, three were short-duration types with small seeds, while the other five were long-duration types with medium to large seeds. Sampling was repeated in the 1986/87 season when seven of the original

Table 3. Results of laboratory screening groundnut varieties for germination (%) and *Aspergillus flavus* infection (%), Msekera Research Station, Zambia, 1986 and 1987.

Variety	1986		1987	
	Germination (%)	*A. flavus* infection (%)	Germination (%)	*A. flavus* infection (%)
Short duration (small seed)				
Tifspan	97	1	86	8
Natal Common	95	10	75	5
Comet	87	1	81	0
Long duration (medium large seed)				
Makulu Red	94	14	73	3
Egret[1]	91	5	-	-
4a/8/2	90	4	70	0
Chalimbana	79	1	55	4
Mean	88	7	71	5
SE	±1.5	±1.1	±2.9	±0.9
MGS-2	69	19	57	17

1. Not tested in 1987.

eight cultivars were tested, 400 seeds of each being sampled and assessed for *A. flavus* infection (%) and germination (%) as described earlier. The results (Table 3) showed that cultivars differed significantly in seed germination and *A. flavus* infection. MGS 2 (M 13), known to be susceptible to *A. flavus*, had the highest percentage of seed infection by *A. flavus* in both years. Germination was also lower in this cultivar than in most others. Makulu Red and Natal Common had more than 10% *A. flavus* infection of seeds in 1986, but only 3–5% infection in 1987, probably because the storage duration in 1987 was only 2 months compared to 6 months in the previous year. There was no apparent association between crop duration, seed size, and *A. flavus* infection. These results support the contention of Mehan et al. (1981) that there is a good possibility of finding varietal resistance to *A. flavus* invasion and/or production of aflatoxin in groundnut seeds.

Studies on Aflaroot Disease

Aspergillus flavus infection of groundnut seeds can result in lowered germination, and poor seedling establishment from aflaroot disease (Chohan and Gupta 1968). In Zambia, the source of seed for most small-scale farmers is their own previous-season crop that is stored at home. As seen from the tests on market samples, *A. flavus* is commonly found in these seeds and it can cause seed rot or seedling disease (aflaroot) both of which affect plant stand in the field. Studies with the cultivar Chalimbana at Msekera Research Station in the 1983/84 and 1984/85 seasons showed that aflaroot disease reduced numbers of pods produced by 70% and kernel yield by 75%.

Four fungicides were tested for their ability to eradicate *A. flavus* infection of seeds of Chalimbana. Benlate® (3g kg^{-1} seed) gave complete eradication and Labilite® (3g kg^{-1} seed) was only slightly less effective. Germination of Benlate®-treated seeds was better than that of nontreated seeds.

The same fungicides were tested as seed protectants in a field trial in the 1986/87 season. Seed treatment with Labilite® (active ingredient: 20% thiophanate methyl + 50% manganese ethylene bisodium dithiocarbonate) or Benlate® increased germination (%), but yields did not differ significantly between treatments. From these studies, it is clear that treating groundnut seed with Benlate® or Labilite® at a rate of 3g kg^{-1} seed would help control *A. flavus* infection and enhance seed germination.

For the Future

Considering the lack of available information, future efforts in Zambia should be directed towards the following aspects:

- Varietal resistance to *A. flavus* invasion and aflatoxin contamination appears to be a promising area of research. Assistance from international institutes such as ICRISAT in the form of stable resistant sources that could be tested in the Zambian environment would be important.
- To increase awareness of the importance of the aflatoxin problem among farmers and consumers, efforts should be taken to focus their attention on the potential health hazards caused by aflatoxin.

Acknowledgments

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Discussion

J. Kannaiyan. In Zambia we found that pods from untreated plants showed more scarification of shells than did pods from plants sprayed with fungicides to control foliar diseases. Seeds from scarified pods were of lower quality than seeds from undamaged pods, and we considered them to pose a greater aflatoxin hazard.

J.I. Pitt. Concerning your technique for looking for *A. flavus* infection, were the kernels surface sterilized before they were plated, or were they plated without sterilization?

J. Kannaiyan. They were plated without surface sterilization. We followed the International Seed Testing Association's blotter-test method and the incubation temperatures ranged from 25 to 30°C.

J.I. Pitt. To determine seed invasion by *A. flavus* it is absolutely essential to surface sterilize the seed and to plate them on a suitable medium because there are always going to be stray *A. flavus* spores around that have no relevance whatever in terms of *A. flavus* invasion or aflatoxin production.

P. Subrahmanyam. You mentioned that losses from aflaroot disease were as high as 70%. This could be an over-estimation as we have found losses of plant stand of around 25% in West Africa, but the losses in population were not reflected in yield losses because of compensation effects.

J. Kannaiyan. I agree. We were estimating loss of plant stand not direct crop yield loss. However, the potential for yield loss in conditions where compensation is limited is high.

J.I. Pitt. Is the disease known as aflaroot really due to *A. flavus* or to some other pathogen?

D. McDonald. In many parts of the world, and particularly when groundnuts are grown on light sandy soils, crown rot and seedling rots caused by *A. flavus* and *A. niger* are of common occurrence. Aflaroot is a special case in which the strain of *A. flavus* causing the seedling disease is aflatoxigenic. Aflatoxins translocated within the seedling from the infection site cause shoot chlorosis and root necrosis in diseased plants.

K.K. Shreshta. You mentioned that Benlate® controls *A. flavus* seed infection. It is true that any systemic fungicide can control seedborne diseases, but do you have any idea of the aflatoxin contamination in such treated samples - whether it is low or high? I have examined treated seed of groundnut and found it contained a high level of aflatoxin.

J. Kannaiyan. Treated seeds were not subjected to aflatoxin analysis. We do not know if aflatoxin is increased or decreased by seed treatment. Treated seed is meant only for sowing and not for human consumption.

Aflatoxin Contamination of Groundnuts: Control Strategies in Malawi

C.T. Kisyombe[1]

Abstract

In Malawi the rains start in October and finish in April so long-duration groundnut cultivars are harvested under dry conditions. These dry conditions favor rapid postharvest drying of groundnut pods thus limiting the opportunity for seed invasion by Aspergillus flavus *and* A. parasiticus *and aflatoxin contamination. Aflatoxin contamination of groundnuts is not a problem in the country. However, certain practices used by smallholder farmers to process groundnuts in readiness for sale create conditions that favor the rapid development of* A. flavus *and* A. parasiticus *and possible aflatoxin contamination of groundnuts. These practices include moistening groundnut pods in order to soften the shell for ease of handshelling. The Agricultural Development and Marketing Corporation (ADMARC) purchases shelled and graded nuts from smallholder farmers, and electronically sorts and tests the nuts for aflatoxin contamination at the Liwonde Groundnut Factory. The process of handshelling and handgrading of groundnuts by smallholder farmers, followed by re-grading, and aflatoxin testing of the nuts has earned Malawi a reputation as a source of high-quality groundnuts for the confectionery trade. Research needs to be done to incorporate resistance to* A. flavus *and* A. parasiticus *and aflatoxin contamination in the already established commercial cultivars, coupled with education for farmers on proper handling methods for processing groundnuts prior to marketing.*

Résumé

Contamination des arachides par l'aflatoxine et les stratégies de lutte au Malawi : *Au Malawi, les pluies débutent en octobre et continuent jusqu'en avril, ainsi les cultivars d'arachide à long cycle sont récoltés sous conditions sèches. Ceci permet de sécher les gousses sans grand risque d'invasion des graines par* Aspergillus flavus *et* A. parasiticus *ainsi que de contamination par les aflatoxines. En fait, la contamination par les aflatoxines ne pose pas de problème au Malawi. Cependant, certaines pratiques traditionnelles de traitement de la récolte avant la vente créent des conditions qui favorisent le développement rapide de ces microorganismes et une contamination éventuelle par les aflatoxines Par exemple, les gousses sont humectées afin d'assouplir la coque pour le décorticage manuel. L'Agricultural Development and Marketing Corporation (ADMARC) achète les arachides décortiquées et triées auprès des paysans, et les soumet à un triage électronique pour éliminer les arachides contaminées et à des analyses dans son usine de Liwonde. Ce processus de décorticage et de triage manuels par les paysans suivi d'un tri supplémentaire et des analyses des aflatoxines a valu au Malawi une bonne réputation pour ses arachides de confiserie. Il faut des recherches permettant d'incorporer la résistance à* A. flavus *et à* A.

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parasiticus *ainsi qu'à la contamination par les aflatoxines dans les cultivars commerciaux. Il convient également de former les paysans aux méthodes éfficaces de traitement des arachides avant la vente.*

Resúmene

La contaminación del cacahuate con aflatoxinas : Estrategias para para combatirla en Malawi : *En Malawi, las lluvias empiezan en octubre y terminan en abril, de manera que los cultivares de cacahuate de largo ciclo vegetativo se cosechan bajo condiciones de estiaje. Estas condiciones favorecen el rápido secado en el período postcosecha de las vainas, limitándose consecuentemente la oportunidad de invasión de las semillas por* Aspergillus flavus *y* A. parasiticus *y su posible contaminación con aflatoxinas. La contaminación con aflatoxinas de los cacahuates no es problema en este país. Sin embargo, algunas de las prácticas seguidas por los agricultores en pequeños predios, en el procesado de los cacahuates previo a su venta, crean condiciones que favorecen el rápido desarrollo de* A. flavus *y* A. parasiticus *y su posible contaminación con aflatoxinas. Estas prácticas incluyen el humedecimiento de las vainas de los cacahuates, para ablandar las cáscaras y facilitar la remoción de las mismas en forma manual. La Empresa de Desarrollo y Comercialización Agrícolas (Agricultural Development and Marketing Corporation, ADMARC) compra cacahuates descascarados y clasificados por los agricultores de pequeños predios, para a continuación reclasificar los mismos electrónicamente y efectuar análisis de su contenido de aflatoxinas, en la planta cacahuatera de Liwonde. El proceso de descascarar y clasificar los cacahuates en forma manual por parte de los agricultores de pequeños predios, seguido por la reclasificación y análisis de su contenido de aflatoxinas, ha ganado para Malawi una buena reputación como una fuente de cacahuates de alta calidad, en la industria de fabricación de dulces. Se necesita realizar investigaciones para incorporar resistencia genética a* A. flavus *y* A. parasiticus, *y a la contaminación con aflatoxinas, en los cultivares ya comercialmente establecidos, simultáneamente con la educación de los agricultores sobre métodos adecuados de manejo durante el procesamiento de los cacahuates, previo a su envío al mercado.*

Introduction

Groundnuts (*Arachis hypogaea* L.) are a rich source of protein in Malawi where they are the fourth most important export crop after tobacco, tea, and sugar and provide 25% of the smallholder farmers' cash income. Groundnut also supplies 50% of the edible oil requirement. The groundnut tops (haulms) are a rich source of protein for cattle and other livestock when used as hay.

The main groundnut cultivars grown in Malawi are long-duration, large-seeded confectionery types. Chalimbana and the newly released cultivar Chitembana are grown in the plateau areas (>1000 m altitude) which are cool and moist in the growing season.

Mani Pintar, a variety with a variegated red and white testa, and Mawanga, a newly released cultivar with a variegated pink and white testa, are grown for oil extraction in the medium altitude areas (500–1000 m altitude) along the lake shore where it is warm and humid in the growing season.

Malimba is a small-seeded, early-maturing variety suited for hot, humid, low-altitude areas (<500 m altitude) with erratic rainfall.

The objective of the Groundnut Pathology research program is to investigate ways and means of increasing the yield and improving the quality of groundnuts through plant disease management.

Aspergillus flavus Link ex Fries and *A. parasiticus* Speare reduce the quality of groundnuts and render them unfit both for human or livestock consumption. To produce nuts of high quality it is necessary to prevent *A. flavus* and *A. parasiticus* from attacking groundnuts (Mercer and Kisyombe 1978). Emphasis so far has been placed on advising smallholder farmers to follow good cultural practices and in particular to take care to dry groundnuts soon after harvest, and in shelling and grading of groundnuts prior to marketing (Ministry of Agriculture and Natural Resources, In press).

Production, Marketing, and Export

The groundnut-growing season in Malawi begins in October and ends in April. In the main groundnut-producing areas of the country (Fig.1), where more than 70% of the crop is produced, there is no rain after April and the dry weather conditions favor rapid drying of pods. However, the disadvantage of drying in the windrow is that the nuts may dry too rapidly and the kernels become brittle. Farmers moisten their groundnut pods to make up for the excessive loss in weight and also to soften the groundnut shells so that they are easy to shell by hand. The Agricultural Development and Marketing Corporation (ADMARC) purchases kernels by weight.

Farmers are advised to refrain from moistening their nuts. The ADMARC classifiers reject all groundnuts with high moisture contents and those which have a mixture of high- and low-grade nuts. Moldy nuts are also rejected by ADMARC. When a smallholder farmer offers groundnuts with high moisture content, he is advised to dry them before selling. Groundnuts are regraded if they have a mixture of high- and low-grade nuts.

Groundnut purchases by ADMARC from smallholder farmers are divided into grade A,—the premium (high) grade; and grade B (low)—shrivelled and split nuts. Grade A nuts are destined for confectionery purposes, and B grades are used either for oil extraction or as livestock feed.

All groundnuts purchased by ADMARC are transported to the Liwonde Groundnut Factory where they are electronically sorted, and debris and other foreign matter are removed. The graded nuts are fumigated with either methyl bromide or Phostoxin® and the groundnut stacks are sprayed with pirimiphos methyl (Actellic®) prior to shipment to an importing country (Ministry of Agriculture and Natural Resources 1977).

The Aflatoxin Analysis Laboratory adjacent to the factory takes representative groundnut samples from each sack. When the aflatoxin content is high, the groundnuts in the sack are regraded and more samples are taken for analysis.

A groundnut consignment is exported only when it has no or negligible levels (below 5 $\mu g\ kg^{-1}$), of aflatoxin contamination. Generally, Malawi groundnuts have no or negligible levels of aflatoxin contamination, chiefly because the nuts are shelled and graded by hand.

Smallholder farmers are unaware that moistening unshelled groundnuts can lead to contamination of the nuts with aflatoxins. However, they are aware that moldy groundnuts result from late harvesting and improper drying, and that they lose money if the nuts are of poor quality.

Groundnut oil is used to prepare cooking oil, margarine, and soap. There is no danger of aflatoxin contamination because any aflatoxin present is removed during the oil-extraction process in modern oil mills. However, emphasis is placed on production of high quality

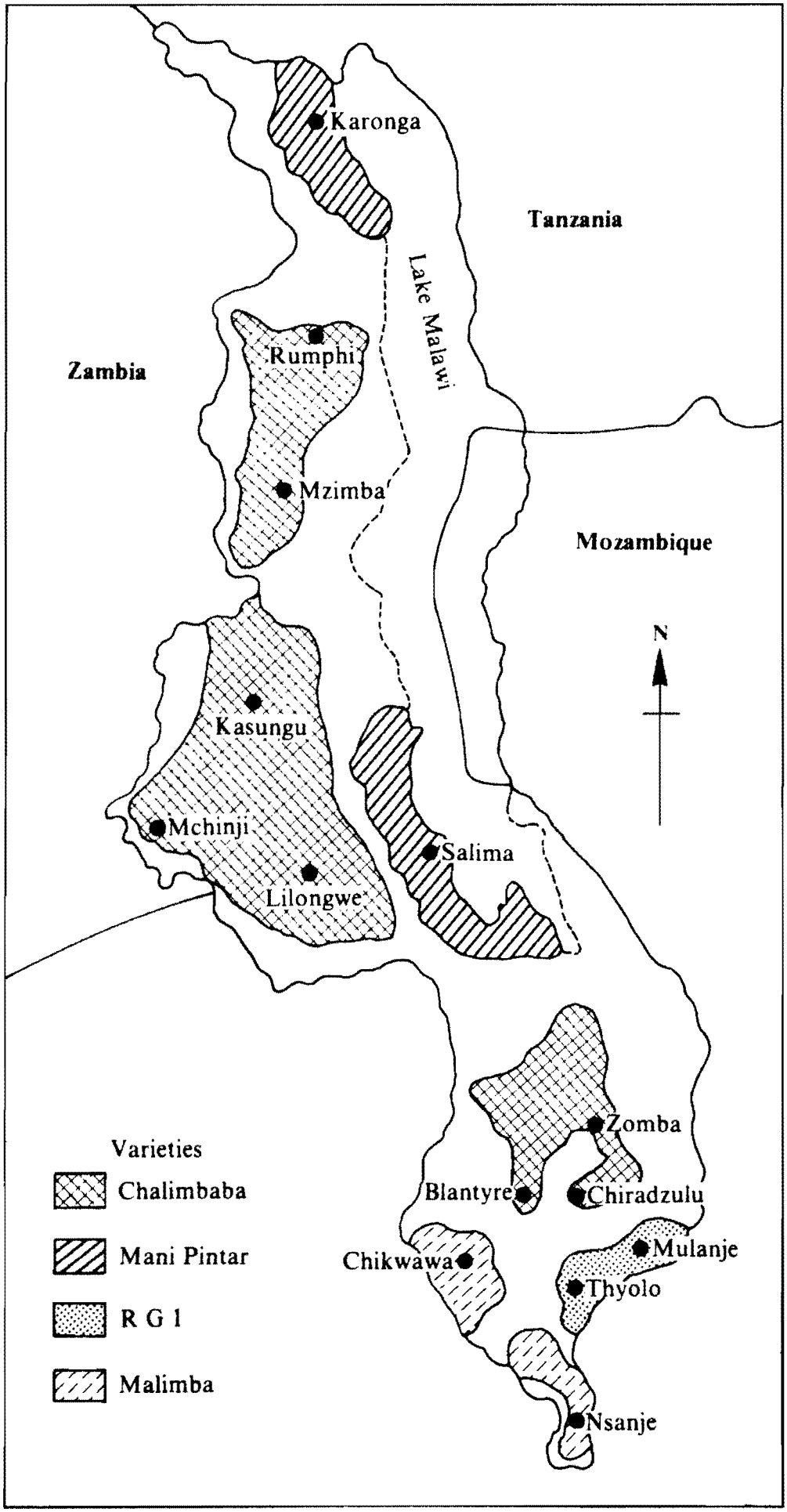

Figure 1. Areas of groundnut production and varieties grown in Malawi.

groundnuts that can be utilized as seed or for the confectionery trade (Mercer and Kisyombe 1978).

Malawi has established itself as a good source of large-seeded groundnuts suitable for the confectionery trade. Therefore, research is needed to incorporate resistance to aflatoxin contamination in these cultivars (Kisyombe et al. 1985). Smallholder farmers need to be further educated by the Agricultural Extension Services on the harmful effects of aflatoxin contamination of groundnuts.

Strategies for the Control of Aflatoxin Contamination

- Sow groundnuts with the first planting rains to optimize the use of soil moisture for growth and to avoid drought late in the growing season.
- Practice crop rotation to reduce the incidence of soilborne pathogens and possible aflatoxin contamination.
- Harvest when the majority of the pods have attained maturity.
- After lifting, dry groundnuts rapidly and store them in a clean and dry store.
- Do not moisten groundnuts prior to shelling by hand.
- Avoid using moldly groundnuts and nuts from gleanings for human consumption or livestock feed (Pettit 1984).

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Discussion

R.C. Nageswara Rao. You mentioned that SAC-58 produced the highest yield in the world and also had a high oil percentage. Can you give some figures of yield obtained and oil percentage of this variety?

C.T. Kisyombe. The local name for SAC-58 in Mawanga. It yields more than 4 000 kg (4 t), kernels ha^{-1}. The maximum oil content is 50%.

Status and Management of Aflatoxin in Groundnuts in Nigeria

S.K. Manzo and S.M. Misari[1]

Abstract

A committee to coordinate action on the aflatoxin problem in Nigeria was constituted in 1961 with representatives from four ministries, the Institute for Agricultural Research (IAR), Zaria, the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigeria Marketing Board. This committee was charged with the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards its elimination. IAR was to investigate the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI was to look at the problem from the time of storage until produce was exported or consumed. NSPRI, therefore, routinely monitored groundnuts in storage pyramids to determine aflatoxin levels before export. Meanwhile, IAR investigated the time of invasion of groundnut kernels by Aspergillus flavus, *and when, and under what conditions it produced aflatoxins. An interplay of temperature, relative humidity, drought, and erratic rainfall situations and maturity of the crop at lifting was found to affect seed invasion by* A. flavus *and aflatoxin contamination of groundnut in the field and store. In the wetter areas of the Southern Guinea Savanna which have long rainy seasons, aflatoxin contamination of groundnuts is mainly a postharvest problem; whereas in the major groundnut-growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is more important preharvest. Insect infestations and wetting of stored groundnuts increase aflatoxin contamination.*

Research information from IAR and NSPRI still provides the basis for recommendations on the handling of groundnut to minimize or prevent aflatoxin contamination. Vegetable oil and feed mill companies routinely submit their groundnut and other feed materials for aflatoxin analysis as there is great awareness among the companies, people, and governments of Nigeria of the dangers posed by aflatoxin to poultry, livestock, and humans. Nigeria is a consignatory to the African Groundnut Council's resolution to export only groundnuts whose aflatoxin content does not exceed the maximum permissible limit of 200 $\mu g\ kg^{-1}$ for animal feed set by the European Economic Community (EEC).

None of the commercially grown groundnut cultivars in Nigeria is resistant to A. flavus *invasion and aflatoxin contamination of seeds. Breeding materials from both domestic and exotic sources are being screened for resistance while other improved management practices are being used or researched.*

Résumé

Problème et maîtrise des aflatoxines au Nigéria : *En 1961, un comité a été créé pour coordonner les actions de lutte contre le problème des aflatoxines au Nigéria. Ce comité regroupe des*

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représentants de quatre ministères, et des organismes de recherche et de commercialisation dont : l'Institute for Agricultural Research (IAR) de Zaria, le Nigerian Stored Products Research Institute (NSPRI), et le Northern Nigeria Marketing Board. Ce comité a la responsabilité d'évaluer l'importance du problème d'aflatoxines des arachides et d'initier les actions visant à son élimination ainsi que de les coordonner. l'IAR a été chargé d'étudier la contamination depuis la culture, jusqu'au stade où la production est vendue par les paysans. Le NSPRI prend le relai en examinant le problème depuis le stockage jusqu'à l'exportation ou la consommation. A cette fin, le NSPRI a effectué des contrôles systématiques afin de déterminer le niveau d'aflatoxine dans les arachides stockées en pyramides avant l'exportation. L'IAR a étudié l'époque d'invasion des graines d'arachide par Aspergillus flavus, *d'une part, et les conditions ainsi que le moment de la production des aflatoxines, d'autre part. L'interaction des différents éléments tels que la température, l'humidité relative, la sécheresse et l'irrégularité des pluies ainsi que la maturité des plantes à la récolte excerce une influence sur l'invasion par* A. flavus *et la contamination par les aflatoxines des arachides aux champs et stockées. Dans les régions plus humides des savanes sud-guinéennes à longue saison des pluies, la contamination pose plus de problèmes après la récolte, tandis que dans les savanes nord-guinéennes et soudanaises plus sèches le problème se produit avant la récolte.*

L'infestation par les insectes et l'humectation des arachides stockées favorisent la contamination par les aflatoxines.

Les acquis des recherches effectuées par l'IAR et le NSPRI ont permis de formuler les recommendations sur le conditionnement des arachides afin de minimiser ou d'empêcher la contamination. Les fabriques d'huile et de tourteaux soumettent régulièrement leurs arachides et autres produits aux analyses des aflatoxines, puisque les sociétés, la population, et le gouvernement du Nigéria sont de plus en plus sensibilisés aux dangers encourus par la volaille, les animaux et l'homme. Le Nigéria est un cosignataire de la résolution ratifiée par le Conseil africain de l'arachide, d'exporter uniquement les arachides dont la teneur en aflatoxines ne dépasse pas la limite autorisée de 200 $\mu g\ kg^{-1}$ imposée par la CEE.

Toutes les variétés commerciales d'arachide au Nigéria sont sensibles à l'invasion par A. flavus *et à la contamination par les aflatoxines. Le matériel en sélection obtenu de sources locales et exotiques est évalué pour la résistance. D'autres pratiques culturales améliorées sont également retenues ou à l'étude.*

Resúmene

La situación actual y control de aflatoxinas en el cacahuate en Nigeria : *En 1961 se constituyò un comité para coordinar las acciones sobre el problema de las aflatoxinas en Nigeria, con representantes de cuatro ministerios, el Instituto de Investigaciones Agrícolas (IAR), el Instituto Nigeriano de Investigación sobre los Productos Almacenados (NSPRI) y el Consejo de Comercialización del Norte de Nigeria. A este comité se le asignó la responsabilidad de evaluar la magnitud del problema de las aflatoxinas en cacahuates en el país, y de iniciar y coordinar acciones conducentes a su eliminación. El IAR debía investigar la contaminación con aflatoxinas de la cosecha de cacahuates hasta el momento en que el producto era vendido por los agricultores, mientras que el NSPRI debía estudiar el problema desde el momento de iniciarse el almacenamiento hasta que el producto se exportara o se consumiera. El NSPRI, por lo tanto, vigilaba de una manera continua las estibas de almacenamiento, para determinar los niveles de aflatoxinas antes de la exportación del producto. Mientras tanto, el IAR investigaba la velocidad de invasión de los granos de cacahuate por* Aspergillus flavus *y cuándo ocurría esto, así como bajo qué condiciones se producían las aflatoxinas. Se encontró que las interacciones entre temperatura, humedad relativa, sequía, situaciones de precipitación errática y la madurez del cultivo en el momento de la cosecha, afectan la intensidad de la invasión de la semilla por* A. flavus *y la contaminación con aflatoxinas tanto en el campo, como en los almacenes. En las partes más*

húmedas de la sabana en el sur de Guinea, que tiene temporadas lluviosas prolongadas, la contaminación con aflatoxinas en los cacahuates es principalmente un problema después de la cosecha, mientras que en las principales áreas productoras que están situadas en las regiones más secas del norte de Guinea y la sabana sudanense, el problema generalmente es más agudo antes de la cosecha. Las infestaciones con insectos y el humedecimiento de los cacahuates almacenados aumentan la contaminación con aflatoxinas.

La información derivada de las investigaciones del IAR y del NSPRI continuan proporcionando las bases para las recomendaciones sobre el manejo de los cacahuates, para minimizar o evitar la contaminación con aflatoxinas. Las compañías fabricantes de aceite vegetal y torta alimenticia para el ganado, regularmente envían muestras de sus cacahuates y otros materiales alimenticios para el ganado, para la determinación de sus contenidos de aflatoxinas, en vista de que existe plena conciencia entre las compañías, personas y gobiernos de Nigeria, de los peligros que constituyen las aflatoxinas para las aves, el ganado y los humanos. Nigeria es uno de los cosignatarios de la resolución del Consejo Africano del Cacahuate, de exportar solamente cacahuates cuyo contenido de aflatoxinas no excede el límite permisible de 200 $\mu g\ kg^{-1}$, fijado por la Comunidad Económica Europea (CEE). Ninguno de los cultivares de cacahuate sembrados comercialmente en Nigeria es resistente a las invasiones de A. flavus *y la contaminación de las semillas con aflatoxinas. Materiales genéticos, tanto de fuentes domésticas como exóticas, se están probando para determinar su resistencia a* A. flavus, *y mientras se utilizan o se investigan otras prácticas mejoradas de manejo del cultivo.*

Introduction

Nigeria lies approximately between latitudes 49°N and 14°N and longitudes 3°E and 14°E providing a range of climatic conditions and allowing great variation in crops and agricultural practices in the different zones. Variations exist in the total amount and the spread of rainfall and the duration of the rainy season (Table 1). The coastal swamps and forest areas receive the highest rainfall (1550–4100 mm $annum^{-1}$ and have the longest rainy season (250 days). This is followed by the central areas of the country which constitute the Southern (6–8°N) and Northern (8–11°N) Guinea Savannahs. North of the Northern Guinea Savannah (10–13°N) lies the Sudan Savannah. In the northeastern corner (12–14°N) of the country lies the Sahel Savannah.

Table 1. Amount of rainfall and duration of rainy season in various ecological zones of Nigeria.

Ecological zone	Latitudes °N	Total annual rainfall (mm)	Extent of rainy season	Length of rainy season (days)
Sahel Savannah	12–14	< 500	Jun–mid Sep	< 90
Sudan Savannah	10–13	500– 900	May/Jun–Sep/Oct	90–130
Northern Guinea Savannah	8–11	900–1400	mid Apr–mid Oct	130–190
Southern Guinea Savannah	6– 8	1000–1650	Apr–Oct	190–250
Rain forest	5– 7	1550–2550	Mar–Nov	250
Coastal swamps	4– 6	2300–4100	Mar–Nov	250

In Nigeria, groundnut is grown as far south as the Southern Guinea Savannah and as far north as the Sahel Savannah. Sowing dates are determined by the onset of the rains (April to mid-June from south to north of the country), but sowing may be delayed because of priority being given to cereals (for food) over groundnuts. Early sowing is desirable so that the crop can mature and be harvested before the end of the rainy season whilst the soil is still moist. Because of the unpredictable nature of the rainy season it is not always possible to avoid rain falling on groundnuts that have been lifted and windrowed in the field, this situation being most common in the Southern Guinea Savannah. Such unpredictable conditions expose the lifted groundnuts to infection by *Aspergillus flavus* Link ex Fries and hence increase the chances for aflatoxin contamination. Peasant farmers cannot afford sophisticated or costly drying facilities and depend on the sun and wind to dry their harvested groundnuts in the windrows or on mats. In the Southern Guinea Savannah area where there is considerable cloud cover at harvest time, it is not always possible to dry groundnuts naturally and rapidly so the risks of aflatoxin contamination are higher than in the drier Sudan and Northern Guinea Savannahs where the bulk of Nigeria's groundnuts are produced. Late sowing exposes the crop to the aphid vectors of groundnut rosette virus disease, and requires harvesting long after the rains have ceased. This results in yield losses due to rosette, poor pod fill, termite attack, and difficulties in lifting from dry, caked soils.

Early sowing has been recommended to farmers as a measure to minimize infection of groundnut by the groundnut rosette virus disease. While this may solve the problem of rosette, it increases the risk of the groundnut maturing before the rains stop, which leads to drying problems and a greater risk of *A. flavus* infection and aflatoxin contamination of the seed.

Groundnut is a very important food and cash crop, especially in the northern parts of Nigeria. Before the early 1970s, Nigeria was a major exporter of groundnuts, especially to western Europe. The government of Northern Nigeria and the entire Federation relied very heavily on groundnut as a major source of foreign exchange (Table 2). Anything that affected the groundnut industry was considered a major threat to the growth and survival of the country's economy and appropriate action had to be promptly taken to avert that threat.

Awareness of the Aflatoxin Problem in Nigeria

A team from the Tropical Products Institute, London, UK, visited Nigeria in 1961 to alert the nation and those concerned with the production and export of groundnuts to the threat posed by aflatoxin, a toxin produced by the fungus *A. flavus*. The government of the then Northern Nigeria immediately set up a committee consisting of representatives from the Ministries of Agriculture, Health, Animal and Forest Resources, and Economic Planning. Included in this committee were representatives from the Institute for Agricultural Research (IAR), Samaru, the Nigerian Stored Products Research Institute (NSPRI) and the Northern Nigeria Marketing Board (NNMB). The committee's terms of reference were to assess the extent of the aflatoxin problem in the country, and to guide and coordinate action aimed at its elimination. The IAR, Samaru was assigned the responsibility of investigating the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI was to investigate the problem from the time of storage until the produce was exported or consumed.

Fungal Contamination before Maturity and Lifting

Although *A. flavus* and related species are ubiquitous in groundnut-growing areas throughout the world, little was known in Nigeria about field and storage contamination of groundnuts, the

Table 2. Contribution of groundnuts to Nigeria's foreign trade, 1961-1978[1].

Year	Total value of exports (Nm)[2]	Value of groundnut products exported (Nm)	Percentage of groundnut export
1961	348.0	74.4	21.0
1962	337.2	77.2	22.0
1963	379.2	56.4	22.8
1964	429.2	94.0	21.9
1965	536.8	106.0	19.8
1966	568.2	110.3	19.4
1967	483.6	93.6	21.6
1968	422.2	104.6	24.8
1969	636.3	103.4	16.3
1970	885.4	77.8	8.8
1971	1293.3	38.8	3.0
1972	1434.2	35.9	2.5
1973	2278.4	86.8	3.8
1974	5794.8	22.2	0.4
1975	4929.3	0.8	0.01
1976	6751.1	3.6	0.05
1977	8673.5	1.9	0.02
1978	6064.4	-	0.00

1. Source: Federal Office of Statistics 1967, 1975; Central Bank of Nigeria, 1979.
2. Nm = million Nira.

conditions that encourage infection by *A. flavus*, or the production of aflatoxin, before the reports by Lancaster et al. (1961) and Sergeant et al. (1961). Initial research at IAR centred on field infection of groundnut by *A. flavus* and the conditions that favored the infection and the production of aflatoxin from pod development through maturity, harvesting, and drying of the nuts. Scientists in the 1960s and 1970s established that several interrelated factors including soil moisture, temperature and relative humidity strongly influenced *A. flavus* infection and development in groundnut pods before and after harvest (McDonald and Harkness 1964, 1965, 1967).

It was found that the developing groundnut fruit (pod) exerted a strong geocarposphere effect; *Penicillium funiculosum* Thom and *A. flavus* were abundant in the geocarposphere, especially at 15 and 17 weeks after sowing, respectively. As many as 10^4 propagules g^{-1} of soil were reported from a study of soil populations of *A. flavus*. The percentages of *A. flavus* of the mycoflorae of groundnut fields and geocarposphere soil were highest 14–18 weeks after sowing, i.e., during the later stages of pod development (McDonald 1969, 1970a, 1976).

Infection of developing pods by *A. flavus* is not common in Nigeria (McDonald 1970a). However, from 90 days after sowing (DAS) to maturity, *A. flavus* was isolated weekly from 4–14% of the groundnut shells. Only about 1% of the seed was infected by *A. flavus* during this period.

Invasion of groundnut pods and seed by *A. flavus* and other fungi usually occurred during curing when the crop was lifted near maturity (McDonald and Harkness 1964).

Fungal Contamination at Maturity and Lifting

Groundnut pods and seeds were usually free of *A. flavus* infection and aflatoxin contamination at maturity except when the pods were damaged either mechanically or by soil arthropods (McDonald 1964, 1976). Following lifting and windrow curing, there was an increase in seed invasion. Seeds from pods picked shortly after lifting and sundried on mats showed little increase in fungal infection during drying. Pods left in the ground after reaching maturity showed a gradual increase in fungal invasion of the seeds. *A. flavus, Macrophomina phaseolina* (Tassi) Goid, *Penicillium* spp, and *Fusarium* spp were the predominant fungi in shells and seeds, with *A. flavus* being the most common (McDonald 1970a, 1970b, Diener 1973, Diener et al. 1982).

Even though shells were commonly infested by fungi at normal maturity and lifting, little or no *A. flavus* was found in seed from intact pods (McDonald 1970a, 1970b). When groundnuts are grown under drought stressed conditions however, a relatively higher incidence of *A. flavus* infection occurred as the pods approached maturity within the soil (McDonald 1970a). This phenomenon has been confirmed by other workers in the USA (Dickens et al. 1973, Davidson et al. 1983).

Fungal Contamination after Lifting

Once the groundnut crop is mature, it should be lifted without delay in order to avoid further preharvest development of mycotoxigenic fungi in the pods.

Data from field trials over several years showed that except in broken pods *A. flavus* did not infect the kernels and aflatoxin did not appear in the groundnut until at least 6 days after lifting (McDonald 1966, 1970b). It was suggested (McDonald 1966) that seeds of groundnut pods still attached to the haulms have higher moisture contents than seeds in detached pods and so have some resistance to penetration by *A. flavus*; but as the seed moisture content drops below a particular level, and the seed has passed into a low metabolic state, the resistance decreases. The death of the testa during curing and drying may also contribute to this loss of resistance to seed infection. Seeds in broken and insect-damaged pods are directly exposed to invasion by *A. flavus* and other soil fungi (McDonald and Harkness 1965, McDonald 1970b, 1976). The quicker natural drying was achieved after lifting from the soil, the less was the *A. flavus* infection and aflatoxin contamination and vice versa. An increase in contamination with *A. flavus* and other fungi occurred with passage of time after lifting, the rate of increase being higher in seeds from broken pods (McDonald and Harkness 1964). Table 3 summarizes the effect of damage to the groundnut pods on *A. flavus* and other fungal invasion of the seeds from lifting to drying.

A close look at the relationship of time to aflatoxin formation in groundnuts after lifting has also revealed that groundnuts free of the toxin at digging contained detectable toxin within 48 h (Bampton 1963). McDonald and A'Brook (1963) found that artificial drying after 4–6 days of windrow drying in the field resulted in toxin-free seed, but samples left for 8–12 days in windrows before artificial drying, or sundried for 10–16 days, were moderately contaminated by aflatoxin (25–500 $\mu g\ kg^{-1}$). In other studies, McDonald and Harkness (1964) found that contamination of seed with *A. flavus* and aflatoxin did not occur until at least 5–6 days after lifting. They also reported (McDonald and Harkness 1965) more *A. flavus* and other fungi in seed from slowly dried pods attached to plants than from rapidly dried pods (Table 4).

Table 3. Number of groundnut seeds per 100-seed sample contaminated with *Aspergillus flavus* and other fungi from lifting through drying.

	Fungal contamination of kernels							
	At lifting[2]		At picking		Sundried		Artificially dried	
Pod grade[1]	A.f.	O.f.	A.f.	O.f.	A.f.	O.f.	A.f.	O.f.
PU	0	0	0	1	1	7	23	8
PT	0	1	0	5	3	19	26	12
PB	0	15	0	23	9	36	43	18
GU	0	3	0	2	0	13	0	20
GT	0	3	0	7	0	20	4	32
GB	0	5	0	18	3	35	7	40
PU	0	0	0	1	1	6	14	31
PT	0	1	0	11	0	10	2	24
PB	0	12	0	11	4	32	29	41
GU	0	1	0	2	0	0	0	51
GT	0	3	0	2	0	4	8	31
GB	0	1	0	5	2	15	17	39

1. P = pickings, U = undamaged pods, B = broken pods, G = Gleanings, T = termite damaged pods.
2. A.f. = *Aspergillus flavus*, O.f. = other fungi.
Source: McDonald and Harkness 1964.

Table 4. *Aspergillus flavus* contamination of groundnut seed samples at different times from lifting expressed as percentages of seeds infested.

	Days after lifting										
Pod damage grades[1]	0	3	4	5	6	11	12	14	20	21	24
U	0	0	0	0	0	0	1	0	0.5	0.5	0.5
T	0	0	0	0.3	0	0.5	2	0.5	1	0	1.5
B	0	0	0	0	0	0.5	3.5	1.5	8	3	6

1. U = undamaged pods, T = termite-damaged pods, B = broken pods.
Source: McDonald 1965.

Effect of Pod Moisture Content and Weather Conditions on Aflatoxin Contamination in the Field after Lifting

High solar radiation and temperature, and low relative humidity have a profound effect on endogeocarpic microflora present in fresh mature pods. At lifting, pod and kernel moisture can range from as high as 48% to below 15% when drying occurs within the soil prior to harvest (Pettit 1986). McDonald and Harkness (1963) found that when groundnuts with high moisture contents are lifted and placed in windrows on the soil surface, rapid invasion of the kernels by *A. flavus* and subsequent aflatoxin contamination can occur. This finding has been confirmed

by other workers (Bampton 1963, McDonald and A'Brook 1963, Burrell et al. 1964). Rapid fluctuations in seed moisture content during drying in windrows can result in aflatoxin contamination, especially when rain falls on partially dried groundnuts (Burrell et al. 1964, Troger et al. 1970). Rewetting groundnuts after drying greatly increases the susceptibility of seeds to *A. flavus* infection and aflatoxin contamination (McDonald 1976, IAR 1984).

Effect of Pest Damage to Pods and Seeds on Contamination by Toxigenic Fungi

Insects and millipedes may damage groundnut pods prior to lifting and so predispose them to invasion by *A. flavus* (Raheja 1975, Misari et al. 1980). Pod-scarifying termites such as *Amitermes* spp, *Microtermes* spp, and *Odontotermes* spp weaken the pods and render them vulnerable to invasion by *A. flavus* and other fungi (Feakin 1973). Johnson and Gumel (1981) reported that pod scarification by *Microtermes lepidus* was more frequent in the drier zones of the Sudan Savannah than in the wetter Southern Guinea Savannah zones. Pod scarification was also more common in dead plants (Perry 1967). Examination of market samples of groundnuts showed that the incidence of scarified pods rarely exceeded 5%, but that more than 85% of the seeds from scarified pods were infected by fungi (Johnson and Gumel 1981).

Groundnuts are attacked by about 48 insect species during storage in Nigeria (Cornes 1973). Agboola and Opadokun (1982) reported that the increase in temperature and moisture content of stored groundnuts brought about by insect infestation could predispose them to fungal attack. About 31 species of fungi including *A. flavus* have been isolated from stored groundnuts (Broadbent et al. 1969). This calls for effective pest control in stored groundnuts.

Varietal Resistance and Aflatoxin Contamination

McDonald and Harkness (1963) carried out a survey on the 1960 groundnut crop to determine the extent of toxicity in the cured and dried produce from various locations in Northern Nigeria. There was no definite evidence of any varietal differences and although locations varied considerably in numbers of toxin-contaminated samples and degree of toxicity, this may have been due to different curing and drying conditions. The condition of the shell (undamaged, termite-damaged, or broken), as determined at lifting, was found to have a marked influence on seed infection by *A. flavus* and other fungi, and on aflatoxin contamination.

Attempts were made to screen varieties in both the laboratory and the field for pod or seed resistance to invasion by *A. flavus* , and to development of aflatoxin. Early trials (McDonald 1966) were not successful in identifying good sources of resistance. All lines tested were found to be susceptible to attack by *A. flavus* but there were some varietal differences due to testa pigments and possibly other factors. Of the short-duration varieties Spanish 205 and Natal Common had the least kernel infection by *A. flavus* and other fungi, while of the long-duration varieties, 48-14 and Samaru 38 were the least infected. Resistance to seed infection by toxigenic fungi has been linked to specific features of the testa and pod wall that retard the penetration of *Aspergillus* spp and other fungi. Research in Nigeria has shown that groundnut testae containing high levels of tannins tended to inhibit the growth of *A. flavus* , but that once the fungus established itself this inhibitory effect was overcome (Carter 1970).

Although groundnut varieties resistant to *A. flavus* invasion and aflatoxin formation potentially afford the most effective and economical means of control, the degree of success in developing resistant commercial cultivars has not been encouraging (Diener et al. 1982). The

sources of resistance developed at ICRISAT in India may provide an answer to the problem. Several *A. flavus*-resistant germplasm lines have been received from ICRISAT and are currently being evaluated in Nigeria. Some entries show promising levels of resistance to locally occurring aflatoxin-producing strains of *A. flavus*. Further investigations are under way. Some of these accessions will be utilized in breeding programs aimed at developing agronomically acceptable *A. flavus*-resistant cultivars for Nigerian groundnut-growing areas.

Aflatoxin Contamination as a Function of Postharvest Quality and Handling of Groundnuts

Long before the discovery of aflatoxin contamination of groundnuts by *A. flavus*, the Produce Inspection Laws of Nigeria had already stipulated definite physical quality standards for exportable grades of groundnuts. This followed the conclusive demonstration that sound, whole, mature groundnut seeds have a higher quality, and are less easily attacked by pests than are defective, broken, or otherwise damaged seeds. "Special grade" groundnut has a whole-seed content of at least 70%, while a whole-seed content as low as 65% was considered "subgrade". Groundnut with less than 65% whole-seed content was rejected and given a "no grade" mark. The maximum permissible content of mineral matter is 0.2%, and of total impurities is 1%. A "moldy" nut content of greater than 0.5% is not acceptable.

Halliday (1965) reported that moisture content at the time of purchase in the northern groundnut-producing areas of Nigeria is normally low and therefore, the danger of *A. flavus* growth between postharvest drying and receipt into storage is negligible. This was reaffirmed by Broadbent et al. (1969) who reported that safe storage at a moisture content of 3-4% could be achieved in the drier areas of the north. In the wetter riverine areas groundnuts are harvested under rainy conditions. The moisture content at purchase is very much higher, and the danger of *A. flavus* growth before receipt into storage is consequently higher than in the drier north. It is estimated that about 50% of the burlap bags of groundnuts at the buying stations of the drier north contain less than 50 $\mu g\ kg^{-1}$ aflatoxin when bagged. Only about 15-20% of the bags contain aflatoxin in excess of 250 $\mu g\ kg^{-1}$. The level and incidence of aflatoxin in groundnuts in the riverine areas is very much higher. A cheap crop drier suitable for the small farmer in these areas has been developed (A'Brook 1963, 1964, 1965, McDonald and A'Brook 1963).

Public Health Implications of Aflatoxin in Groundnuts

Many staple foods and feeds in Nigeria are usually contaminated with mycotoxins (McDonald 1976, Nwokolo and Okonkwo 1978). Groundnut and groundnut products are among the high-risk foods because of their susceptibility to contamination by aflatoxin. NSPRI revealed that levels of aflatoxin in groundnut cake from the Kano oil mills were low, averaging 250 $\mu g\ kg^{-1}$ with a range of 50 to 1000 $\mu g\ kg^{-1}$. Higher values up to 380 $\mu g\ kg^{-1}$ were however recorded in the 1973 drought year (McDonald 1976). The aflatoxin B_1 levels in groundnut cake ranged from 600 to 1100 $\mu g\ kg^{-1}$, and in crude groundnut oil from 200 to 500 $\mu g\ kg^{-1}$ (Nwokolo and Okonkwe 1978), showing that the bulk of the aflatoxin remains in the cake and only a small amount gets into the oil. The level of aflatoxin B_1 was found to be approximately four times that of B_2 in some Nigerian groundnuts (Opadokun 1978). Abalaka (1984) recorded higher levels of the aflatoxins B_1 and B_2 than the aflatoxins G_1 and G_2 in virtually all products and by-products of groundnut from edible oil-extracting plants and poultry feed mills. The aflatoxin levels in poultry feeds

produced in the mills were found to be relatively low, but were considered sufficient to constitute a health hazard.

Aflatoxins may work synergistically with other carcinogens to produce the high incidence of primary liver cancer in men under the age of 40 in Nigeria (Nwokolo and Okonkwo 1978). In the Guinea Savannah of Nigeria, there is a high incidence of primary liver cell carcinoma (PLCC) (Anthony 1977, Fakunle et al. 1977). Recent studies in Nigeria revealed high levels of aflatoxins B_1, B_2, G_1, and G_2 in sera of patients with PLCC (Onyemelukwe and Ogbadu 1981, Onyemelukwe et al. 1982). The contribution of foods and feeds to the total aflatoxin load in human patients has not been conclusively investigated. However, Abalaka and Eronini (1987) detected as much as 120–510 $\mu g\ kg^{-1}$ of aflatoxins B_1, B_2, G_1, and G_2 in marketed meat samples in Zaria. This is in excess of the 30 $\mu g\ kg^{-1}$ limit fixed by the World Health Organization (1979), which categorizes these samples as unfit for human consumption. Since the animal protein intake of Nigerians is on the increase, and since meat derived from animals fed on materials susceptible to mycotoxin contamination forms a major bulk of this protein, studies on the levels of aflatoxin in tissues and organs of animals could be helpful in determining the sources of aflatoxin in sera of patients with PLCC. The risk is high in animals maintained on feeds formulated from mycotoxin-contaminated ingredients (Abalaka and Elegbede 1982, Abalaka 1984). Though the incidence of liver cancer in a number of countries has been epidemiologically correlated with mycotoxin contamination of foods (Linsell and Peers 1977, Krishnamachari et al. 1975), and because aflatoxin produces experimental liver tumors in laboratory animals (Lancaster 1968) the 120–510 $\mu g\ kg^{-1}$ levels recorded in market meat samples constitute a significant health hazard to consumers (Abalaka and Eronini 1987). Earlier investigations by Abalaka and Elegbede (1982) and Abalaka (1984) revealed that edible oils (including groundnut) were significantly contaminated with aflatoxins. Though no direct evidence exists to show that small amounts of aflatoxin constitute a health hazard to man, regular ingestion of minute doses of such potent carcinogens from different sources predisposes individuals to other infections or modification of immune systems in favor of hepatitis B virus (HBV) (Lutwich 1975).

Recently kwashiorkor and marasmus have been linked directly to high aflatoxin levels normally detected in children with such conditions (personal communication from Dr G. Ogbadu, Biochemistry Department, Ahmadu Bello University, Zaria). Naturally occuring aflatoxins have been found in Nigerian cowpeas, soyabean, millet, maize, rice, sorghum, and cassava (Bassir and Bababunmi 1971, McDonald 1976, Nwakolo and Okonkwo 1978, Elegbede 1978, Dada 1979, Opadokun et al. 1980, Nduka and Ogbadu 1980, Salifu 1981, Gbodi et al. 1986), and in milk (Opadokun et al. 1980). The fact that weaning cereal foods are often fortified with milk powder, and soyabean and groundnut flours calls for caution on the parts of food processors, nutritionists, and the general public.

Management of Aflatoxin in Groundnut

The following methods that are simple and capable of being understood and applied by the Nigerian farmer have evolved from cooperative research efforts in the country.

Groundnut plants should be harvested as soon as the crop is mature and care should be taken to avoid damaging the pods during lifting and handling in the field. Broken, damaged, and rotten pods should be removed and destroyed. When groundnuts are decorticated before sale, attempts should be made to sort out and destroy discolored and moldy seeds. In areas where the crop is harvested under wet conditions, the pods should be picked from the plants within 3 days of lifting and sundried to less than 7% moisture content in shallow layers on mats or on other

suitable materials. Alternatively, the pods could be picked and then artificially dried. Dried material should be stored carefully and kept dry and free from insect infection and rodent attack. Clean, dry, disinfested vehicles adequately protected against accidental wetting should be used to transport the crop.

The high-technology methods of aflatoxin detoxification have not been fully adopted by the Nigerian oil mills and food processors, but research work on irradiation (Ogbadu 1979, Ogbadu and Bassir 1979, Ogbadu 1980, 1981) and chemical detoxification (Abalaka 1985) has shown some promise.

As a consignatory to the African Groundnut Council's resolution to produce and/or export only groundnut with aflatoxin content not exceeding the maximum permissible limit set for intra-African trade or the European Economic Community, Nigeria instituted a campaign in 1964 to inform farmers about the aflatoxin problem. This has yielded positive results, due to implementation of the above recommendations which have remained unchanged and only revalidated by later research findings.

Prospects of Aflatoxin Research in Nigeria

In view of the importance of groundnut and the potential hazards to man and animals posed by aflatoxin, it is clear that Nigeria should continue research on aflatoxin, including the routine monitoring of aflatoxin contamination of groundnut and its products. Research on aflatoxin will not be limited to groundnuts but will extend to other crops, especially those used for food and animal feeds. Limitations in research facilities because of lack of funds currently hinder research on aflatoxins and it is hoped that as soon as Nigeria fully recovers from its present economic depression, normal research activities will resume.

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Groundnut Aflatoxin Problems in Tanzania

M.D. Raya[1]

Abstract

Groundnuts are grown in most parts of Tanzania but the bulk of the crop is grown in the southeast of the country. The crop is exclusively grown by small-scale farmers mainly for local markets. Groundnut research in Tanzania started at Nachingwea in the late 1940s. In early 1970 with assistance from the Overseas Development Administration (ODA), UK, groundnut research work was transferred to Naliendele, Mtwara in southeast Tanzania. Apart from a little research at Sokoine University of Agriculture, Morogoro, most of the research work on groundnut breeding, agronomy, and crop protection is done at Naliendele.

Earlier efforts on crop protection were devoted to foliage diseases and insects. Recently it was realized that aflatoxin contamination of groundnut was one of the major factors reducing groundnut quality in the country. The National Groundnut Improvement Program has decided to start research on the problem to develop effective control measures.

Résumé

Problème des aflatoxines des arachides en Tanzanie : *L'arachide est cultivée dans la plupart des régions en Tanzanie, mais surtout dans le sud-est du pays. Il s'agit d'une production paysanne qui est destinée aux marchés locaux. La recherche sur les arachides a débuté vers la fin des années 40 à Nachingwea. Au début de 1970 cette opération a été transférée à Naliendele (Mtwara) dans le sud-est, grâce à l'appui de l'Overseas Development Authority (ODA) en Grande-Bretagne. Désormais la plus grande partie des recherches sur l'arachide dans les domaines de la sélection, de l'agronomie et de la défense des cultures est entreprise à Naliendele, à l'exception de certains travaux qui sont conduits à l'Université de Sokoine (Morogoro).*

Les premières recherches sur la défense des cultures ont porté sur les maladies foliaires et les insectes. La contamination par les aflatoxines a récemment retenu l'attention du fait de la réduction de qualité de la production arachidière du pays. Le National Groundnut Improvement Program a entamé des recherches poussées visant à élaborer des méthodes de lutte efficaces.

La plupart des recherches nationales conduites auparavant étaient destinées aux luttes contre les maladies foliaires et aux insectes ravageurs. Ce n'est que très récemment qu'on s'est rendu compte de l'importance de la contamination des arachides par les aflatoxines comme un facteur limitant la qualité arachidière du pays. Le Programme national d'amélioration de l'arachide a donc décidé d'initier des recherches approfondies sur ce problème afin d'élaborer des stratégies de lutte efficaces. A cette fin, les concours de l'ICRISAT et du Programme d'appui à la recherche collaborative sur l'arachide (CRSP) seront bien accueillis.

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Problemas de aflatoxinas en el cacahuate en Tanzania : *Los cacahuates se siembran en casi todas partes de Tanzania, pero la mayor parte del cultivo está sembrado en la región sureste del país. El cultivo es sembrado exclusivamente por agricultores con pequeños predios, principalmente para los mercados locales. Las investigaciones en Tanzania, sobre el cacahuate, se iniciaron en Nachingwea, a fines de la década de 1940. A principios de 1970, con la ayuda de la Dirección del Desarrollo en Ultramar (Overseas Development Administration, ODA) del Reino Unido, se trasladaron las investigaciones sobre el cacahuate a Naliendele, Mtwara, en el sureste de Tanzania. Con excepción de una pequeña cantidad de investigación que se realiza en la Universidad Agrícola Sokoine, en Morogoro, la mayor parte de las investigaciones realizadas sobre el mejoramiento genético del cacahuate, aspectos agronómicos y la protección del cultivo se desarrollan en Naliendele.*

Nuestros primeros esfuerzos en la proteccion de cultivos fueron dirigidos a enfermedades foliares e insectos. Racientemente, se evidenció que la contaminación por aflatoxina del cacahuate era uno de los principales factores en la reducción de la calidad del cacahuate en el país. El Programa Nacional de Mejoramiento del cacahuate ha decidido iniciar una cuidadosa investigación del problema para desarrollar medidas efectivas de control. La asistencia a nuestro programa por parte del ICRISAT y el Programa Colaborativo de Apoyo en la Investigacion del Cacahuate (CRSP), será bienvenida.

Present Status and Perspectives of Aflatoxin Research in Mozambique

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Abstract

In Mozambique, there is a high correlation between the incidence of primary liver cancer and the consumption of aflatoxin-contaminated food. Some work has been done to assess and minimize the aflatoxin problem.

Institutions such as the Instituto Nacional de Investigación Veterinaria (INIV) and the Laboratorio Nacional para la Higiene de Agua y Alimentos (LNHAA) are involved in the analysis of food products, for both animals and humans. The main technique used for determination and quantification of aflatoxins is thin layer chromatography (TLC) although high performance liquid chromatography (HPLC) is also available at the LNHAA.

In 1981, 17 food products, comprising a total of 313 samples were tested and it was found that 16 samples were contaminated with aflatoxin B_1, 10 with B_2, 4 with G_1, and 3 with G_2. It was found that 87-100% of the groundnut, beer, rice, and maize samples tested were contaminated. The aflatoxin contamination levels in the groundnut samples ranged from 3 to 5500 $\mu g\ kg^{-1}$, aflatoxin B_1, being the main contaminant (Casadei et al. 1981).

An analysis program is investigating the possible correlation between the consumption of contaminated food and the possible presence of aflatoxin M_1 in human breast milk.

The possibility of further work involving the INIV, LNHAA, and the Faculdade de Agronomia, Universidade Eduardo Mondlane, Groundnut Improvement Project is being studied to include an agronomic component and to formulate practical recommendations for small farmers and traders.

Résumé

Etat actuel et avenir de la recherche sur les aflatoxines au Mozambique : *Au Mozambique, on a constaté une forte corrélation entre l'incidence du cancer hépatique primaire et la consommation d'aliments contaminés par les aflatoxines. Des études ont été effectuées en vue d'évaluer et de réduire ce problème.*

Les produits alimentaires de consommation animale et humaine sont analysés par les organismes tels que le National Institute for Veterinary Research (INIV) et le National Laboratory of Water and Food Hygiene (LNHAA). La principale technique utilisée pour la détermination et le dosage des aflatoxines est la chromatographie en couche mince (TLC); le LNHAA se sert également de la chromatographie liquide haute performance (HPLC).

Lors d'une étude d'analyse portant sur 313 échantillons de 17 produits alimentaires, Casadei et al. (1981) ont trouvé que 16 échantillons étaient contaminés par l'aflatoxine B_1, 10 autres par B_2, 4

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Introduction

The contamination of foodstuffs by aflatoxins produced by aflatoxigenic strains of *Aspergillus flavus* is a problem that is being taken more and more seriously by people all over the world. Since the discovery of the double-carcinogenicity of aflatoxins in the People's Republic of China (Li Mingxin et al. 1979), people are deeply concerned about the possible relationship between *A. flavus* in food and liver cancer, especially as China has a high national incidence of this disease. Approximately 100 000 people die of liver cancer in China each year, and it is the third most frequent cancer of the nine that occur in China, after stomach and oesophageal cancers (Li Bing and Li Junyao 1980). It has been confirmed that aflatoxins are among the main factors inducing liver cancer (Wang Yaobing et al. 1983). Consequently, the Chinese government pays great attention to the problem of aflatoxin contamination, and research in this area receives high priority. Special cooperative research groups have been set up and many studies have been made on the carcinogenicity of aflatoxins, their occurrence in food, methods to protect food from mold damage, and detoxification of foods. This paper gives an evaluation of the aflatoxin problem in groundnut in The People's Republic of China, and indicates how the contamination may be prevented.

Aflatoxin Contamination in Groundnut and Groundnut Oil

Aspergillus flavus is very widely distributed in nature; groundnuts can be contaminated by aflatoxin in all parts of the country, but the levels of aflatoxin contamination vary depending on the location and the climate.

From 1973 to 1977, a general survey of aflatoxin B_1 contamination in food was made in 24 provinces by the Ministry of Public Health of the People's Republic of China (PHI 1983). More than 14 000 samples of maize, groundnut, rice, wheat, and various legumes were analyzed for aflatoxin B_1 contamination. The contamination in groundnut samples was 26.3% and in groundnut oil samples 47.3%. The aflatoxin B_1 contamination was serious in south-central China, moderate in east and northwest China, and negligible in northeast China (Table 1).

The percentage of aflatoxin B_1 contamination gradually decreased as latitude increased. Aflatoxin B_1 contamination in groundnut (41.7%) and groundnut oil (68.1%) in southern China was high, but there was almost no contamination in northern China. The climate in southern China and in the Yangtze valley is warm and damp, the rainfall is above 750 mm when the

Table 1. Aflatoxin B_1 contamination in groundnut and groundnut oil in three regions of the People's Republic of China, 1973/74.

Sample	Region	Latitude	Samples	Positive samples (Number)	Positive samples (%)
Groundnut seed	Northern	39–45°N	33	0	0
	Central	24–31°N	60	18	30.0
	Southern	21–27°N	528	220	41.7
Groundnut oil	Northern	39–45°N	234	11	4.7
	Central	24–31°N	11	3	29.3
	Southern	21–27°N	533	363	68.1

Table 2. Aflatoxin B_1 contamination in groundnut and groundnut oil in southern provinces of the People's Republic of China, 1973–77.

Sample	Province	Samples	Positive samples (Number)	Positive samples (%)
Groundnut seeds	Guangdong	221	46	20.8
	Guangxi	528	220	41.7
	Fujian	44	27	61.4
	Hunan	60	18	30.0
	Hubei	48	21	43.8
Groundnut oil	Guangdong	142	125	88.0
	Guangxi	409	399	97.6
	Fujian	27	27	100.0

temperature is above 15°C, and the average relative humidity is above 80%; all factors advantageous to mold growth. In southern China, groundnuts are therefore easily contaminated by aflatoxin after harvest and in storage (Table 2).

Studies have indicated that aflatoxin contamination could be reduced or prevented, even in southern China, by following good cultural practices. In 1976/77, according to a report by the Sanitation and Antiepidemic Station of Guangxi Province the groundnuts grown by production teams carrying out the recommended preventive measures did not contain aflatoxin B_1 or contained only low levels. However, aflatoxin B_1 contents in groundnuts from production teams that did not carry out the preventive measures were several times higher than the legal limit.

Permitted Levels of Aflatoxin B_1 in Food in China

Based on the present level of aflatoxin contamination in food at home and abroad, and taking into account the toxicity and carcinogenicity of aflatoxins to humans and animals, in 1981 the Chinese government formally issued the following legal criteria for aflatoxin B_1 levels in different foods :

Maize, groundnuts, groundnut oil $< 20\ \mu g\ kg^{-1}$
Maize and groundnut products (converted raw material) $< 20\ \mu g\ kg^{-1}$
Rice, other edible oils $< 10\ \mu g\ kg^{-1}$
Other grains, beans, and fermented foods $< 5\ \mu g\ kg^{-1}$
Infant milk substitutes–no detectable aflatoxins.

Studies on the Relationship between *A. flavus* Infection and Aflatoxin Contamination

In order to prevent and control aflatoxin contamination it was necessary to understand the process by which groundnuts were infected by toxigenic strains of *A. flavus*. It was found that pre- and postharvest stages in crop production were the main periods of seed infection and aflatoxin contamination, but that significant contamination could also occur in storage. The period from harvest to entry into storage was the most important for aflatoxin contamination.

Sheng Juechen (1979) isolated 299 strains of *A. flavus* from soil samples taken in groundnut

fields in Fujian Province, and 146 were found to be aflatoxigenic. Further investigation showed that there was a clear positive correlation between the percentage of groundnuts infected by aflatoxigenic *A. flavus* and the level of aflatoxin B_1 contamination in groundnut. Ruan Zhenxi and Wu Niaoban (1985) found that 67% of the aflatoxin contamination in groundnuts occurred before harvest and that aflatoxin B_1 contents ranged from 12.5 to 200 $\mu g\ kg^{-1}$. The level of aflatoxin B_1 contamination increased if the pods could not be dried rapidly after harvest. The Moldproof and Detoxicating Cooperative Group of Fujian Province (1977) reported 22% *A. flavus* invasion of groundnut seeds before harvest under drought-stress conditions, but only 4% in seeds from a nondrought-stressed crop. After 2–3 days of postharvest drying in the sun, the percentage of seeds infected by *A. flavus* showed a slight increase when their moisture contents were 7–8%, but a significantly greater increase when seed moisture content was 8–9% after 5–6 days drying in the shade. They pointed out that percentage seed infection reached a maximum before storage. Therefore, the best way of preventing or reducing aflatoxin contamination in groundnut is to dry the product as rapidly as possible after harvest.

It is also important to store groundnuts in shell. According to a study by Xu Huaiyou and Li Rongtao (1983) seeds, especially those shelled by machine, are more contaminated with aflatoxin B_1 than those left in the pods. Xu Huaiyou and Li Rongtao (1986) observed that the seed testa of groundnut had a very important function in preventing infection by *A. flavus*. The detected percentage of *A. flavus* inside the seed was reduced by 32–74% because of the protection of the testa.

It is therefore important to avoid drought stress before harvest, to dry rapidly after harvest, and to store groundnuts in shell at low moisture content and at low temperature.

Correlation between Aflatoxin B_1 Content and *A. flavus* Invasion in Stored Groundnuts

In a study of the correlation between the aflatoxin B_1 content of seed, and percentage *A. flavus* infection of groundnut seeds in storage, it was shown that the aflatoxin B_1 content did not vary with the detected percentage of *A. flavus* in seeds (Table 3).

Table 3. Correlation between aflatoxin B_1 content and *Aspergillus flavus* detected (%) in groundnuts stored in Sichuan and Shandong Provinces of the People's Republic of China.

Location	Detected *A. flavus* (%)		Aflatoxin B_1 content ($\mu g\ kg^{-1}$)
	Seed surface	Inside seed	
Sichuan Province			
Neijian	68.0	100.0	12.5
Neijian	12.0	0	7.8
Neijian	8.0	0	5.8
Ziyang	32.0	66.0	9.5
Ziyang	6.0	2.0	6.7
Ziyang	32.0	100.0	3.0
Shandong Province			
Qixia	64.0	10.0	16.7
Jiaonan	80.0	84.0	2.7

There was no correlation between aflatoxin B_1 content of seed and the percentage of seeds infected by aflatoxigenic *A. flavus* (Table 4). This might be due to a critical moisture content needed for production of aflatoxin. If the moisture content of stored groundnuts is less than 10% and the storage temperature is low, even though there are many spores of *A. flavus* on the groundnut there will not be serious aflatoxin contamination. It is possible that, because there are many fungi, bacteria, and actinomycetes on the seed surfaces, there may be antagonism among these microorganisms under natural conditions. The Food School of Zhengzhou reported that when rice was inoculated with *A. flavus* alone its aflatoxin B_1 content was 40 000 $\mu g\ kg^{-1}$, but when inoculated with *A. fumigatus, A. nidulans, A. niger*, and *Penicillium islandicum* in addition to *A. flavus*, its aflatoxin B_1 content was only 200 $\mu g\ kg^{-1}$.

Table 4. Correlation between aflatoxin B_1 content and groundnut seed infected by *Aspergillus flavus* (%) in the Xiamen region of the People's Republic of China.

Location (County)	Seeds infected (% 100 grains^{-1})		Aflatoxin B_1 content ($\mu g\ kg^{-1}$)
	A. flavus	Aflatoxigenic *A. flavus*	
Jiangtou	27	17.0	20
Jiangtou	12	5.5	50
Jiangtou	15	6.0	75
Xinlin	31	16.0	75
Tongan	34	6.5	8
Tongan	24	13.0	25
Tongan	20	12.0	500

Breeding for Resistance to *A. flavus* and Aflatoxin Production in Groundnut

Good results have been attained in preventing mold growth on groundnut, and on detoxification of groundnuts and groundnut products in China, especially by removing aflatoxin B_1 from groundnut oil. But the best approach to prevent and control aflatoxin contamination is by utilizing genetic resistance to select or breed cultivars that resist seed infection by *A. flavus* and/or aflatoxin production. In 1987, work on breeding resistant groundnut cultivars was initiated at the Institute of Oil Crops of the Chinese Academy of Agricultural Sciences with financial aid from the National Natural Science Foundation of China (NSFC) and the Science and Technology Committee of Hubei Province. Screening germplasm for resistance to *A. flavus* is in progress. In view of the abundant germplasm resources in China, it is hoped that selection and breeding of groundnut cultivars with resistance to aflatoxin contamination will make rapid progress.

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Aflatoxin Contamination of Groundnuts at the Post-production Level of Operation in the Philippines

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Abstract

The results of surveys in the Philippines have shown that the farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 μg kg^{-1} aflatoxin. During windrowing, aflatoxin levels increased at the rate of 1.5 μg kg^{-1} per day. In farm storage aflatoxin contamination continued to increase at the rate of 1.4 μg kg^{-1} per day. Aflatoxin contamination was significantly higher during the main cropping season than the second cropping season.

At the traders' level, groundnut samples taken from various middlemen contained 35.0 μg kg^{-1} aflatoxin. On the other hand, samples taken from the wholesalers' newly procured groundnuts contained 188 μg kg^{-1} aflatoxin. Groundnuts that had been in the wholesalers' warehouse for more than 3 months contained 275 μg kg^{-1} aflatoxin.

At the processors' level, raw materials for confectionery groundnuts (roasted and fried) contained 7.73 μg aflatoxin kg^{-1} groundnuts intended for peanut butter contained 17.13 μg kg^{-1}, and rejected groundnuts had 120.6 μg kg^{-1}.

Aflatoxin contamination could start during harvest. Aflatoxin content climbed to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.

Résumé

Contamination des arachides par les aflatoxines après la récolte aux Philippines : *Pendant la principale campagne agricole, le taux d'aflatoxine augmente significativement entre la récolte et le stockage sur l'exploitation. Les arachides récoltées ont une teneur moyenne de 3,16 μg kg^{-1} d'aflatoxine. Ce pourcentage progresse à raison de 1,5 μg kg^{-1} par jour pendant le séchage en andains. Lors du stockage, la contamination continue à augmenter à raison de 1,4 μg kg^{-1} par jour. La contamination est significativement supérieure pendant la campagne principale par rapport à la deuxième campagne.*

Au niveau du commerce, des échantillons d'arachides collectés auprès de plusieurs intermédiaires présentent une teneur de 35,0 μg kg^{-1} d'aflatoxine. Par contre, les échantillons des nouvelles arachides recueillis auprès des grossistes contiennent 188 μg kg^{-1} d'aflatoxine. Les arachides ayant été stockées pendant plus de trois mois dans les entrepôts des grossistes contiennent 275 μg kg^{-1} d'aflatoxine.

En ce qui concerne le traitement industriel, les taux d'aflatoxine sont les suivants : la matière première pour les arachides de confiserie (grillées et frites), 7,73 μg kg^{-1}; les arachides destinées à la production de beurre, 17,13 μg kg^{-1}; les arachides rejetées 120,6 μg kg^{-1}.

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La contamination déclenchée pendant la récolte augmente significativement au cours de la vente et du traitement industriel. Cette augmentation continue est attribuée à un séchage insuffisant des arachides après la récolte.

Resúmene

La contaminación con aflatoxinas del cacahuate, después de la cosecha, en Filipinas : *Al nivel de predio rural, aflatoxina aumentó significativamente entre la cosecha y almacenamiento, durante el período de la cosecha principal. Al cosecharse, los cacahuates contenían, en promedio, 3.16 $\mu g\ kg^{-1}$ de aflatoxinas. Durante el tiempo en que secaban las hileras de plantas con las vaínas en el campo, los niveles de aflatoxinas aumentaron a razón de 1.5 $\mu g\ kg^{-1}$ por día. Durante el almacenamiento en el predio agrícola, la contaminación con aflatoxinas continuó aumentando a razón de 1.4 $\mu g\ kg^{-1}$ por día. La contaminación con aflatoxinas fue significativamente mayor durante el período de cosecha principal que durante el segundo período de cosecha.*

Al nivel comercial, muestras de cacahuates recolectados en varias empresas intermediarias tuvieron contenidos de 35.0 $\mu g\ kg^{-1}$ de aflatoxinas. Por otro lado, muestras recolectadas de lotes de cacahuate recién recibidos por comerciantes mayoristas, contenían 188 $\mu g\ kg^{-1}$ de aflatoxinas. Los cacahuates que estuvieron en los almacenes de los comerciantes mayoristas durante más de tres meses contenían 275 $\mu g\ kg^{-1}$ de aflatoxinas.

Al nivel de los procesadores, las materias primas utilizadas para la elaboración de cacahuates como confites (tostados o fritos), contenían 7.73 $\mu g\ kg^{-1}$ de aflatoxinas; los cacahuates destinados a la elaboración de mantequilla de cacahuate, 17.13 $\mu g\ kg^{-1}$, y los cacahuates desechados, tenían un contenido de 120.6 $\mu g\ kg^{-1}$.

La contaminación con aflatoxinas podía iniciarse en el momento de cosecha. El contenido de aflatoxinas se incrementó a un nivel significativamente alto durante el manejo comercial y el procesado de las semillas. Este aumento continuo fue atribuído a que los cacahuates no se secaron lo suficiente después de la cosecha.

Introduction

Groundnut is now considered a major commodity in the Philippines. Although a great deal of research is being done to improve its production, postproduction technology has remained undeveloped.

One of the major postproduction (postharvest) problems of groundnut is aflatoxin contamination. A 1970 survey by the Food and Nutrition Research Council (FNRC) of the Philippines revealed that all processed groundnuts in the country had aflatoxin contamination levels >30 $\mu g\ kg^{-1}$ (FNRI 1975). In another survey made by Santamaria et al. (1972) groundnuts ranked second in terms of aflatoxin contamination with levels ranging from 14 $\mu g\ kg^{-1}$ to 985 $\mu g\ kg^{-1}$. Freshly dug groundnuts contained the lowest level of aflatoxin (14 $\mu g\ kg^{-1}$). Similar levels were observed in some groundnut samples taken from traders' warehouses. No definite pattern of increase in aflatoxin contamination was observed.

Efforts to prevent aflatoxin contamination were initiated with research on aflatoxin detoxification. However, prevention of contamination is believed to be crucial at the start of aflatoxin formation. Before any control strategy can be developed, it is important to determine where aflatoxin formation starts, and the extent of contamination in the various postharvest operations.

This study was undertaken to determine the cause and extent of aflatoxin contamination in groundnuts after harvest, and is part of an initial program to improve the groundnut postharvest industry in the Philippines.

Methods

The survey focused on groundnut samples taken at different times after harvest. It was divided into three levels: on-farm (farmers), off-farm (traders), and processors. The three levels were independent of each other in view of the difficulty in monitoring the commodity from farmers to traders to processors.

On-farm Survey

The survey was undertaken in two seasons in two provinces (Quirino and Isabela) where relatively large quantities of groundnuts are produced. It covered harvesting, windrowing, stripping, drying, and farm storage operations. The main crop, when yields are high, is harvested in May and June, i.e., during the dry season. The wet-season crop, harvested in September and October is grown mainly for seed, and yields are low.

The survey started during the latter part of the dry-season harvest. Two farmer-cooperators were identified in each province. During the wet-season harvest, 6 farmer-cooperators were identified in Isabela province, while only 2 farmer-cooperators were identified in Quirino province due to the limited volume of groundnuts harvested there.

The initial farm samples were taken at random from newly harvested groundnuts. Succeeding samples were taken during every postharvest operation (windrowing, stripping, drying, storage) practiced by the farmers. During windrowing, samples were taken 48 or 72 h after harvest. Final sampling during this operation was done just before stripping.

It was observed that drying and storage operations were done simultaneously by some farmers during the dry-season harvest. Samples in storage were taken 48 to 72 h after stripping.

During the wet-season harvest, windrowing was seldom practiced. Farmers strip the groundnuts after harvest and immediately dry them. Samples were taken 48 h after stripping and every 2 days thereafter until the groundnuts were dry. Sampling in farm storage was done once only because of the small amounts of groundnuts stored.

Off-farm Survey

From the farm, groundnuts are either purchased by middlemen or wholesalers. Groundnuts purchased by middlemen are in turn sold to wholesalers. Thus sampling at the traders' level was further subdivided into middlemen and wholesalers. Five middlemen were identified at random in Isabela. Sampling in the middlemen's storage was done once only because of the fast turnover of stocks.

Two wholesalers were identified in Isabela, and in Quirino the identified wholesaler was a farmers' cooperative (Maddela Peanut Planters Cooperative). Sampling at the wholesalers' level was undertaken during procurement, in storage, after shelling, and during the classification of groundnuts for size and quality.

In storage, at the wholesaler's level, samples were initially taken from accessible portions of the pile. To be able to take samples from the inner portion of the pile, sampling was scheduled to coincide with the issue of groundnuts to the sheller.

Processor's Survey

Two processing sites were identified, at Metro Manila and Pangasinan. Cooperators were selected from these sites, and five major processors were identified for sampling.

Analyses

Each sample consisted of 3 to 5 kg groundnuts, thoroughly mixed to ensure homogeneity. Samples were divided into three parts for the following analyses:

Physical

Three 100-g samples each of unshelled and shelled groundnuts were taken and the following quality parameters measured:

a. unshelled groundnuts:
- damaged pods
- discolored pods

b. shelled groundnuts:
- immature nuts
- discolored nuts

Moisture content was measured using the fractional distillation process (Brown Duvell method) on shelled groundnuts.

Microbial

Shelled groundnuts were used to determine microbial infection(%). The 5-g samples were surface sterilized using 10% sodium hypochlorite for 1 min, rinsed three times with sterile distilled water, and blot-dried on sterile filter paper in a petri dish. Five kernels were placed equidistantly on previously plated Malt Salt Agar (MSA) medium and incubated at room temperature for 48 h to 72 h.

Aflatoxin

The samples were extracted with chloroform, filtered, and evaporated almost to dryness. The extracted samples were spotted onto a glass plate thinly coated with an absorbent. The plate was placed in a developing tank with a solvent for 40 min to allow the samples to move through at 23–25° or until aflatoxin B_1 reached Rf 0.4–0.7. The plate was then dried and examined under ultraviolet light. The presence of aflatoxin was determined by comparing fluorescence intensity and Rf value of sample patterns with standards. The Rf value of aflatoxin in samples should be the same or only slightly different from the aflatoxin B_1 standard spots.

Results and Discussion

The critical factors in the production of aflatoxin are substrate moisture content and relative humidity(RH). According to Diener and Davis (1967), 85% is the limiting RH for aflatoxin production by *Aspergillus flavus* in groundnuts over a 21-day storage period.

The survey during the dry-cropping season revealed that freshly dug groundnuts contain an average of 3.16 $\mu g\ kg^{-1}$ aflatoxin. Aflatoxin contamination increased at the rate of 1.5 $\mu g\ kg^{-1}\ day^{-1}$ during windrowing which lasted for 3 to 6 days. Aflatoxin contamination further increased in storage at the rate of 1.4 $\mu g\ kg^{-1}\ day^{-1}$ (Fig.1). It was also observed that aflatoxin contamination in groundnuts did not differ significantly between provinces.

The continued increase in aflatoxin at the farm level during the main cropping season may be attributed to the high RH during the survey period (Table 1) and farmers' insufficient drying of the groundnuts after harvest. The farmer-cooperators in this study seldom dried their groundnuts in the sun before storage. Instead they were spread on the floor inside their houses, resulting in slow drying of the nuts. McDonald and Harkness (1965) reported that slow drying results in higher infection by *A. flavus* and other fungi.

Table 1. Relative humidity during dry (May-Jun) and wet (Sep-Oct) season harvests and corresponding decrease in moisture content in groundnuts. Pooled data, Isabela and Quirino Provinces, the Philippines, 1985.

	Dry-season harvest		Wet-season harvest	
Days after harvest	Relative humidity (%)	Moisture content (wet basis) (%)	Relative humidity (%)	Moisture content (wet basis) (%)
0	84.0	38.0	67.0	38.0
3	82.7	34.7	66.5	30.9
6	82.7	21.8	67.5	18.0
9	80.5	18.9	68.6	15.0

The percentages of groundnut seeds yielding *A. flavus* were lower than the percentage seeds yielding other isolated fungi (Table 2), i.e., *Diplodia* spp, *Penicillium* spp, and *Fusarium* spp. The percentage of groundnut seeds yielding *A. flavus* was higher in Quirino than in Isabela, where RH is higher and conditions were generally wetter at the time of the study. The correlation between *A. flavus* infection and aflatoxin contamination levels was very low.

The amount of damaged and discolored pods, and discolored nuts was observed to increase from harvest to farm storage (Table 2). These parameters were also found to have very low correlation with aflatoxin contamination levels.

Ironically, the aflatoxin contamination level of groundnuts on the farm during the wet season was almost negligible (Fig. 1). This low level could be explained by the differences in the farmers practices and the prevailing conditions during the wet season. The RH at the time of the study ranged from 70% to 85% in Isabela and 50% to 65% in Quirino provinces.

The decrease in moisture content in groundnuts during the wet-season harvest was faster than in the dry-season harvest (Table 1), probably because of the prevailing RH. In addition, the

Table 2. Physical and microbial analyses of groundnuts sampled during dry- and wet-season harvests. Pooled data, Isabela and Quirino Provinces, the Philippines, 1986.

Analysis (%)	Days after harvest			
	0	3	6	9
Dry-season harvest 1986				
Seeds yielding *Aspergillus flavus*	10.0	15.0	8.0	2.0
Seeds yielding other fungi[1]	24.0	38.0	67.0	62.0
Damaged pods	4.0	1.0	4.0	7.5
Discolored pods	0.0	2.0	1.0	3.0
Discolored nuts	5.0	4.0	4.0	10.0
Wet-season harvest 1986				
Seeds yielding *Aspergillus flavus*	3.0	20.0	5.0	8.0
Seeds yielding other fungi[1]	10.0	35.0	58.0	43.0
Damaged pods	8.0	9.0	7.0	8.0
Discolored pods	3.0	4.0	5.5	6.0
Discolored nuts	1.3	1.1	1.6	2.0

1. *Diplodia* spp, *Aspergillus niger*, *Fusarium* spp, *Penicillium* spp.

farmers postharvest treatment of the crop differed depending on the season. Groundnuts from the crop harvested during the wet season were intended for seed, and the yields were low (464 kg ha^{-1}). These groundnuts were immediately stripped and dried. On the other hand, the crop harvested during the dry season had a higher yield (895 kg ha^{-1}), and the groundnuts were windrowed before stripping. Groundnuts dried faster when immediately stripped and sun-dried than when windrowed. It is likely that during windrowing the groundnuts tend to reabsorb moisture from the field.

The farmers were reluctant to sun-dry groundnuts once the moisture content of the nuts reached 18% because they are sold by weight. This reluctance may be due to the fact that there is a significant decrease in weight when groundnuts are dried thoroughly to about 8% moisture content, resulting in decreased revenue for the farmers. The farmers' lack of awareness about aflatoxin contamination may also explain their attitude towards drying.

Very few damaged and discolored pods, and discolored nuts were observed during the wet-season harvest (Table 2), and these parameters were found to have a low correlation with aflatoxin contamination levels.

Farm sampling was independent of trader sampling because it was difficult to monitor produce from the farmers to the traders. The aflatoxin contamination level was significantly higher in traders' samples than in farm samples (Fig. 2).

Groundnut samples taken from middlemen contained an average of 35.0 $\mu g\ kg^{-1}$ aflatoxin, but the newly purchased groundnuts sampled from wholesalers contained, on an average, 188 $\mu g\ kg^{-1}$ aflatoxin. Moisture contents at the time of sampling at wholesalers ranged from 7% to 10%. At this point, conjectures can only be made to explain the higher levels of aflatoxin contamination in the traders' samples. It is possible that the groundnuts they purchased already contained high levels of aflatoxin, considering that they were slowly dried on the farm. Another possibility is that the moisture content of groundnuts, although considered dry at purchase, may still be favorable for aflatoxin formation, since groundnuts with moisture contents of 16% were considered by traders to be dry.

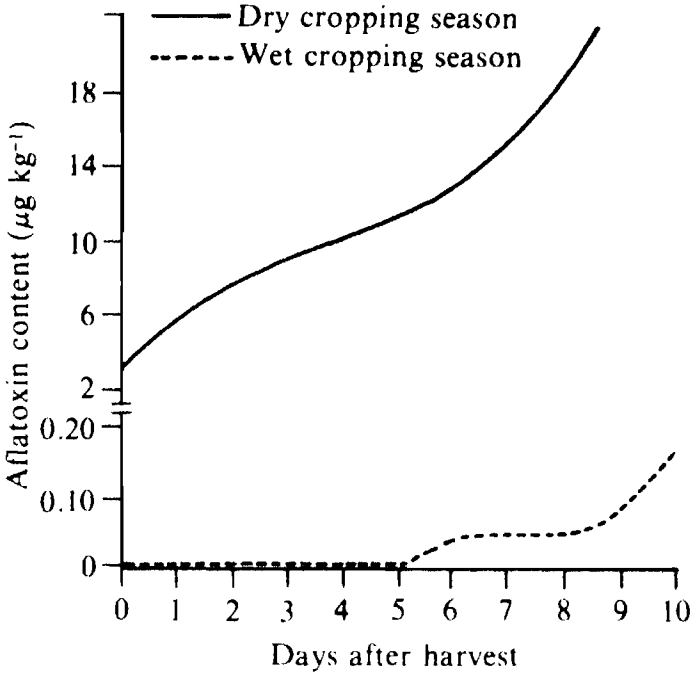

Figure 1. Comparative build-up of aflatoxin contents in groundnuts on the farm during the dry- and wet-harvest seasons, Isabela and Quirino provinces, the Philippines, 1985.

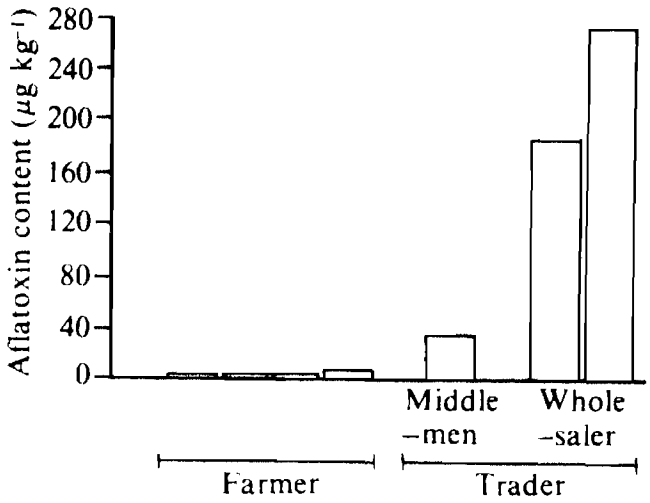

Figure 2. Aflatoxin contents in groundnuts at farmer and trader levels, Isabela and Quirino provinces, the Philippines, 1985.

Groundnut samples stored in warehouses for more than 3 months had aflatoxin contamination levels ranging from 8.7 μg kg^{-1} to 989 μg kg^{-1}, with an average of 275 μg kg^{-1}. Although the moisture content of these samples was already low (5-8%), the significant increase in aflatoxin contamination in groundnuts stored for more than 3 months indicates that storage conditions were favorable for aflatoxin formation.

Groundnuts sampled from the traders were more infected with *A. flavus* than those from the farm (Table 3). There were fewer groundnut seeds infested with *A. flavus* than with other fungi.

The percentage of damaged and discolored pods was higher at the traders' level than on the

Table 3. Physical and microbial analyses of groundnuts sampled from farms, middlemen, and wholesalers, Isabela and Quirino Provinces, the Philippines, 1985.

			Wholesaler[3]	
Analysis (%)	Farm[1]	Middleman[2]	At procurement	After storage
Seeds yielding *Aspergillus flavus*	8.0	30.0	24.0	24.0
Seeds yielding other fungi	42.0	72.0	77.0	80.0
Damaged pods	6.3	12.0	13.0	17.0
Discolored pods	3.0	20.0	14.0	19.0
Discolored nuts	3.0	13.0	8.0	8.0

1. Pooled data, both provinces, two seasons, May-Oct, 1985.
2. Pooled data from five middlemen, Isabela Province.
3. Pooled data, both provinces.

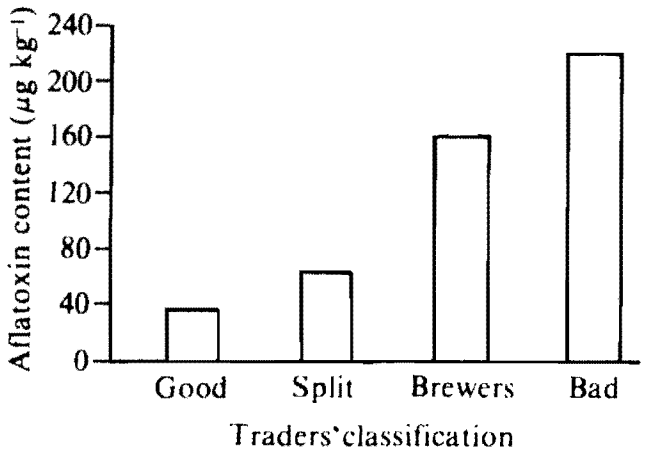

Figure 3. Trader's classification of groundnuts and corresponding aflatoxin content, Isabela province, the Philippines, 1985.

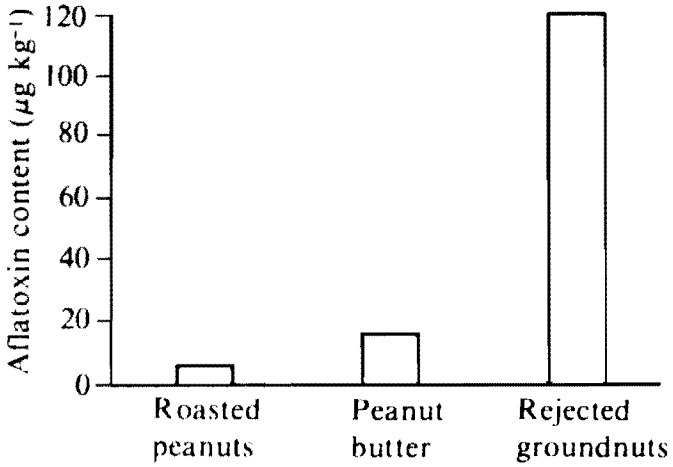

Figure 4. Aflatoxin content of groundnuts intended for roasting, peanut butter, and rejected groundnuts, Isabela and Quirino province, the Philippines, 1985.

farm, but the percentage of discolored nuts was almost similar at both farm and traders' levels (Table 3).

Wholesalers shell and classify groundnuts before distribution. They are classified as good, split, "binlid" (brewers), and bad. Splits are good quality nuts that cleave during shelling, brewers are small-sized, poor quality nuts. In general, the good quality nuts had lower aflatoxin contents than the rejects or poor quality nuts (Fig. 3). Classification based on the condition of the kernels can eliminate aflatoxin contaminated discolored nuts. However, this needs further verification because the correlation analysis revealed that the percentage of discolored nuts did not correlate well with their level of aflatoxin contamination.

At the processor's level groundnuts intended for roasting and frying contained 7.73 $\mu g\ kg^{-1}$ aflatoxin while those intended for peanut butter manufacture contained 17.13 $\mu g\ kg^{-1}$ aflatoxin (Fig. 4). Rejected groundnuts which are usually used for oil extraction or animal feeds, contained 120 $\mu g\ kg^{-1}$ aflatoxin.

The aflatoxin contamination levels in groundnuts at the processors' level were below the limit set by the Food and Drug Administration (FDA) of the Philippines, which is 30 $\mu g\ kg^{-1}$. These relatively low levels could be explained as follows:

- Groundnuts sampled from the processors came from various places in northern Luzon, therefore, these may not necessarily include produce from the trader-cooperators who participated in the study.
- Most of the cooperators who participated in this study have licenses from the Food and Drug Administration (FDA) to process groundnuts. To be able to obtain a licence, their stocks of raw and processed groundnuts must not contain more than 30 $\mu g\ kg^{-1}$ aflatoxin.
- Licensed processors ensure that groundnuts are dry before storage, and just before processing, the groundnuts are further segregated into good and bad.

Summary and Recommendations

Aflatoxin contamination starts at harvest. The level continues to increase after harvest and is significant at trader and processor level. Drying of groundnuts on farms was found to be

insufficient to control infection by *A. flavus*. Moreover, windrowing after harvest exposes the groundnuts to the danger of reabsorbing moisture and this increases the chance of infection by *A. flavus*. The key to prevention of aflatoxin contamination is to dry the groundnuts immediately after harvest. This requires knowledge of the drying rate to enable rapid drying without impairing groundnut quality. If windrowing is inevitable, it is recommended that inverted windrowing be practiced. Previous researchers (Santamaria et al. 1972) have recommended this method to slow down aflatoxin build-up.

The absence of a definite criterion on the safe moisture level for storage results in a significant increase in levels of aflatoxin at the trader and processor levels. The practice of sorting into good and bad quality groundnuts before distribution or processing reduces the level of aflatoxin contamination in good quality nuts.

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Discussion

T.O.M. Nakayama. Who is responsible for monitoring the level of aflatoxin in groundnut in the Philippines?

R. Quitco. The Food and Drug Administration (FDA) of the Philippines monitors aflatoxin in peanut butter only; they don't monitor other products, as we did in our survey. We found very low levels of aflatoxin in those groundnut products intended for roasting or for peanut butter at the farm level. But we found the very high alfatoxin levels in samples from the wholesalers. One factor is that before the groundnut processors can process the nuts into peanut butter they have to get a licence from the FDA, so they have to keep down the aflatoxin level. When they buy the nuts they dry and then sort them. If the nuts are stored for a long time they sort them again. They also illegally sell quality nuts processed into roasted peanuts coated with sugar.

J.I. Pitt. In Australia, it appears that the safe moisture content for long-term storage to avoid spoilage is about 8 to 9%. To prevent aflatoxin formation, it can be considerably higher than that, around 11 to 12%, the level at which aflatoxin can be formed.

R. Quitco. And, how long will the storage be?

J.I. Pitt. Seven months, perhaps even longer. There is no real time period that is safe, it varies a little with different countries and different cultivars.

R.J. Cole. The safe moisture levels in the USA are considered to be 9-10%, below 7% or 6% you will loose quality.

R. Quitco. How about the oil content, don't you destroy it once you discharge.

R.J. Cole. No.

J.I. Pitt. I will make one more comment about the wet and dry seasons. Modern experience clearly indicates that you would expect to have far more aflatoxin in your dry-season crop then in your wet-season crop. It is a really good case for growing more groundnuts in your wet season because aflatoxin is much less likely to be formed.

R. Quitco. Actually, we had a bad harvest during the second cropping season. I really don't know the agronomic behavior of groundnuts, but based on observations we found that during the wet-cropping season the harvest is very poor, the pods are very small, and some pods do not contain nuts. In contrast, during the dry season, the pods are very large and they all have nuts inside. It is said that this is because of too much water during the wet season which encourages vegetative growth. During wet-cropping seasons farmers plant on sloping plots and achieve better harvests than on flat land with flooded soils.

T. Shantha. How do the farmers determine the moisture content of the produce?

R. Quitco. The matter of determining moisture content is somewhat relative. They take some nuts from a sack and crack them, if they crack, then they are considered to be dry. We took some samples that were considered to be dry and found that their moisture content was still 16 to 14%.

D. Caley. I have a question about the temperature at which you determine the moisture content for preventing damage from aflatoxin. What temperature are you talking about?

J.I. Pitt. I think you are really looking at the safe moisture level for long-term storage at temperatures up to 25 or perhaps even to 30° C. But to store groundnuts at such high temperatures to avoid aflatoxin will raise other problems.

D. Caley. We have constantly been pressurised to raise moisture content, and unfortunately we store sacks in stacks covered by black tarpaulins. The stack temperature goes up to 40° C at the top outside surface.

J.I. Pitt. This kind of storage is of course, very dangerous because of the moisture distribution from one part of the stack to another. I think if you are going to store in sacks under tarpaulins you should be really looking at a lower moisture level rather than anything else, because you have a real problem of moisture movement from one part of the area to another with changes in diurnal temperature. Under those conditions, I think you really need to be looking at a little lower moisture content, certainly 1% lower at least.

R.D. Coker. This is a serious matter in Senegal, when you place groundnuts in these large stacks at 6–8% moisture and cover them with a tarpaulin then insect activity and moisture content increase. The major problem I see here is each individual groundnut may have a different moisture content depending on its age, and immature nuts tend to pick up atmospheric moisture faster than mature ones do. These factors also affect insect and fungal activity.

Aflatoxin Contamination of Groundnuts in Pakistan

I.A. Rana[1]

Abstract

Groundnut samples from various parts of Pakistan were analyzed for aflatoxin content, and no fresh samples contained the toxin. However, 6-15% of the roasted peanuts from areas other than Khuzdar were contaminated. The aflatoxin content of the contaminated samples varied from 24 to 800 μg kg^{-1}. All the tested samples of roasted peanuts from Khuzdar were contaminated with aflatoxins.

Résumé

Contamination des arachides par les aflatoxines au Pakistan : *Les échantillons d'arachide recueillis dans plusieurs parties du Pakistan ont été analysés pour leur teneur en aflatoxine. Aucun échantillon d'arachides nouvelles ne contient de toxine. Tous les échantillons d'arachides grillées provenant de Khuzdar et 6-15% des échantillons provenant des autres régions sont contaminés. La teneur en aflatoxine de ces échantillons varie de 24 à 800 μg kg^{-1}.*

Resúmene

La contaminación con aflatoxinas del cacahuate en Pakistán : *Muestras de cacahuate procedentes de varias partes de Pakistán se analizaron para determinar su contenido de aflatoxinas, y ninguna de las muestras de cacahuate fresco contenía toxinas. Sin embargo, entre el 6 y el 15% de las muestras de cacahuate tostado, procedentes de otras áreas, no del Khuzdar, estaban contaminadas. El contenido de aflatoxinas de las muestras contaminadas varió de 24 a 800 μg kg^{-1}; en contraste, todas las muestras de cacahuate tostado analizadas, procedentes de Khuzdar, estaban contaminadas.*

Introduction

Groundnut cultivation in Pakistan started in 1948, and it is now the second largest oilseed crop in the country: every year approximately 60 000 ha are sown. It is grown mostly under rainfed conditions and on river banks in almost all the provinces except Baluchistan. About 92% of the total crop area lies in the province of Punjab, 7% in the North West Frontier Province, and 1% in

1. Principal Scientific Officer, National Agricultural Research Centre, Food Technology Department, PO National Institute of Health, National Park Road, Islamabad, Pakistan.

Citation: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1989. Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India: ICRISAT.

Sind. Nearly 87% of the total area sown to groundnut in the Punjab is in the three districts of Rawalpindi, Jhelum, and Attock. The sub-district of Swabi in the North West Frontier Province and the districts of Sanghar and Larkana in Sind Province are important areas of groundnut cultivation (Khan and Qayyum 1986).

Tropical conditions in Pakistan, harvesting practices, postharvest storage conditions, high temperature, high moisture levels during the monsoon, unusual rains, and floods not only cause damage to standing crops and stored grains, but also create conditions conducive to fungal invasion and production of mycotoxins.

Aflatoxins

Aflatoxins are a group of toxic, crystalline, highly fluorescent, bis- furano-coumarine metabolites (B_1, B_2, G_1, and G_2) produced by some strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Fishbein 1979). When ingested these metabolites cause liver damage which can be fatal (IARC 1976).

Nizami and Zuberi (1977) correlated the high incidence of liver cancer (3.6% of total cancer cases) in Karachi with a high degree of aflatoxin contamination (42%) in foodstuffs and attributed it to the hot and humid climate in this region.

In another study, Nizami et al. (1986) examined urine samples from 200 apparently healthy subjects and detected aflatoxin in the urine of 9.5% of the subjects from the lower, and 5% from the upper socioeconomic groups. Liver cancer was also more common in less-privileged individuals. The presence of aflatoxin in the urine was not related to the age or sex of the subjects studied. The incidence of liver cancer was lower in the cooler northern areas of Pakistan than in the warmer Sind Province, and was highest in Karachi.

Shah et al. (1981) studied aflatoxin content in samples of wheat, raisins, nuts (including groundnut), maize etc., collected from various parts of Pakistan (Table 1). Twenty percent of maize and 70% of maize gluten samples were found to be contaminated with aflatoxin. Roasted peanuts showed levels of contamination much higher (800 $\mu g\ kg^{-1}$) than the permissible limit (20–25 $\mu g\ kg^{-1}$).

Sheikh et al (1983) studied the aflatoxin content of industrially produced groundnut cake and found that it was not only loaded with dust and dirt, but also contained amounts of aflatoxin B_1,

Table 1. Aflatoxin contamination of foods surveyed in Pakistan, 1981.

Materials sampled	Samples	Samples containing aflatoxins	Aflatoxins identification		Aflatoxin concentration ($\mu g\ kg^{-1}$)
			B_1	B_2	
Wheat and wheat flour	54	-	-	-	-
Roasted groundnuts	16	1	+	+	800
Raw groundnuts	7	-	-	-	-
Raisins	11	-	-	-	-
Maize	5	1	+	+	500
Milk	1	-	-	-	-
Total samples	97	2			

Source: Shah et al. (1981).

(1200 $\mu g\ kg^{-1}$), well above the permissible level of 30 $\mu g\ kg^{-1}$. Extraction of the meal with alcohol considerably reduced afaltoxin contamination but after extraction contamination was still above the permissible level.

Begum et al. (1985) sampled roasted groundnuts from local markets in Lahore, Khuzdar, Quetta, and Murree and obtained raw groundnut samples from the Assistant Botanist, Rawalpindi to examine the distribution of aflatoxins. The moisture contents and the extent of aflatoxin contamination in these samples are presented in Table 2. The moisture contents of roasted samples ranged between 4.06% and 9.47%, while in raw groundnuts moisture content varied from 5.60 to 7.55%. The samples were infected with the fungi responsible for the production of aflatoxins. Forty percent of the groundnut samples procured from Lahore markets were infected by *Aspergillus flavus* and their highest moisture content was 9.47%. None of the samples from Quetta were infected by *A. flavus*, but all the samples from Khuzdar were contaminated with this fungus, as was one sample from Murree. The presence of *A. flavus* spores in a sample is an indication that under favorable conditions these could germinate and produce aflatoxins. Raw groundnuts supplied from Rawalpindi were all intact and did not show evidence of infection by *A. flavus* but had a high proportion of shrivelled and moldy kernels. Groundnut samples obtained from Lahore market had high percentages of damaged and shrivelled kernels.

Table 2. Aflatoxin contamination in groundnuts surveyed in Pakistan, 1985.

Locality	Moisture content (%)	Fungus-contaminated, damaged, shrivelled, and discolored kernels (%)	Kernels with greenish-yellow fluorescence (%)	Samples containing aflatoxins	Contamination (%)	Aflatoxin content ($\mu g\ kg^{-1}$)
Lahore	5.63–9.47	5.80–13.23 (2.70)	0.83–5.12	3	15	373
Quetta	5.50–6.32	7.12– 8.24 (1.25)	0.80	-	-	-
Khuzdar	6.92–8.20	7.00– 7.80 (0.56)	2.10	5	100	84
Murree	4.06–5.12	5.00– 5.50 (0.35)	1.50–2.00	-	-	-
Rawalpindi (Raw groundnuts)	5.60–7.55	0.42–14.17	0.50	-	-	-

1. Numbers in parentheses are standard deviations.
Source: Begum et al (1985)

On exposure to UV light some of the roasted kernels showed a critical bright greenish yellow fluorescence, which is a presumptive indicator of the presence of alfatoxins in agricultural commodities (Bothast and Hesseltine 1975). Maximum fluorescence was seen in samples from Lahore markets of which 15% were contaminated with aflatoxins.

All the samples from Khuzdar were contaminated. A very low percentage of the kernels from Quetta, Murree, and Rawalpindi showed critical fluorescence under UV light but these samples did not show detectable aflatoxin contamination. The maximum concentration of aflatoxins was detected in samples from Lahore markets (200–800 $\mu g\ kg^{-1}$). The European Economic Community (EEC) has set a tolerance limit of 20–25 $\mu g\ kg^{-1}$ (FAO 1977). It is thus evident that

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all the contaminated samples except one had aflatoxin contents exceeding the permissible tolerance limit.

The Pakistan Council of Scientific and Industrial Research (PCSIR) Central Laboratories, Karachi, have analyzed 150 samples of groundnuts collected from various parts of Sind and Baluchistan Provinces and found aflatoxin levels of up to 300 $\mu g\ kg^{-1}$ (Mansoor et al., personal communication).

The above studies show that aflatoxin contamination of groundnuts is an important problem in Pakistan likely to affect the health of both humans and livestock, and urgent measures should be taken to improve the harvesting, storage, and processing conditions of groundnut so as to eliminate or reduce the chances of aflatoxin contamination. The measure of the concern is indicated by the establishment in Pakistan of two pilot detoxification plants with the assistance of the Overseas Development Natural Resources Institute (ODNRI), UK.

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National Monitoring and Control Program on Mycotoxins in Brazil

M. Sabino[1]

Abstract

The west of São Pãulo State is a region of high temperature and humidity and is the principal groundnut-growing area of Brazil. Survey results of groundnut samples collected in the rainy season (313 samples) and dry season (83 samples) in that State showed that on an average 48-74% of the samples collected from the west and northeast regions contained 5-22 500 μg aflatoxin B_1 kg^{-1}.

This survey reconfirmed the extent and level of occurrence of aflatoxins in groundnut in Brazil and showed that a mycotoxin problem exists. Suggestions and recommendations were made to the relevant authorities as a resul. of the survey.

Résumé

Programme national sur le contrôle et la maîtrise des mycotoxines au Brésil : *La partie occidentale de l'Etat de São Pãulo est une région caractérisée par des températures et une humidité élevées. C'est la principale zone arachidière du Brésil. Les résultats d'analyses portant sur des arachides collectées pendant la saison des pluies (313 échantillons) et pendant la saison sèche (83 échantillons) ont démontré que 48 à 74% d'échantillons des régions ouest et nord-est de cet Etat contenaient entre 5 et 22 500 μg kg^{-1} d'aflatoxine B_1.*

Cette étude a confirmé l'importance de ce problème au Brésil. Des recommandations ont été proposées aux autorités brésiliennes à la suite de cette étude.

Resúmene

El Programa Nacional de Vigilancia y Control de Micotoxinas en Brasil : *La parte occidental del Estado de São Pãulo, que es una región con altas temperaturas y alta humedad, es la principal zona productora de cacahuates en Brasil. El estudio de los resultados obtenidos durante la temporada de lluvias (en 313 muestras) y durante el estiaje (en 83 muestras) dentro de ese estado, indicaron que en promedio de 48 a 74% de las muestras recolectadas en las regiones occidental y del noreste contenían de 5 a 22500 μg kg^{-1} de aflatoxina B_1.*

Este estudio reconfirmó la extensión y la frecuencia con la cual ocurren las aflatoxinas en cacahuates en Brasil, y demostró definitivamente que existe en ese país un problema de micotoxinas. Se hicieron propuestas y recomendaciones al respecto a las autoridades pertinentes, como resultado del estudio.

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Introduction

There have been several studies on aflatoxin contamination of groundnuts in Brazil (Fonseca 1969, Purchio 1969, Fonseca and Del Nery 1970, Pregnolatto and Sabino 1969, Sabino 1980, Sabino et al. 1982). In years when rain is continuous during harvest, 60–80% of the crop may be contaminated with aflatoxin as a result of poor drying. Contamination is less in the dry- season crop but poor drying and storage sometimes result in mold growth and toxin formation. The principal groundnut-producing area is in the west of São Pãulo state, a region of high temperature and humidity. Most of the groundnuts produced annually in Brazil come from the west and northeast regions of this State.

In 1980, the Sociedade Brasileira de Microbiologia (SBM) had a workshop on mycotoxins in which researchers, analysts, and representatives from the food and feed industries met to discuss the biology of the toxigenic fungi, conditions favoring the production of mycotoxins, methods of detecting mycotoxins is food and feeds, and control measures. The main objective of the workshop was to develop guidelines for research on mycotoxin problems in Brazil.

Even though Brazil had taken action in 1976 on defining the legal limit for aflatoxin contamination in food, little was known about the extent of the mycotoxin problem. Isolated groups of researchers (Tango et al. 1966, Purchio 1969, Fonseca and Del Nery 1970, Della Rosa 1979, Sabino 1980, Sabino and Correa 1981, Sabino et al. 1982) had detected and identified mycotoxins in foods and agricultural commodities, and studied methods to prevent aflatoxin formation, as well as the veterinary and toxicological aspects of mycotoxicosis.

Our workshop was very fruitful and we decided to implement a National Monitoring and Control Program on Mycotoxins. Food and feed industries are also participating in this program. As a preliminary step, the Monitoring and Control Program conducted a survey to determine the extent and level of contamination by a given mycotoxin in groundnuts and maize, the survey was conducted according to the methods presented at the FAO Conference on Mycotoxins held in Nairobi in 1977 (FAO 1979).

In Brazil, a tropical country where the climate favors the growth of aflatoxin-producing fungi, very high levels (1–3000 $\mu g\ kg^{-1}$) and incidence (60%) of aflatoxin contamination in food and feed have been reported (Sabino 1980, Sabino 1983, Prado 1983, Scussel and Rodrigues-Amaya 1985).

Products offered for export have been rejected by importing countries because of aflatoxin contamination. This is the major reason why Brazilian authorities concluded that aflatoxin regulatory activity is necessary.

By Resolution no. 34/76, published on 1 Jan 1977, in the Diario Oficial da Uniao, Brazil, the Comissao Nacional de Normas e Padroes para Alimentos (CNNPA) of the Health Ministry established tolerance levels for aflatoxins B_1 and G_1: $30\mu g\ kg^{-1}$ for food (domestic consumption), and has recommended levels of $50\mu g\ kg^{-1}$ for feed (animal consumption).

National Mycotoxin Control Program Objectives

1. To ensure that public and animal health is safeguarded and that the appropriate authorities are well informed.
2. To ensure that agricultural products offered for export are not rejected by importing countries because of mycotoxin contamination.

3. To promote a system of enlightened education, and to train producers, manufacturers, wholesale distributors, and consumers on the detection and prevention of mycotoxin contamination.

Stages in the Implementation of a National Program

Stage I

1. The establishment of sampling procedures.
2. The establishment of analytical methods.
3. Training in aflatoxin analysis.
4. The establishment of laboratories for aflatoxin analysis.
5. The provision of aflatoxin check samples to ensure the quality of aflatoxin analysis.

Stage II

To conduct a survey to determine the extent and level of occurrence of aflatoxin in selected foods and present it at the workshop on mycotoxins in groundnut and maize held at São Pãulo in 1980.

Stage III

The establishment of a National Monitoring and Control Program on aflatoxins of those commodities determined in Stage II.

Stage IV

To expand the program to other mycotoxins and commodities.

Sampling Methods

Samples were collected during harvest in the groundnut-producing regions of São Pãulo Sate by the Department of Agriculture. Data refer to the following cropping seasons:

1. Collection period: Jan/Feb/March 1981 (rainy-season crops)
   Area collected: 26 Regions
   Total number of samples: 313.
2. Collection period: May/June/July 1981 (dry-season crops)
   Area collected: 14 Regions
   Total number of samples: 83.

Twenty samples were taken (one from each 200-g bag). The 4-kg samples were mixed thoroughly and divided into four 1-kg samples, A, B, C, and D, and analyzed in the laboratory.

Results and Discussion

The results of the survey on rainy-season groundnut crops from 26 regions are summarized in Table 1. Samples collected from the regions of Marilia Pirapozinho, Adamantina, Dracena, Pompeia, Tupa, Presidente Prudente (west São Pãulo State) and Santo Anastacio (northeast São Pãulo State) showed that 48 to 73.5% of samples contained aflatoxin B_1 at levels of between 5 and 22 500 $\mu g\ kg^{-1}$.

The survey of groundnuts from west São Pãulo State showed that 61.2% of these samples were contaminated with aflatoxin. Nine percent contained levels above 5000 $\mu g\ kg^{-1}$ aflatoxins B_1 and

Table 1. Incidence of aflatoxins (B_1 + G_1) in groundnut in São Paulo, Brazil, rainy-season crop 1981.

Regions	Total samples	Number positive	No. of samples containing aflatoxin ($\mu g\ kg^{-1}$) >30[1]	>500	>1000	>5000	Average content of positives ($\mu g\ kg^{-1}$)	Range ($\mu g\ kg^{-1}$)
West	152	93	71	44	36	14	2391	0–30 000
Northwest	50	30	22	10	7	5	1971	0–12 832
Northeast	111	49	40	23	13	8	2664	0–20 423

1. Aflatoxins tolerance limit in Brazil is 30 $\mu g\ kg^{-1}$ (B_1 + G_1).

G_1, and the average level in all contaminated samples was 2391 $\mu g\ kg^{-1}$. Analysis of 111 samples collected from northeast São Paulo State showed that 44% were contaminated with aflatoxin. Seven percent contained more than 5000 $\mu g\ kg^{-1}$ aflatoxins B_1 and G_1, and the average level in all these contaminated samples was 2664 $\mu g\ kg^{-1}$ (Table 1).

A summary of the survey results for groundnut samples taken from the dry-seasn crop is given in Table 2. The 83 samples orginated from 14 regions. That number was insufficient for conclusions to be drawn on the relative incidence and levels of aflatoxins. But, the lower aflatoxin content when compared with the rainy-season crops was noted. However, the exceptions, i.e., those samples collected from Marilia (west São Paulo State) showed high levels of aflatoxins: 991, 21 666, and 33 500 $\mu g\ kg^{-1}$; and two samples collected from Pompeia contained 870 and 10 750 $\mu g\ kg^{-1}$.

Table 2. Incidence of aflatoxins (B_1 + G_1) in groundnut in São Paulo State, Brazil, dry-season crop 1981.

Regions	Total samples	Aflatoxin contamination: Not detectable	Number positive	Samples containing aflatoxin ($\mu g\ kg^{-1}$) >30[1]	>100	Average of positives ($\mu g\ kg^{-1}$)	Range ($\mu g\ kg^{-1}$)
West	55	40	15	9	7	4555	0–33 500
Northwest	13	11	2	2	0	66	0– 67
Northeast	15	13	2	0	0	10	0– 10

1. Aflatoxin (B_1 + G_1) tolerance limit in Brazil is 30 ($\mu g\ kg^{-1}$).

Conclusions

This survey confirmed the extent and level of occurrence of aflatoxin contamination of groundnuts in Brazil.

Suggestions for Future Action

1. Exert pressure on the relevant authorities to ensure that:
   - aflatoxin analysis of groundnuts and groundnut products for direct human, or anima consumption, should be obligatory, and
   - legislation on mycotoxins should be revised.
2. Motivate agriculture technicians to educate farmers on the problems of mycotoxins.
3. Increase government authorities' awareness of their responsibilities, and define more clearl these responsibilities, particularly with respect to the buying and utilization of groundnut
4. Conduct national-level training courses at laboratories capable of conducting analyses f aflatoxins.
5. Augment laboratory facilities and their analytical capabilities.
6. Develop surveillance for other mycotoxins based on research findings.
7. Encourage good cultural practices to minimize field, harvest, and postharvest contaminatior of groundnuts.

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Discussion

K.J. Middleton. Is the dry-season groundnut crop in Brazil irrigated?
M. Sabino. No irrigation is given.
T. Shantha. Very high levels of aflatoxins in groundnut seeds were quoted for Brazil. What did these seed samples (that contained 10 000 $\mu g\ kg^{-1}$ of aflatoxin) look like? Were they of an appearance that would render them acceptable in the market?
M. Sabino. The higher levels were exceptional. Seed samples that had 1000 or 2000 $\mu g\ kg^{-1}$ aflatoxin looked quite good, but those with very high levels (10 000 $\mu g\ kg^{-1}$ or more) looked very bad.

Session V

Removal of Aflatoxins

Control of Aflatoxin in Groundnut Products with Emphasis on Sampling, Analysis, and Detoxification

R.D. Coker[1]

Abstract

The control of the occurrence of aflatoxin in groundnut products requires a combination of quality control and decontamination procedures. Recent work at the Overseas Development Natural Resources Institute (ODNRI) has focussed upon the development of efficient sampling, sample preparation, aflatoxin assay, and chemical detoxification procedures.

The use of selected mathematical models to describe the distribution of aflatoxin in groundnut kernels, roasted peanuts, peanut butter, and groundnut cake has been investigated in order to facilitate the design of statistically sound sampling plans for these commodities. A subsampling mill has been developed, in collaboration with a UK company, which enables representative, comminuted subsamples to be rapidly produced from large samples of groundnut kernels.

Methods have been elaborated for the accurate analysis of the aflatoxin content of groundnut products utilizing bonded-phase cleanup procedures in combination with high performance liquid chromatography (HPLC) and high performance thin-layer chromatography (HPTLC) quantification methods. The application of enzyme-linked immunosorbent assay (ELISA) methods to the analysis of peanut butter has also been extensively examined.

A procedure for the detoxification of groundnut cake using ammonia gas at high temperatures and moderate pressures has been developed, and preliminary toxicity trials have been completed using ammoniated material generated by a 50-kg capacity reaction vessel. A 1-t h^{-1} capacity reaction vessel is under construction and trials will begin in India in 1988.

Résumé

Maîtrise des aflatoxines dans les produits d'arachides — échantillonnage, analyse et détoxification : *La maîtrise des aflatoxines contenues dans les produits arachidiers nécessite un contrôle de qualité accompagné des processus de décontamination. Les récents travaux entrepris par l'Overseas Development Natural Resources Institute (ODNRI) sont axés sur la mise au point de méthodes efficaces d'échantillonnage, de préparation des échantillons, d'analyse des aflatoxines et de détoxification chimique.*

Certains modèles mathématiques permettant de décrire la distribution des aflatoxines dans les graines d'arachide, les arachides grillées, le beurre d'arachide et les tourteaux, ont été étudiés afin de faciliter la conception des plans d'échantillonnage pour ces produits. Un broyeur-échantillonneur a été mis au point en collaboration avec une société anglaise pour la production rapide de sous-échantillons broyés représentatifs à partir de grands échantillons de graines d'arachide.

Des méthodes ont été perfectionnées pour une analyse précise de la teneur en aflatoxine des produits d'arachide en utilisant des procédés de purification sur phases greffées ainsi que les

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méthodes quantitatives de la chromatographie liquide haute performance (HPLC) et de la chromatographie en couche mince haute performance (HPTLC). L'application des méthodes ELISA à l'analyse du beurre d'arachide a été également examinée.

On a mis au point un processus de détoxification des tourteaux à l'aide de gaz ammoniac à forte température et sous pression moyenne. Les premiers essais de toxicité ont été effectués sur du matériel ammoniaque produit dans un réacteur d'une capacité de 50 kg. Une unité semblable en cours de construction aura une capacité de traiter 1 t h^{-1}; les premiers essais sont prévus en Inde en 1988.

Resúmene

El control de las aflatoxinas en productos derivados del cacahuate, con especial atención al muestreo, análisis y eliminación de las toxinas : *El control de la frecuencia con la cual ocurren las aflatoxinas en los productos derivados de cacahuates requiere de una combinación de procedimientos para controlar la calidad y eliminar las toxinas. Los trabajos recientes del Instituto para el Desarrollo de Recursos Naturales en Ultramar (Overseas Development Natural Resources Institute, ODNRI) se han centrado en el desarrollo de procedimientos de muestreo eficientes, de preparación de las muestras por analizarse, de análisis de aflatoxinas, y la eliminación de las toxinas por medios químicos.*

Se ha investigado el uso de modelos matemáticos seleccionados para describir la distribución de las aflatoxinas en los granos de cacahuate, cacahuates tostados, mantequilla de cacahuate y torta de cacahuate, con la finalidad de facilitar el diseño de planes de muestreo estadísticamente fundamentados para el muestreo de estos productos comerciales. Se ha desarrollado un molino submuestreador, en colaboración con una empresa del Reino Unido, que permite la obtención de submuestras representativas finamente molidas, en forma rápida, a partir de muestras grandes de granos de cacahuate.

Se han desarrollado métodos para el análisis preciso del contenido de aflatoxinas en productos derivados del cacahuate, utilizando procedimientos de extracción o "limpieza" por ligamento de fases en combinación con cromatografía líquida de alto rendimiento (HPLC) y cromatografía de capa delgada de alto rendimiento (HPTLC), como métodos de cuantificación. La aplicación de métodos inmunoenzimáticos de cuantificación de anticuerpos (ELISA) en el análisis de la mantequilla de cacahuate, también ha sido detalladamente examinada.

Se ha desarrollado un procedimiento para la eliminación de toxinas de la torta de cacahuate, empleándose amoniaco en forma gaseosa, a altas temperaturas y presiones moderadas, y se han realizado pruebas preliminares de toxicidad, usando material producido en un reactor de 50 kilogramos de capacidad. Se encuentra en construcción un reactor de una capacidad de 1 tonelada por hora y las pruebas respectivas se iniciarán en la India, en 1988.

Introduction

In order to protect the health and productivity of both man and animals it is most important that the incidence of aflatoxin in foods and feeds is reduced to an acceptable level. The introduction, by importing countries, of legislation limiting the levels of aflatoxin accepted in groundnut products necessitates the implementation of efficient quality control measures by the exporting countries. For example, legislation proposed in the UK, under the Food Act 1984, will restrict the level of total aflatoxin in processed groundnuts to 10μg kg^{-1} or less. Furthermore, a recent

European Economic Community (EEC) Directive restricts the aflatoxin content of groundnut products used as animal feeds to a maximum of 200 $\mu g\ kg^{-1}$.

The control of aflatoxin consists of surveillance procedures to identify the nature and extent of the aflatoxin problem, together with quality control and detoxification procedures designed to minimize the problem. Furthermore, surveillance and quality control procedures require efficient sampling, sample preparation, and aflatoxin assay methods.

Some of the recent work at the Overseas Development Natural Resources Institute (ODNRI) on the development of sampling, sample preparation, aflatoxin assay, and detoxification methods is summarized in this paper.

Work at ODNRI

Sampling Methods

The use of inefficient sampling methods invalidates the results obtained from surveillance or quality control procedures. Consequently, much care must be taken in the design of statistically based sampling protocols. This is especially important when, as in groundnuts, the distribution of aflatoxin is highly skewed. For example, Cucullu et al (1966) found that only 12 kernels from a sample of approximately 5000 groundnuts contained detectable levels of aflatoxin. Furthermore, the quantity of aflatoxin in the contaminated kernels varied from approximately 0.1 to 220 μg.

The first step in the development of a sampling protocol is the investigation of the distribution of aflatoxin in groundnut products. Studies at ODNRI have included groundnut kernels, in-shell groundnuts, roasted peanuts, peanut butter, groundnut cake, and groundnut meal amongst the commodities investigated.

Groundnut kernels and peanut butter

It is not unusual for 1-kg samples to be used for the quality control of batches of raw groundnut kernels. The sampling error associated with the collection of small samples was illustrated when representative 54-kg sample of raw kernels was randomly divided into twenty 2.7-kg subsamples, each of which was assayed for aflatoxin. Their aflatoxin contents ($\mu g\ kg^{-1}$) were as follows; 202, 1, 16, 0, 5, 0, 0, 94, 0, 159, 46, 0, 39, 1, 14, 43, 0, 1, 0, and 0. It can be seen that although the true sample mean was 31 $\mu g\ kg^{-1}$, the aflatoxin content of the 20 subsamples varied from <1.0 to 202 $\mu g\ kg^{-1}$, and that 8 of the subsamples did not contain detectable aflatoxin.

The distribution of aflatoxin in small, incremental samples of raw groundnut kernels systematically collected from 15 to 20-t batches, has also been investigated. It may be seen from Table 1 that only 1-6% of the 100-g samples contained detectable levels of aflatoxin, while the highest level of contamination was up to 100 times greater than the estimated batch mean (i.e., the aflatoxin content of the aggregate sample). For example, batch B, with an estimated mean of 10 $\mu g\ kg^{-1}$, afforded only one contaminated sample containing 1000 $\mu g\ kg^{-1}$ aflatoxin. Similarly, batch A (estimated mean of only 1 $\mu g\ kg^{-1}$) afforded samples containing aflatoxin up to approximately 100 $\mu g\ kg^{-1}$. Finally, a sample containing up to 3729 $\mu g\ kg^{-1}$ was generated by batch C (estimated mean 38 μg aflatoxin kg^{-1}).

Quality control protocols for raw groundnut kernels must include statistically based sampling methods which can accommodate these "hot-spots" of aflatoxin, and provide samples which are truly representative of the batch. The so-called Tropical Products Institute (TPI) Plan (Coker

Table 1. Distribution of aflatoxin in raw groundnut kernels analyzed at ODNRI, UK, 1982.

Batch	Batch size (samples taken)	Contaminated samples	Aflatoxin content ($\mu g\ kg^{-1}$)	Batch average ($\mu g\ kg^{-1}$)
A	20 t (200 × 100g)	4/200 (2%)	18–97	1
B	15 t (100 × 100g)	1/100 (1%)	1000	10
C	20 t (252 × 100g)	15/252 (6%)	5–3729	38

1984), developed at ODNRI, is a simple, low-cost plan that can be used to determine whether a batch of groundnut kernels has a total aflatoxin content exceeding 30$\mu g\ kg^{-1}$, (30 $\mu g\ kg^{-1}$ being the maximum level of aflatoxin permitted in edible nuts imported into the UK, according to a UK trade agreement between the importers and manufacturers of edible nut products). The TPI Plan requires the collection of a representative 10.5-kg sample, composed of at least 100 incremental samples. The 10.5-kg sample is randomly divided into three 3.5-kg portions and the batch is assumed to contain no more than 30 μg aflatoxin kg^{-1} if any one of the three portions contain 10 $\mu g\ kg^{-1}$, or less. (It is imperative that the complete 3.5-kg subsample is analyzed.) A similar plan has been developed by the United States Department of Agriculture (USDA). The USDA Plan requires an initial sample of approximately 66 kg. If the population of batches under test is of high quality, the consumer and producer risks associated with the TPI and USDA Plans will be very similar. However, for a population of low quality (where, for example, 30% of the batches contain more than 30 μg aflatoxin kg^{-1}) the consumer risk from the TPI Plan will be considerably greater than that from the USDA Plan. Work is in progress to further refine the TPI Plan so that the consumer risk may be reduced for low-quality populations of batches, without disproportionately increasing the possible loss to the producers.

The distribution of aflatoxin in roasted peanuts and peanut butter has also been investigated at ODNRI, in collaboration with the British Peanut Council. The results of the analysis of retail packs and grab-samples of roasted peanuts, systematically collected from the production line, are summarized in Table 2. It is clear that local "hot-spots" of aflatoxin remain after processing (which includes automatic color sorting and roasting). For example, some batches containing estimated mean levels of aflatoxin of 1.0 $\mu g\ kg^{-1}$ or less (batches C-F) afforded individual retail packs contaminated at levels far in excess of the proposed UK legal limit of 10 $\mu g\ kg^{-1}$. Batch H, with a mean level of 10 $\mu g\ kg^{-1}$, afforded one retail pack containing 1938 μg aflatoxin kg^{-1}.

The distribution of aflatoxin in systematically collected jars of peanut butter is shown in Table 3. From these results it is clearly evident that local "hot-spots" of aflatoxin can also occur in peanut butter. For example, for batch B, with an estimated mean aflatoxin content of 6 $\mu g\ kg^{-1}$, the single contaminated sample contained 621 μg aflatoxin kg^{-1}.

The effect of processing on the distribution of aflatoxin in roasted peanuts may be observed by comparing batch C (Table 1) with batch H (Table 2). Raw groundnut kernels were commercially processed, by a combination of blanching, splitting, automatic color sorting, and roasting. For these particular sample populations, the estimated mean aflatoxin content was reduced from 38 to 10 $\mu g\ kg^{-1}$ as a result of processing. However, the seven contaminated samples contained 8, 14, 38, 116, 161, 386, and 1938 μg aflatoxin kg^{-1}.

Table 2. Distribution of aflatoxin in processed groundnut kernels analyzed at ODNRI, UK.

Batch	Batch size[1]	Contaminated samples	Aflatoxin content ($\mu g\ kg^{-1}$)	Batch average ($\mu g\ kg^{-1}$)
A	20	0/200 (0%)	0	0
B	20	1/200 (0.5%)	10	0.05
C	10	1/200 (0.5%)	60	0.3
D	20	6/200 (3.0%)	8–81[2]	1.0
E	15	3/200 (1.5%)	31, 92, 109	1.0
F	20	3/150 (2.0%)	30, 40, 107	1.0
G	15	2/200 (1.0%)	7, 609	3.0
H	20	7/200 (3.5%)	8–1938[2]	10.0

1. All samples 100g, 200 taken from each batch, excepting Batch F (150 × 200 g samples).
2. Aflatoxin level range.

Table 3. Distribution of aflatoxin in peanut butter produced in the UK, 1982.

Batch	Batch size (samples taken)	Contaminated samples	Aflatoxin content ($\mu g\ kg^{-1}$)	Batch average ($\mu g\ kg^{-1}$)
A	15 t (100 × 225g)	3/100 (3%)	66, 297, 396	8
B	7 t (100 × 340g)	1/100 (1%)	621	6

Preliminary work is in progress in collaboration with the Department of Mathematics at Portsmouth Polytechnic, UK, to devise a sequential method of testing the acceptability of batches of processed groundnuts, based upon the Central Limit Theorem. The latter states that if a random variable, x, has a distribution with a mean u and a variance σ^2, and if a sample size n has a mean $\bar{x}$, then $z = \frac{\bar{x}-u}{\sigma/\sqrt{\eta}}$

has an approximately normal distribution. The approximation improves with increasing values of n. A computer program has been used to calculate the values of z after entering each value of x (the aflatoxin content of the nth sample of groundnuts). The application of this method to batch C (Table 1) indicated that the true batch mean could be less than 30 $\mu g\ kg^{-1}$. It is evident, therefore, that a batch of raw groundnut kernels, that may meet the requirements of the UK Trade Agreement, can eventually be split into retail packs (batch H, Table 2) containing levels of aflatoxin far in excess of the anticipated 10 $\mu g\ kg^{-1}$ regulatory limit.

In-shell groundnuts

The quality control of in-shell groundnuts is especially difficult. Samples cannot be collected from sacks by simple spear sampling, and the collected sample must either be decorticated prior

Table 4. Distribution of aflatoxin in in-shell groundnuts imported into the UK, 1981.

Batch size (subsamples taken)	Contaminated subsamples	Aflatoxin content ($\mu g\ kg^{-1}$)	Batch average ($\mu g\ kg^{-1}$)
5 t (400 × approx.120g)	29/400 (7%)	0– 30 (372/400) 31– 100 (5/400) 101– 500 (12/400) 501–1000 (4/400) 1001–3000 (4/400) 3001–5000 (3/400)	70

to analysis, or the analysis result adjusted for the mass of the shells. Furthermore, the efficacy of the electronic color sorting of in-shell groundnuts must be questioned.

The distribution of aflatoxin in a 5-t batch of in-shell groundnuts is shown in Table 4. Four hundred samples were systematically collected, decorticated, and assayed for aflatoxin. The estimated batch mean, based upon an aggregate sample of approximately 40 kg of kernels, was 70 $\mu g\ kg^{-1}$. However, the use of the computer program, outlined above, to compute the z values has indicated that the true batch mean may be as low as 30 to 40 $\mu g\ kg^{-1}$, although still above the permitted level of aflatoxin. The results in Table 4 show that most of the collected samples in this batch were heavily contaminated, one sample containing 4652 $\mu g\ kg^{-1}$ aflatoxin.

Groundnut cake

Work performed in collaboration with the Departments of Pharmacy and Mathematics at Portsmouth Polytechnic has indicated (Jazwinski 1986) that the distribution of aflatoxin in groundnut cake may be described by the three-parameter Weibull model (Ross 1987).

$$f(x) = \frac{\beta}{\eta}\left[\frac{x-\gamma}{\eta}\right]^{\beta-1} \exp\left\{-\left[\frac{x-\gamma}{\eta}\right]^{\beta}\right\}, (\gamma < x < \infty)$$

where;
γ = is the minimum aflatoxin level,
β = is the shape parameter, and
η = is the scale parameter.

These parameters may be estimated from the data by the method of maximum likelihood. The Weibull model is a flexible distribution that is suitable for a continuous random variable, such as aflatoxin. Using this approach, the samples of groundnut cake may be described as (a) those containing no aflatoxin, (b) those containing a low, usually acceptable, level of aflatoxin, and (c) those containing an unacceptably high level of aflatoxin, where the samples in categories b and c may be described by two different Weibull distributions. The observed cumulative distribution of the aflatoxin contents of representative samples of groundnut cake collected from the top, middle, and bottom of the sacks of a consignment, with an estimated mean aflatoxin content of approximately 50 $\mu g\ kg^{-1}$, appeared to be consistent with a single Weibull distribution (Fig. 1) where $\gamma = 6.14$, $\beta = 2.48$, and $\eta = 94.01$

The investigation of the distribution of aflatoxin in further batches of groundnut cake, groundnut meal, and FAQ (Fair Average Quality) groundnut kernels is in progress, with the objective of devising sampling protocols for these commodities.

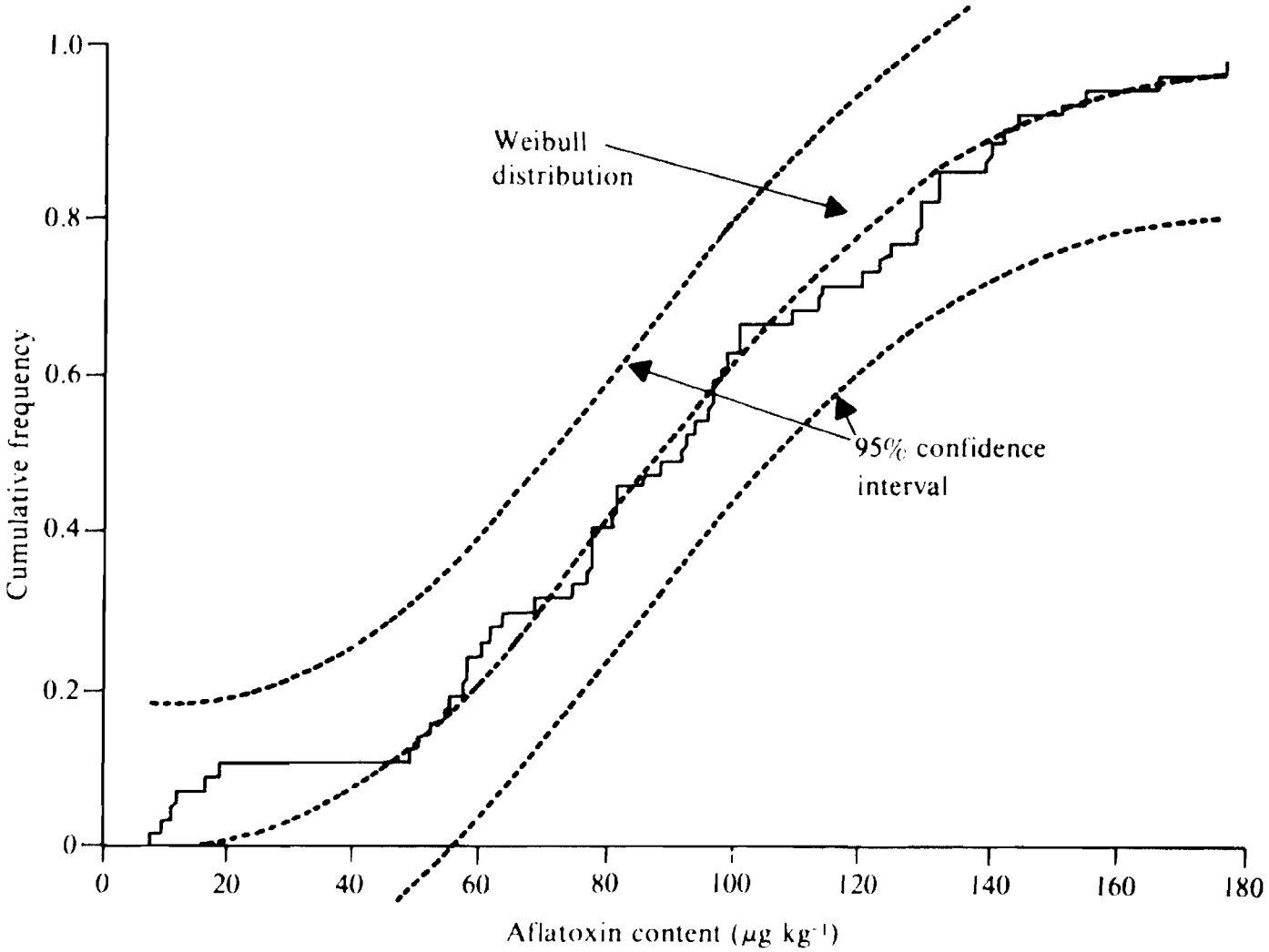

Figure 1. A single Weibull distribution fitted to aflatoxin B_1 data from groundnut cake imported into the UK, 1985.

Sample Preparation

It is necessary to collect large, representative samples for surveillance and quality control purposes. The USDA Plan for the sampling of raw groundnut kernels requires the collection and comminution of three 22-kg samples. It is imperative that the complete sample is comminuted, and that a representative subsample of the comminuted material is assayed for aflatoxin.

The development, in collaboration with commercial partners, of a subsampling mill, that facilitates the simultaneous comminution and subdivision of a range of commodities, is nearing completion at ODNRI. The mill operates on a continuous-flow basis and can handle 22-kg samples of groundnut kernels at a throughput in excess of 1 kg min^{-1}. Free-flowing, representative subsamples are produced that constitute either 5 or 10% by weight of the original sample. It is planned that the mill will be marketed by 1988. The subsamples, weighing approximately 1 kg, may be converted into an homogeneous aqueous slurry (Velasco and Sheldon 1976) of which replicate aliquots, of approximately 100 g are assayed for aflatoxin.

Assay Methods for Aflatoxin

Assay methods used to estimate the aflatoxin content of samples generated by surveillance or quality control procedures should be accurate, precise, rapid, sensitive, selective, and of reasonable cost (Coker 1984). Furthermore, the introduction of automated methods offers improvements in precision and allows the analysis of large numbers of samples that are accumulated, for example, during surveillance studies.

Many aflatoxin assays involve cumbersome column chromatographic and chemical cleanup techniques which may lead to poor accuracy and precision. Recent work at ODNRI has been successful in overcoming this problem. Methods have been developed (Tomlins 1987) which utilize a bonded-phase, cleanup cartridge as a sample enrichment and cleanup device. Various polar and nonpolar phases, chemically bonded to a silica substrate, are available (Coker and Jones 1988).

The ODNRI method has been applied to samples of peanut butter. Typically, 5 mL of an aqueous acetone extract of the sample is treated with lead acetate solution and diatomaceous earth contained within a reservoir attached to a 500-mg bonded phenyl cartridge. The resultant filtrate is passed through the cartridge (that has been conditioned with methanol and water), and the cartridge is then washed with methanolic acetic acid solution followed by water. After the cartridge has been dried by passing air through it, a reservoir containing granular anhydrous sodium sulphate is attached to its base, and the aflatoxin is eluted from the cartridge using chloroform. Using this technique, in combination with a single 12-position vacuum manifold, a single operator can process 120 sample extracts day^{-1}.

High performance thin layer chromatography (HPTLC) is routinely used at ODNRI, in combination with bonded-phase cleanup, for the assay of large numbers of accumulated samples of groundnut products. For example, a bi-directional HPTLC method has been developed (Tomlins 1987), that facilitates the quantification of 200 sample extracts day^{-1}. The extracts are applied using an autosampler, to the HPTLC plate, at a distance of about 2 cm from the top edge, and the plate is developed in dry diethyl ether for 30 min. The top 1 cm of the plate is then removed, the plate rotated through 180° and developed twice in chloroform:xylene: acetone (6:3:1), drying the plate after each development. The aflatoxins are then quantified using a fluorodensitometer. Accurate and precise results have been obtained when the combined bonded-phase/HPTLC method was applied to the analysis of peanut butter and groundnut meal.

Work is in progress in combination with the Department of Pharmacy at Portsmouth Polytechnic, on a comparison of the efficiencies of bonded-phase HPTLC, bonded-phase/HPLC, and enzyme-linked immunosorbent assay (ELISA) procedures, when applied to the estimation of aflatoxin in peanut butter.

Detoxification by Ammoniation

A low-pressure method has been developed at ODNRI (Coker et al. 1985a, b) that facilitates the detoxification of aflatoxin-contaminated groundnut cake and mixed feeds containing groundnut.

The groundnut cake was treated with steam, followed by ammonia gas, then the excess ammonia was purged using a combination of air and steam. Typical operating conditions, for at least 90% detoxification, involved temperatures of the order of 115° C and pressure below 15 psi. A 50-kg capacity reaction vessel has been constructed and successfully tested (Coker et al. 1985a

and b) at ODNRI. A 0.5-t h^{-1} capacity vessel has also been constructed and successfully commissioned for the treatment of maize and poultry feed. A 1.0-t h^{-1} capacity reaction vessel is currently under construction at ODNRI. This will be installed at the Central Poultry Training Institute, Hessarghatta (Bangalore), India in 1988 and used to detoxify groundnut cake and poultry feed.

The toxicity of ammoniated groundnut cake has been assessed, at ODNRI, by feeding ducklings five diets for 22 days. The diets consisted of a control (zero aflatoxin), and diets containing 5% and 25% nontreated cake, and two diets with 5% and 25% ammoniated groundnut cake (Panigrahi, personal communication). All the birds fed on the diet containing 25% nontreated cake died within 14 days. The performances of those ducklings fed diets containing 25% ammoniated cake were slightly reduced compared with those receiving the control diet, and the diet containing 5% ammoniated groundnut cake. On microscopic examination, the livers of five out of nine birds whose diet contained 25% of the ammoniated cake were found to have developed slight bile duct proliferation, and one had marked bile duct proliferation. No abnormal lesions were present in the livers of those birds that received the diet containing 5% ammoniated groundnut cake. The reduced lesions produced by the diets containing ammoniated groundnut cake were consistent with the levels of residual aflatoxin.

Acknowledgments

The studies described in this paper were performed by the members of the Mycotoxin, Animal Feeds, Workshop, and Mechanical Engineering Sections at ODNRI. The collaboration of members of the Departments of Pharmacy and Mathematics at Portsmouth Polytechnic, Portsmouth, UK and the British Peanut Council is also gratefully acknowledged. These studies were partially funded by the UK Ministry of Agriculture, Fisheries, and Food.

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Discussion

R.V. Bhat. Does the UK permit ammonia detoxification of groundnut meal?

R.D. Coker. There is no reason why ammoniated material cannot be imported and used but there are a few questions about the toxicity of the ammoniated groundnut cake, and these will have to be resolved before the big animal-feed compounders are going to show an interest. I am thoroughly optimistic, that in the short or medium term, ammoniation has to be the natural answer.

T. Shanta. The levels of aflatoxin presented by you for Indian groundnuts are rather high. The average level of aflatoxin, especially in export quality groundnuts, cannot be so high. Can you just mention the history, i.e., time lapsed during transport, etc., of the samples?

R.D. Coker. The 20-t batch was imported into the UK by a major UK processor of groundnuts. We estimated the mean level of aflatoxin (using the aggregate sample) at 38 $\mu g\ kg^{-1}$. A recent reassessment of the data using a special computer program, has indicated that the true aflatoxin content of the batch was approximately 30 $\mu g\ kg^{-1}$. A batch of this quality is acceptable in terms of the existing UK trade agreement.

Removal of Aflatoxin Contamination from the Australian Groundnut Crop

M. Read[1]

Abstract

The Australian groundnut crop is significantly affected by aflatoxin in some years because of preharvest drought stress. By a process of selective segregation and sorting, aflatoxin-containing kernels are removed from contaminated lots to satisfy a 15 $\mu g\ kg^{-1}$ (total) regulatory limit. This sorting is made possible by the characteristic discoloration of groundnut flesh caused by Aspergillus *spp growth and the small percentage of aflatoxin-containing kernels. The variance contributions of sampling, sample preparation, and analysis are quoted. Even with very high standards of sampling and analysis, uncertainty in aflatoxin control is significant.*

Résumé

Elimination de l'aflatoxine de l'arachide en Australie : *En Australie, pendant certaines années, la sécheresse avant la récolte des arachides provoque une forte incidence d'aflatoxine. Grâce à la ségrégation séléctive et au triage, les graines contenant de l'aflatoxine sont éliminées des lots contaminés afin de respecter la limite autorisée de 15 $\mu g\ kg^{-1}$ (total). Ce triage est fondé sur la décoloration caractéristique des graines d'arachides contaminées par* Aspergillus *spp et le faible pourcentage des graines contenant les aflatoxines. Les variations dues aux différents éléments tels que l'échantillonnage, la préparation des échantillons et les analyses sont également indiquées. Malgré le niveau très élevé de l'échantillonnage et de l'analyse, il reste toujours une probabilité d'erreur dans le contrôle d'aflatoxines.*

Resúmene

La eliminación de la contaminación con aflatoxinas del cultivo del cacahuate en Australia : *En Australia el cultivo del cacahuate contiene cantidades significativas de aflatoxinas durante ciertos años, debido a la presencia de condiciones de sequía aguda, justamente antes de la cosecha. Mediante un proceso de clasificación y separación selectiva, se pueden eliminar los granos que contienen altos concentrados de aflatoxinas, de los lotes de semillas contaminadas, logrando así no rebasar el límite máximo autorizado de 15 $\mu g\ kg^{-1}$ (total). Esta separación es factible debido a la decoloración característica de los granos de cacahuate, producida por el desarrollo de especies de* Aspergillus, *y el reducido porcentaje de granos que contienen aflatoxinas. Se presentan las contribuciones a la varianza del muestreo, la preparación de las muestras y de análisis. Incluso con normas muy rígidas en el muestreo y el análisis, la incertidumbre en el control de las aflatoxinas es estadísticamente significativa.*

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Introduction

Groundnuts are grown in two distinct climatic regions of Australia (Fig. 1). The major cause of aflatoxin contamination of the Australian groundnut crop is preharvest drought stress in the drier southern region. Drought stress varies greatly from year to year (Table 1). In the humid northern region, periods of high ambient humidity cause less serious postharvest problems.

The psychrometrics of groundnut storage molding have been described by Smith and Davidson (1982). The groundnut hydroscopic equilibrium (Fig. 2) and local humidity data should

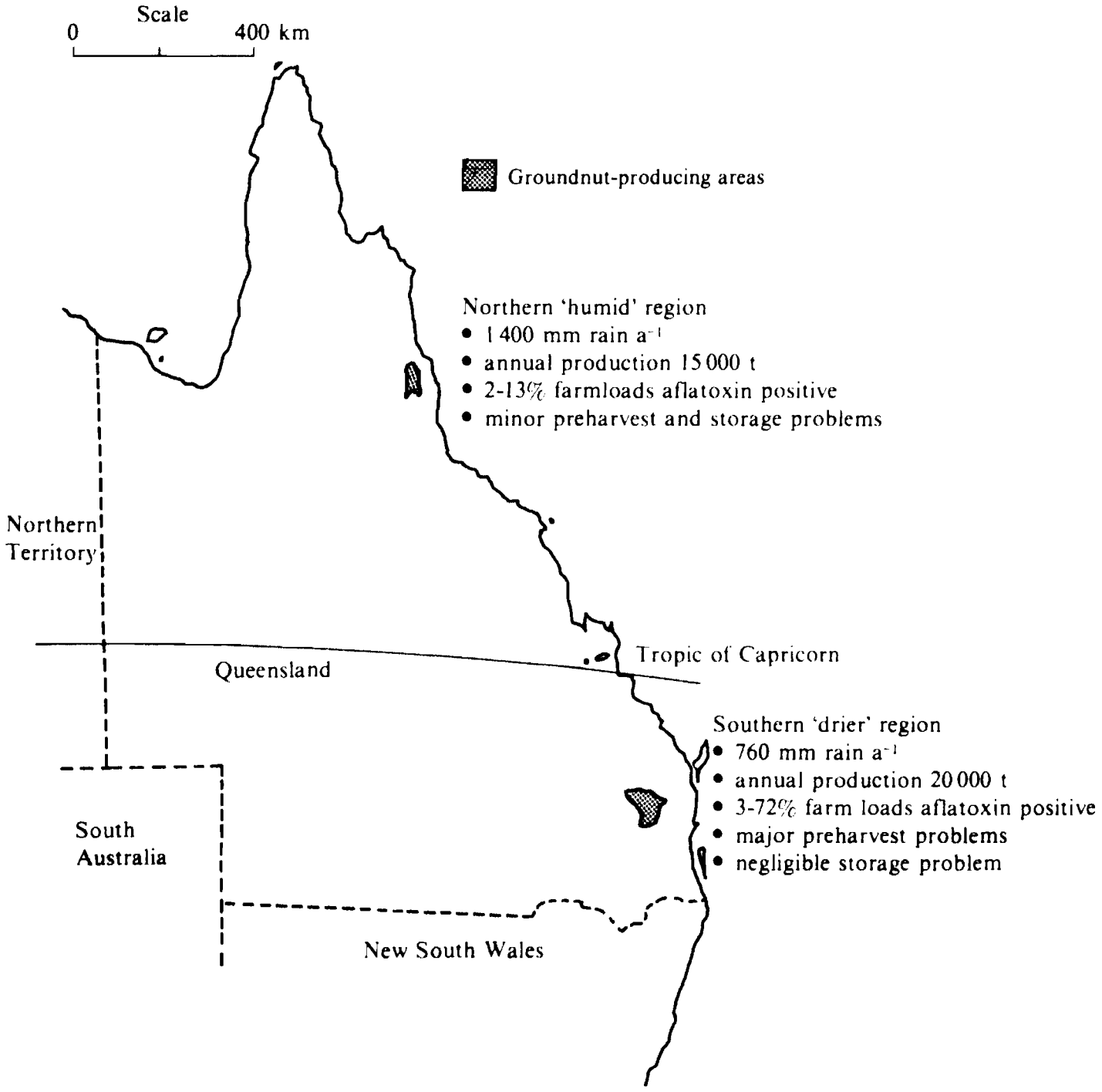

Figure 1. Major groundnut-producing areas of Australia. Virginia bunch varieties grown mainly on friable red volcanic soils.

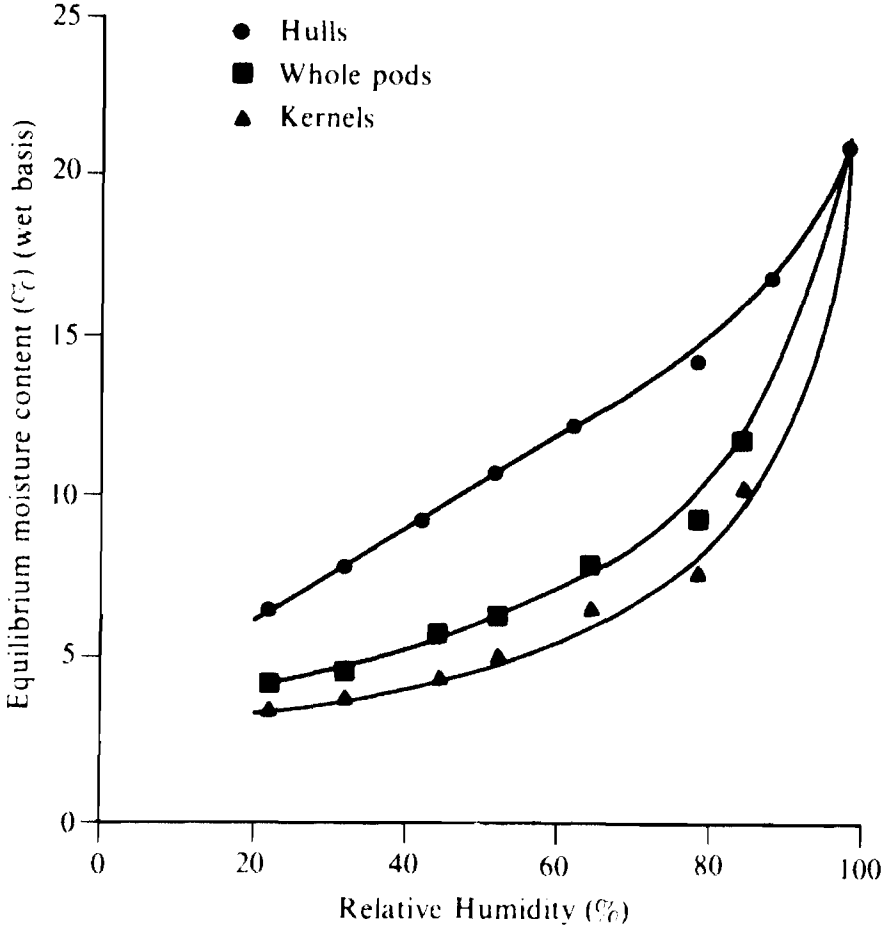

Figure 2. Equilibrium moisture content of groundnut hulls, kernels, and whole pods at 32.2° C.
(Adapted from Smith and Davidson 1982).

always be considered before building storage or processing plants. The best district in which to grow groundnuts may not be the best one in which to store and process them. This paper describes how the Queensland groundnut crop is sampled and tested for aflatoxin contamination by the Peanut Marketing Board, and how contamination is controlled by sorting and quality control.

Table 1. Farmers' truckloads (%) of virginia bunch groundnuts affected by preharvest aflatoxin contamination from two growing regions of Australia, 1978-1987.

	Growing region			Growing region	
Year	Kingaroy (southern)[1]	Tolga (northern)[2]	Year	Kingaroy (southern)[1]	Tolga (northern)[2]
1978	8	2	1983	14	12
1979	5	2	1984	12	11
1980	72	13	1985	12	9
1981	8	5	1986	62	3
1982	3	4	1987	(54)[3]	(6)

1. Average annual rainfall 760 mm.
2. Average annual rainfall 1400 mm.
3. Figures in parentheses are provisional values.

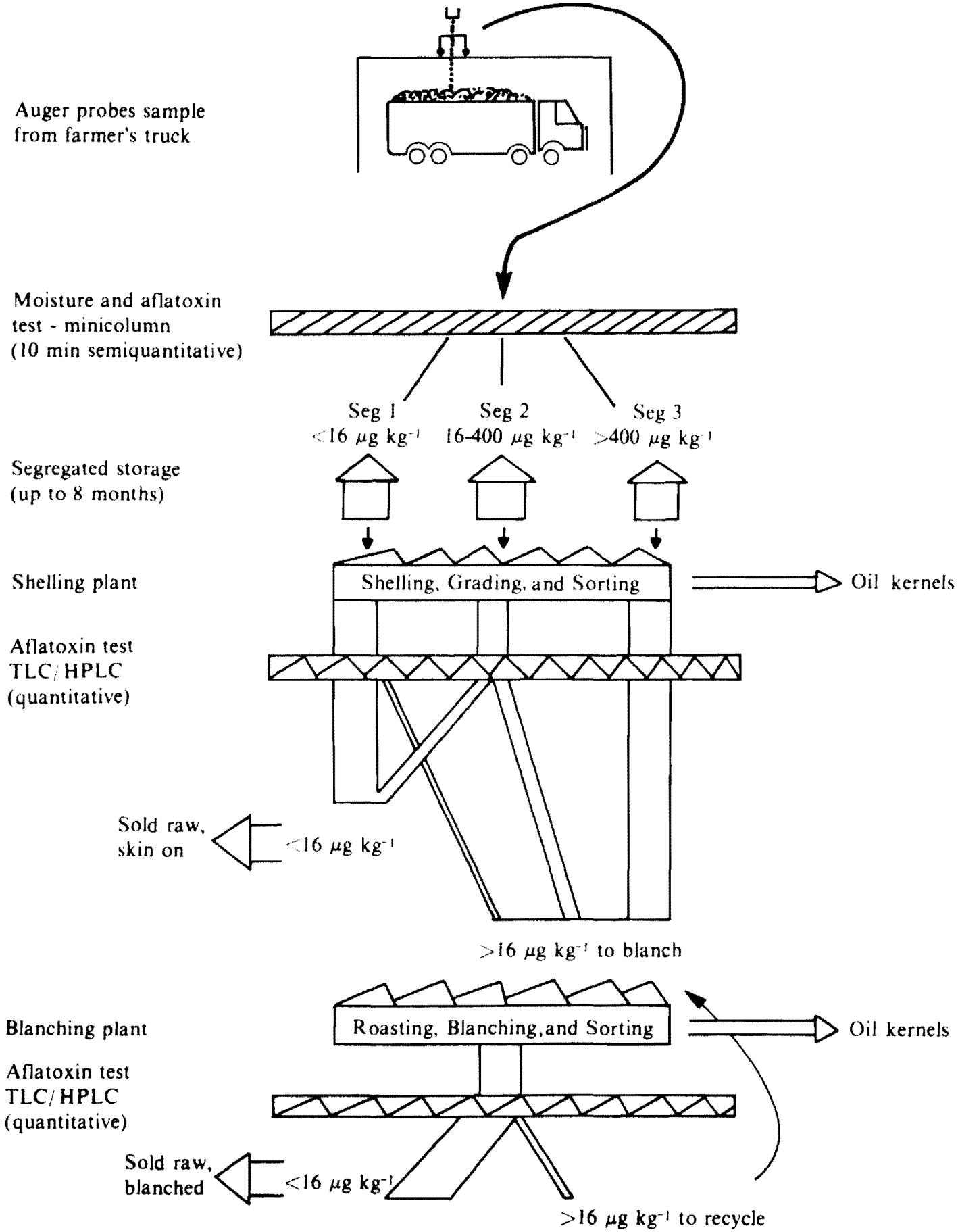

Figure 3. Production flow chart for segregation and sorting of an aflatoxin contaminated groundnut crop, Queensland, Australia.

When a farmer's truckload arrives at a depot, it is first representatively sampled and moisture levels checked. If the nut-in-shell moisture content is below 11%, the truckload is accepted into storage. A minicolumn aflatoxin test is done on the kernels before the truck is tipped and the load is allocated to one of three segregations based on this test result. Segregation No. 1 is for the product with < 16 $\mu g\ kg^{-1}$, segregation No. 2 with 16–400 $\mu g\ kg^{-1}$, and segregation No. 3 with > 400 $\mu g\ kg^{-1}$.

All three segregations are sorted to recover the maximum percentage of product with contamination below the 15 $\mu g\ kg^{-1}$ total aflatoxin regulatory limit. Aflatoxin-positive kernels are diverted into oilstock, the meal from which is primarily used in beef-cattle rations. The intake, segregation, and subsequent handling system outlined in Figure 3 has been evolved to achieve this objective at maximum efficiency for particular market requirements for raw and blanched/roasted kernels.

In an aflatoxin 'audit' study of this system (Baikaloff and Read 1985) over a year's operation, 71% of intake aflatoxin was concentrated into the 16% (by weight) of the crop sent for oil crushing. More than 50% of the aflatoxin in the product sent for blanching/roasting was lost and/or degraded in the operation.

The sorting procedures for both raw (testa on) and blanched/roasted (testa off) kernels combine both electronic color sorters and handpicking of damaged and discolored kernels. This sorting strategy is possible because: firstly the percentage of aflatoxin-positive kernels in a shelled and graded positive lot is small, generally $< 5\%$, with the number of highly positive kernels generally $< 1\%$; and secondly although some positive lots with testa on cannot be "sorted clean", all positive lots can be "sorted clean" after blanching as the combination of *Aspergillus* spp growth and heat always produces a characteristic discoloration. However, not all discolored kernels contain aflatoxin.

Table 2 illustrates the above points. Pickings from Lot 6 were further subdivided into fractions of 6, 12, and 65 $\mu g\ kg^{-1}$, which were 0.16, 0.08, and 0.03% (by weight) of the original lot. While such a distribution is characteristic of preharvest drought-stressed groundnuts, it may not be for crops with significant postharvest problems. The development of specific wavelength UV color sorters would greatly assist the commercial cleanup of aflatoxin-positive lots.

Table 2. Aflatoxin contents of lots of blanched groundnut splits picked to remove typical *Aspergillus* spp discoloration, Kingaroy, Australia, 1982.

		Aflatoxin content ($\mu g\ kg^{-1}$)	
Lot	Weight picked (%)	Pickings	Remainder
1	2.8	1528	Not detected
2	2.9	7900	Trace
3	2.9	2080	Trace
4	1.0	169	Not detected
5	2.0	754	Trace
6	1.3	3226	Trace

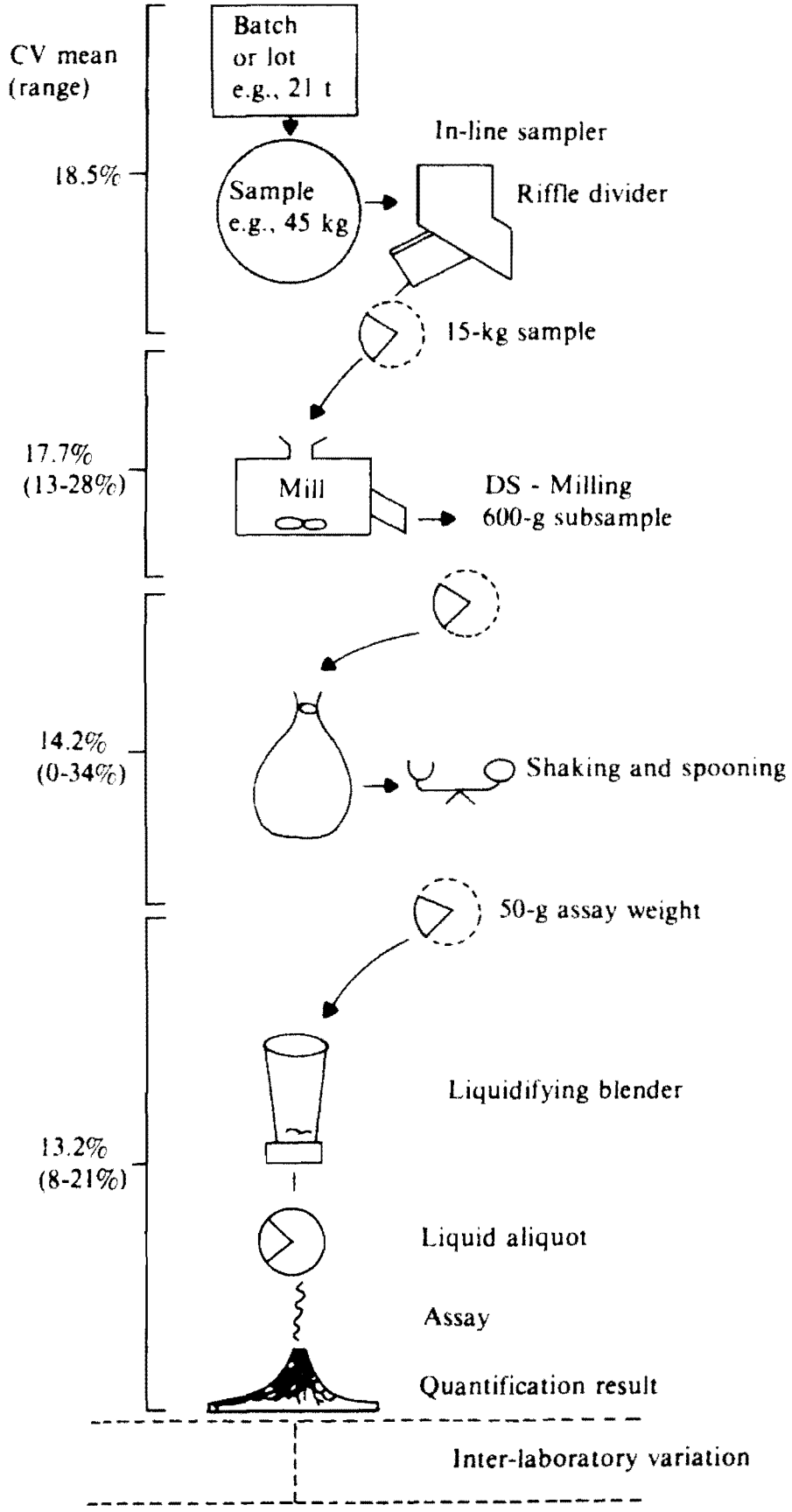

Figure 4. Aflatoxin sampling and analysis scheme used by Peanut Marketing Board (PMB) Australia : sources of variation.

Aflatoxin Testing

As shown in Figure 3, two types of testing are carried out. On receipt, farmers' loads are segregated by a rapid semiquantitative minicolumn test. The method is a modification of the Shotwell and Holaday (1981) procedure, it takes 9.5 mins and costs Aus$ 2.82 (US$ 2.02) per test for labor and materials.

Before the finished product is despatched to customers, it is tested by a more rigorous quantitative thin layer chromotography (TLC) method. Lots can also be assayed by high performance liquid chromotography (HPLC) with a runtime of 4–5 mins, but TLC is thought to be more cost-effective and reliable, and to have equivalent accuracy. The TLC method used is a modification of the method of Pons and Franz (1978). In 1987 the Peanut Marketing Board were placed first for accuracy out of 71 laboratories in the collaborative groundnut aflatoxin program over 8 samples distributed by the Smaller Committee of the American Oil Chemists' Society (AOCS).

Of equal if not greater importance to the assay is sample size, collection, and preparation. Few people in government or industry can come to terms with the novel statistical problems presented by aflatoxin sampling. Horwitz (1982) of the US Food and Drug Administration (USFDA) quotes the interlaboratory coefficient of variation for aflatoxins as 32% of the mean on a "sample". However, a greater variation may be involved when taking a sample to represent a lot. Figure 4 shows the sampling procedure followed by the Peanut Marketing Board amd estimates of the relative contributions of each step to the overall uncertainty in the test result. These estimates give an average total CV of 58% for good-practice, commercial aflatoxin quality control. These values are in reasonable agreement with published figures (Whitaker 1977, Dickens and Whitaker 1979, Whitaker and Dickens 1981) derived by other means. However, even with the best-known statistically designed sampling schemes, and the highest standards of analytical chemistry, commercial disputes as to whether a lot is positive or negative are inevitable.

Conclusions

Aflatoxin contamination is a significant problem to the Australian groundnut industry due to preharvest drought stress. Postharvest contamination is currently controlled by sorting and strict quality control. Despite the best efforts of statisticians and chemists, there is still considerable uncertainty in aflatoxin certification of groundnut lots.

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Discussion

S. Moody. Is the postharvest aflatoxin contamination problem in the northern region of Australia a consequence of preharvest *A. flavus* invasion, or does the fungus only invade seeds after harvest, e.g., during drying in the field, or in storage bins?

M. Read. The problem is a postharvest one, with fungal invasion occurring in the storage bins. The *A. flavus* source is unknown, but the spores are probably omnipresent in groundnut shelling and storage areas. This is a relatively small problem in Australia.

R.V.Bhat. The example of Australia is very relevant to developing countries. The different methods of aflatoxin estimations followed, i.e., minicolumn at the initial stage, and TLC at the final stage are very appropriate for developing countries.

M. Read. Agreed. The minicolumn test as we do it is very quick (9 min) and cheap (US $2 total). Accurate TLC does not require autospotting densiometers as evidenced by our performance in the Smaller program.

C.L.L. Gowda. Does blanching reduce the aflatoxin content of seeds? In the sorting process, you showed that some nuts from the second lot (16–400 $\mu g\ kg^{-1}$ aflatoxin) were brought back to Lot 1 ($<16\ \mu g\ kg^{-1}$ aflatoxin). How could this happen?

M. Read. In the blanching/roasting process aflatoxin contents can be reduced by around 50% and by sorting out the reject nuts it was possible to upgrade lots.

Removal of Aflatoxin B_1 from Peanut Milk by *Flavobacterium aurantiacum*

D.Y-Y. Hao, R.E. Brackett, and T.O.M. Nakayama[1]

Abstract

The potential for using Flavobacterium aurantiacum *NRRL B-184 to remove aflatoxin B_1 from peanut milk was evaluated. Preliminary experiments determined that this bacterium grew in both nondefatted peanut milk (NDPM) and partially defatted peanut milk (PDPM). In neither case was the growth inhibited by the presence of aflatoxin B_1. Other experiments were designed to assess the ability of 10^9 resting (stationary) cells of* F. aurantiacum *to remove aflatoxin B_1 from phosphate buffer (PB), NDPM, and PDPM. After 24 h at 30°C,* F. aurantiacum *decreased aflatoxin B_1 by 40% in PB, 23% in NDPM, and 70% in PDPM. Proteolysis of PDPM before inoculation with* F. aurantiacum *increased recovery of toxin by about 30% over nonproteolyzed samples. This increase in recovery was not observed when NDPM samples were proteolyzed, suggesting that some of the toxin may be bound to the groundnut protein and not be available for removal by* F. aurantiacum.

Résumé

Elimination de l'aflatoxine B_1 du lait d'arachide grâce à *Flavobacterium aurantiacum* : *La capacité de* Flavobacterium aurantiacum *d'éliminer l'aflatoxine B_1 présente dans le lait d'arachide a été étudiée. D'après les résultats des premiers essais, cette bactérie se développe aussi bien dans le lait d'arachide non deshuilé (NDPM) que dans le lait d'arachide demi-deshuilé (PDPM) et son développement n'est nullement inhibé par la présence de l'aflatoxine B_1. D'autres expériences ont été mises au point pour évaluer la capacité de 10 cellules immobiles de* F. aurantiacum *d'éliminer l'aflatoxine B_1 présente dans un tampon phosphaté (PB), le lait NDPM et le lait PDPM. Les taux de diminution de l'aflatoxine B_1 par* F. aurantiacum *après 24 heures à 30°C, sont de 40% (PB), de 23% (NDPM) et de 70% (PDPM). La protéolyse du PDPM avant l'inoculation avec la bactérie a amélioré la récupération de la toxine d'à peu près 30% par rapport aux échantillons non protéolysés. Cependant, la protéolyse des échantillons NDPM n'a pas donné les mêmes résultats, ce qui signifie qu'une partie de la toxine serait liée à la protéine de l'arachide ne permettant pas son élimination par* F. aurantiacum.

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Resúmene

La eliminación de la aflatoxina B_1 de la leche de cacahuate por medio de *Flavobacterium aurantiacum* : *Se evaluó el potencial que tiene el empleo de* Flavobacterium aurantiacum *NRRL B-184 para eliminar la aflatoxina B_1 de la leche de cacahuate. Experimentos preliminares establecieron que esta bacteria se desarrollaba bien, tanto en leche de cacahuate no desgrasada (LCND), como en leche de cacahuate parcialmente desgrasada (LCPD). En ninguno de estos casos fue inhibido el desarrollo por la presencia de aflatoxina B_1. Experimentos adicionales se diseñaron para evaluar la capacidad de una concentración de 10^9 células individuales de* F. aurantiacum, *para la eliminación de aflatoxina B_1, de una solución amortiguada de fosfato (AF), LCND, y LCPD. Después de 24 horas a 30°C.,* F. aurantiacum *redujo en 40% el contenido de aflatoxina B_1 en AF; 23% en LCND, y 70% en LCPD. La proteólisis de LCPD antes de la inoculación con* F. aurantiacum *aumentó la recuperación de toxina en alrededor de un 30% sobre el caso de muestras no proteolizadas, lo cual sugiere que parte de la toxina puede ester ligada a las proteínas del cacahuate, y por lo tanto, no disponible para su eliminación por* F. aurantiacum.

Introduction

One of the most serious and persistent problems facing groundnut growers, processors, and brokers is aflatoxin contamination. As one would expect, this has stimulated much research on ways to prevent and eliminate aflatoxin contamination.

One of the areas of aflatoxin research where a lot of work remains to be done is the detoxification of contaminated products. There has been some work done on this subject, and most of the detoxification methods tried have involved chemical treatments (Doyle et al. 1982). The procedure found most promising and used most often is treatment with ammonia compounds, known as ammoniation.

Biological detoxification has also been tested. Ciegler et al. (1966) at the Southern Regional Research Center have been the most active in searching for microorganisms to accomplish this task. However, of the many cultures they screened, only one bacterium, *Flavobacterium aurantiacum* , was able to completely remove aflatoxin with no residual toxicity.

The Food Technology group at the University of Georgia has been conducting research aimed at using *F. aurantiacum* to detoxify aflatoxin contaminated groundnut products. This paper will report on some general results obtained during this work. The details of this project have been published in scientific journals (Hao and Brackett 1988, 1989).

Materials and Methods

Experimental Design

Initial experiments were designed to determine the stability, growth and survival of *F. aurantiacum* in phosphate buffer and peanut milks. Additionally, the effect of the presence of aflatoxin in the peanut milk on the survival of *F. aurantiacum* was also determined. The purpose of these experiments was to determine optimal conditions for detoxification of nondefatted and partially defatted peanut milks.

In the second phase of this project both peanut milks and buffer were treated with viable *F. aurantiacum* cells and the amount of detoxification determined.

Cultures

Flavobacterium aurantiacum NRRL B-184 was used in all experiments. The stock culture was maintained on trypticase soy agar (TSA) at 5°C. Before use in the experiments, cells were activated by two successive transfers in trypticase soy broth (TSB, Difco®) and incubated at 30°C.

Determination of microbial populations

Populations of *F. aurantiacum* were determined by taking 1-mL samples of test solutions and making appropriate serial dilutions in potassium phosphate buffer (pH 7.2, 0.1 M). One mL of each dilution was deposited and mixed in duplicate TSA pour plates. Colonies were counted after the plates had been incubated for 72 h at 30°C.

Preparation of peanut milks

Florunner type groundnuts were used throughout this study. Nondefatted peanut milk (NDPM) was made by first loosening the skin of groundnut kernels in hot water (79°C) for 90 sec and removing the testae as described by Branch (1984). The kernels were then dried for 24 h at 25°C and NDPM prepared as described by Beuchat and Nail (1978).

Partially defatted peanut milk (PDPM) was prepared by first crushing the whole groundnuts in a Carver Laboratory Press® (Model C, Fred S. Carver Inc., Wisconsin, USA) with 9 to 11 tons of pressure for 45 min. The kernel testae were then removed by a groundnut blancher and PDPM prepared as described by Beuchat and Nail (1978).

Growth and harvest of *F. aurantiacum*

Flavobacterium aurantiacum was grown in both nondefatted and partially defatted milks, by inoculating 0.2 mL of a 24-h culture (about 10^7 cells) into duplicate 250 mL Erlenmeyer flasks containing 100 mL of autoclaved (121°C for 10 min) milks. Populations of *F. aurantiacum* cells were periodically determined. In addition, the pH was monitored with a combination pH probe and meter during the growth period.

Stationary phase cells of *F. aurantiacum* were harvested by first inoculating about 10^7 cells into duplicate 250 mL Erlenmeyer flasks containing 50 mL TSB. After 24-h incubation at 30°C, the TSB containing the cells was centrifuged at 5000 × g for 15 min after which the supernatant fluid was discarded. The pellet was washed with potassium phosphate buffer and recentrifuged. The supernatant fluid was again discarded and the pellet was resuspended in 0.5 mL phosphate buffer. This final suspension was used in all survival experiments.

Preparation, extraction, and analysis of aflatoxin

Aflatoxin B_1 (Sigma®, St. Louis, Missouri, USA) was prepared as a stock chloroform solution (5 mg mL^{-1}) and stored at -20°C in volumetric flasks covered with aluminum foil. All aflatoxin handling was carried out in a darkened area.

Aflatoxin B_1 was extracted from duplicate samples using the method described by Ciegler et al. (1966). Extracts were then evaporated to dryness using a rotary evaporator, and the residue was redissolved in 5 mL of dimethylene chloride. The extracts were then subjected to the cleanup procedure described by Hurst et al. (1984). Cleaned samples were dried under nitrogen and then

reconstituted with high pressure liquid chromatography (HPLC)-grade benzene-acetonitrile (98:2) to known volumes. Aflatoxin was isolated using thin layer chromatography (TLC); using chloroform:acetone (9:1) as developing solvent. Aflatoxin B_1 was quantified using a FOCI® spectrofluorometer with a TLC scanner attachment (Farrand Optical Col. Inc. New York). The spectrofluorometer excitation wavelength was 365 nm and emission wavelength was 445 nm. Results are expressed as means of duplicate readings from duplicate samplings.

Treatment of phosphate buffer, NDPM, and PDPM with *F. aurantiacum*

Samples of potassium phosphate buffer, NDPM, and PDPM were each divided in half. One half was used to determine survival of *F. aurantiacum* in the presence of aflatoxin B_1 and subsequent detoxification. The other half was used as a control for survival studies. Aflatoxin B_1-contaminated milks were prepared by adding 10 μL of the aflatoxin stock solution to the milks so that the final concentration of aflatoxin B_1 was 1 $\mu g\ mL^{-1}$. Contaminated phosphate buffer was similarly prepared except that the final concentration of aflatoxin was 5 $\mu g\ mL^{-1}$. Then 50-mL portions of control and contaminated solutions were placed in duplicate 250-mL Erlenmeyer flasks and heated at 121°C for 10 min to sterilize the solutions and remove any chloroform present that resulted from the addition of aflatoxin B_1.

A cell suspension (0.2 mL, about 10^9 cells) of a 48-h culture of *F. aurantiacum* was added to both control and treatment solutions and all solutions were incubated at 30°C in an incubator-shaker. Solutions were agitated continuously at 200 rpm. Cell populations and aflatoxin B_1 concentrations were quantified after 0, 1, 2, 6, 12, and 24 h.

Effect of proteolysis of groundnut milks on aflatoxin B_1 recovery

Protein contents of NDPM and PDPM were determined by the micro-Kjeldahl method (AOAC 1980) before they were used in the proteolysis experiments. The milks were then contaminated with aflatoxin B_1 as described above. Triplicate samples of contaminated milks were taken before and after autoclaving for quantification of aflatoxin B_1. After autoclaving, samples were allowed to equilibrate for 24 h at 30°C and then triplicate samples were taken. The milks were then treated with protease, after which triplicate samples were taken for aflatoxin analyses.

The protease used in these experiments (type XIV, 5.8 Units mL^{-1}; Sigma®, St. Louis, Missouri) was prepared by suspending 7.5 mg of protease in 7.5 mL of water; and sterilized by filtration (0.22-μm filter). Proteolysis was done by adding 5 units of protease to 100 mL milk and incubating both proteolyzed and control (non-proteolyzed) milks at 20°C for 48 h, after which aflatoxin content was determined.

Results

Flavobacterium aurantiacum grew well in both NDPM and PDPM (Fig. 1). However, populations of viable cells increased more slowly in PDPM during the first 48 h. The presence of up to 1 $\mu g\ mL^{-1}$ of aflatoxin B_1 did not affect the growth of *F. aurantiacum* in either of the groundnut milks (Fig. 2). The presence of aflatoxin B_1 did not reduce the viability of resting cells in either of the milks, or in the phosphate buffer (Fig. 3). These results suggest that this bacterium could survive well in either liquid during subsequent detoxification trials.

A major problem in comparing the degree of degradation among the samples was the low recovery of aflatoxin from the samples at time 0. These initial recoveries averaged only 30%, for

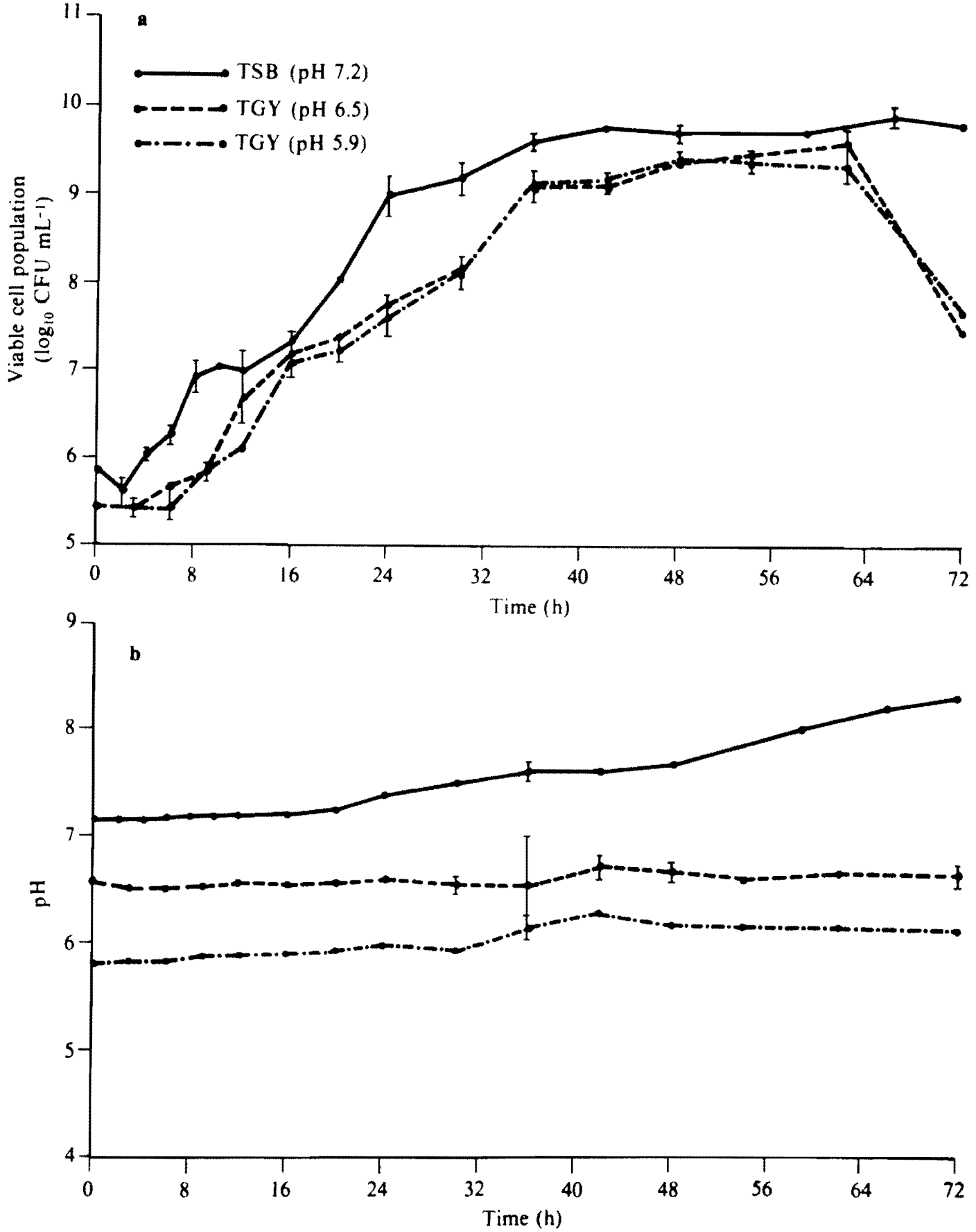

Figure 1a. Growth of *Flavobacterium aurantiacum* in trypticase soy broth (TSB, pH 7.2), and tryptone-yeast extract-glucose broth (TGY, pH 5.9 or 6.5), b. Associated pH changes.

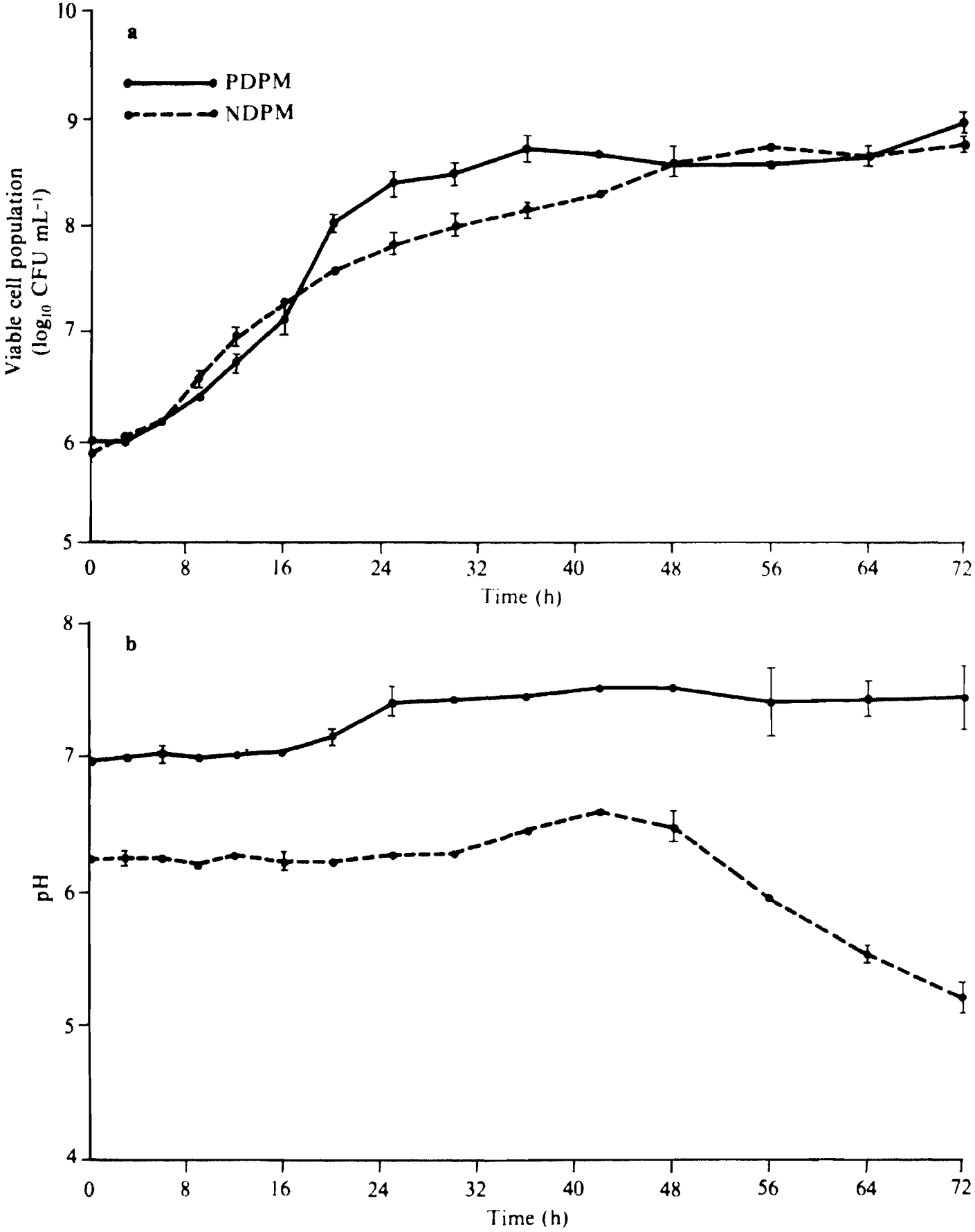

Figure 2a. Growth of *Flavobacterium aurantiacum* in nondefatted peanut milk (NDPM) and partially defatted peanut milk (PDPM), b. Associated pH changes.

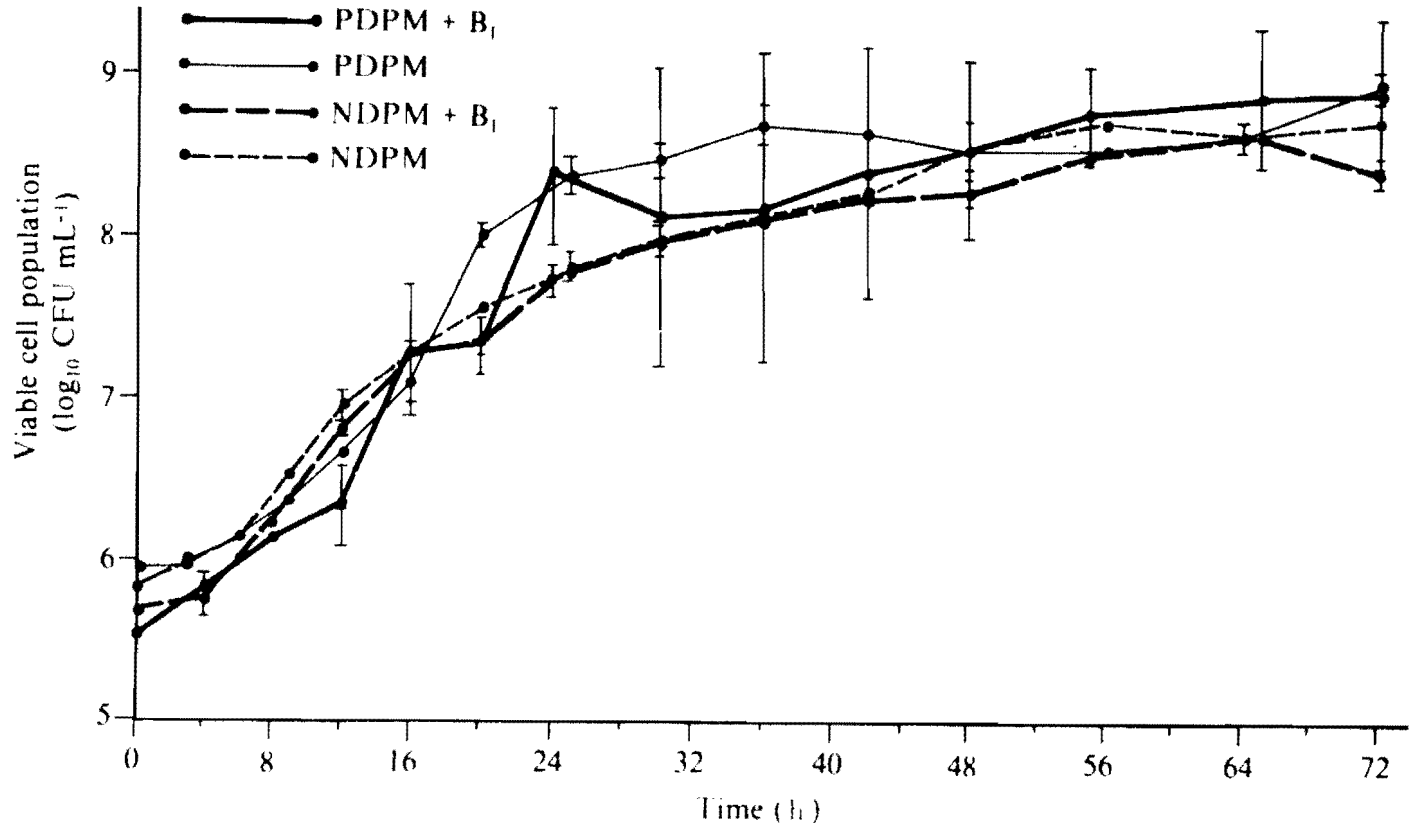

Figure 3. Growth of *Flavobacterium aurantiacum* in nondefatted peanut milk (NDPM) and partially defatted peanut milk (PDPM) with (+B_1) or without aflatoxin B_1.

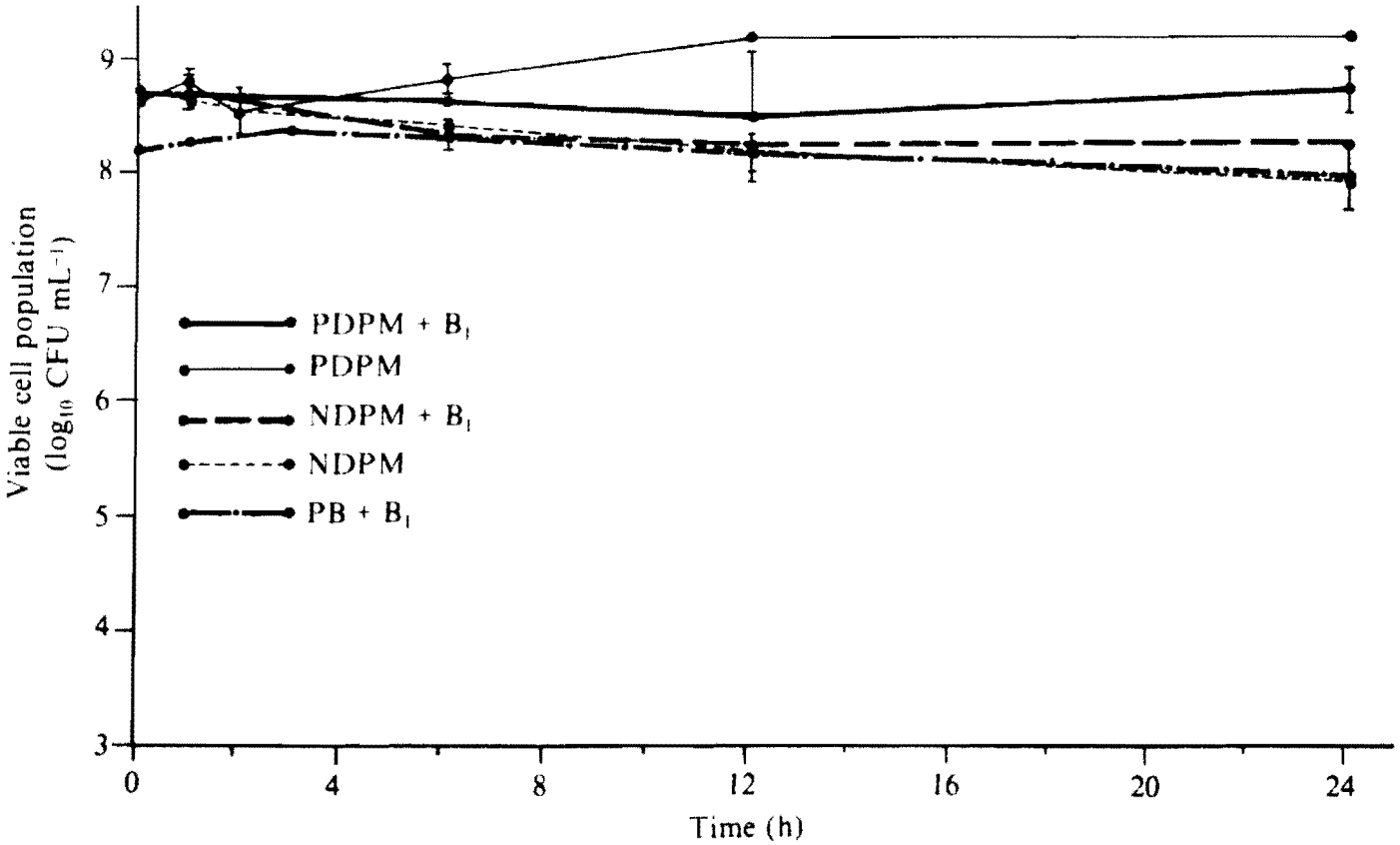

Figure 4. Survival of *Flavobacterium aurantiacum* in phosphate buffer (PB, pH 7.0) and nondefatted peanut milk (NDPM) and partially defatted peanut milk (PDPM) with (+ B_1) or without aflatoxin B_1.

phosphate buffer, 51% for PDPM, and 82% for NDPM. At later times, recovery increased for all samples, and then decreased in all samples except for those of PDPM that had not been inoculated with *F. aurantiacum*. Because recoveries differed for the three media, the removal of aflatoxin is reported as percentage of aflatoxin B_1 recovered at time 0.

The addition of *F. aurantiacum* resulted in a reduction of the aflatoxin concentrations in both milks (Fig. 4), but the degree of reduction differed among the solutions. Reduction was greatest in PDPM (74%), followed by phosphate buffer (40%), and NDPM (23%). In comparison, recovery of aflatoxin B_1 from contaminated milks not inoculated with *F. aurantiacum* did not significantly change during the experiment.

Results from other studies (Brackett and Marth 1982) have suggested that the binding of aflatoxins to protein may affect the efficiency of their recovery during solvent extraction. The low initial recovery of aflatoxin B_1, and the apparent increase in recovery over time (Fig. 4) suggested that a similar phenomenon might be happening in milks. This stimulated us to determine the effects of proteolysis on aflatoxin recovery. Results of these experiments (Fig. 5) indicated that proteolysis increased recovery of aflatoxin B_1 from PDPM by about 35%. However, proteolysis did not enhance recovery of aflatoxin B_1 from NDPM (Fig. 6) possibly because the protein content of this milk (18.2 mg mL^{-1}) was much less than that of PDPM (35.1 mg mL^{-1}). Thus, one would expect less opportunity for binding to protein. However, because of

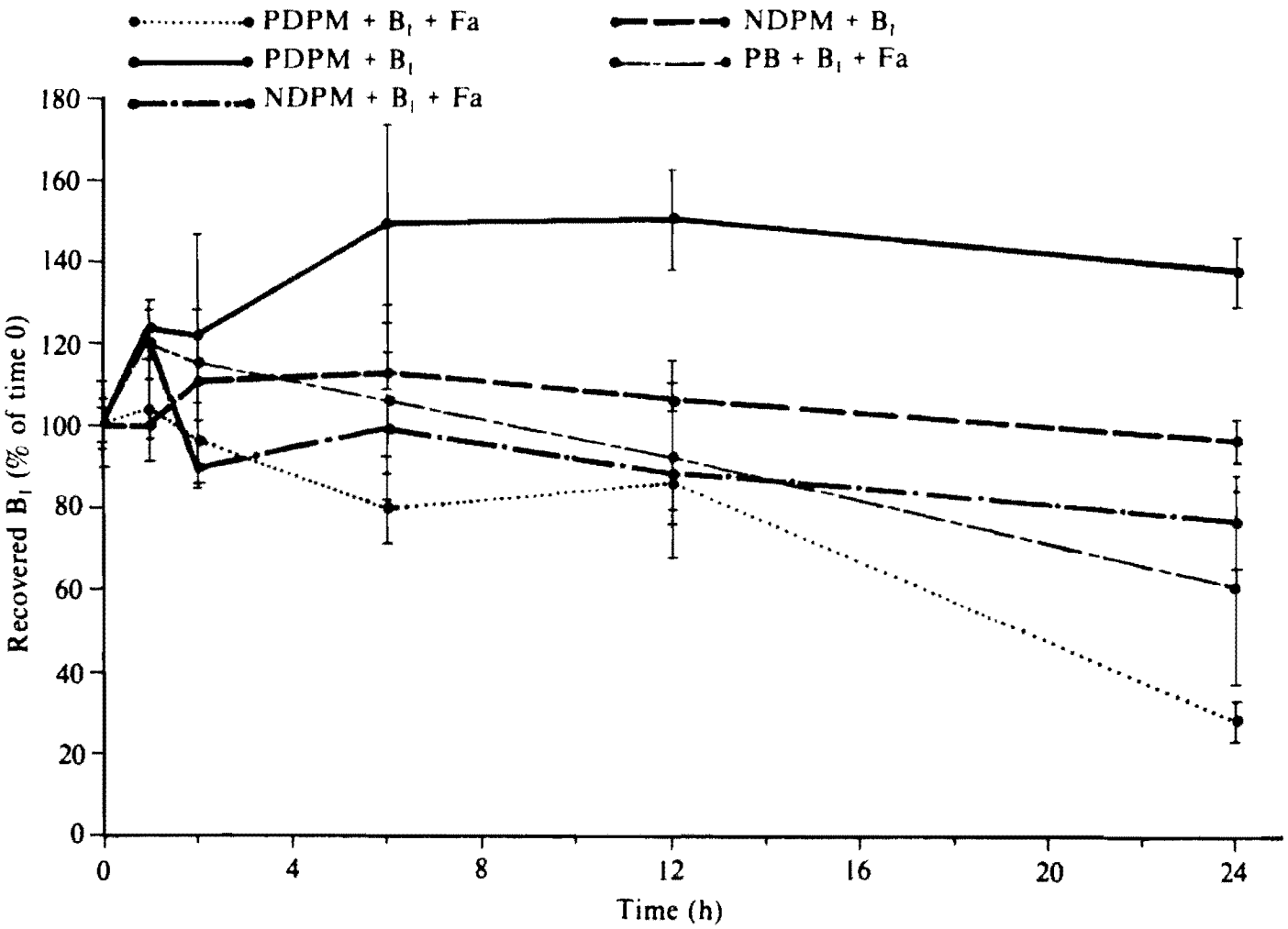

Figure 5. Comparison of aflatoxin B_1 (+ B_1) removed by *Flavobacterium aurantiacum* (+ Fa) in phosphate buffer (PB) and nondefatted peanut milk (NDPM) and partially defatted peanut milk (PDPM). Data reported as the percentage of B_1 recovered at time 0.

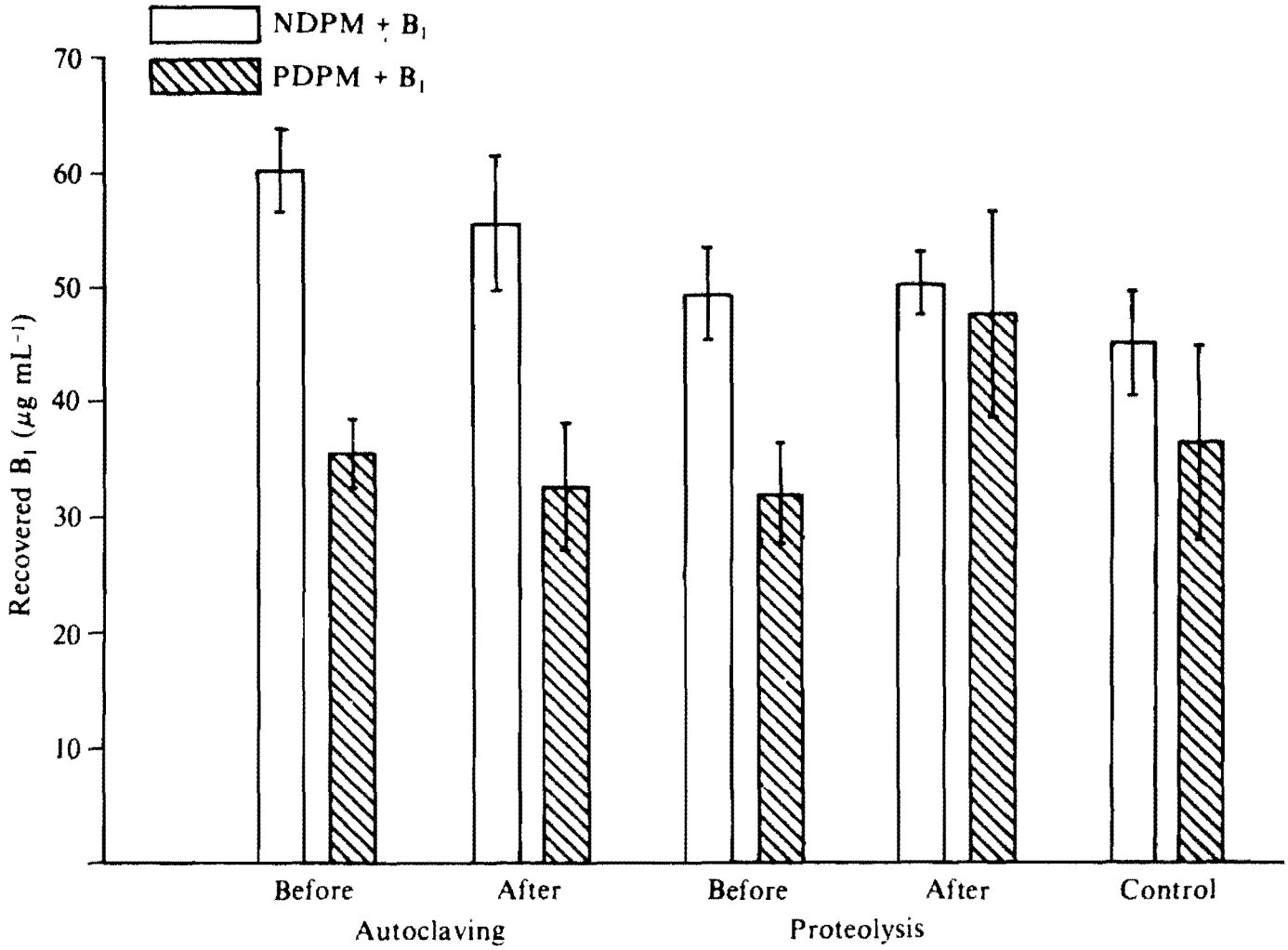

Figure 6. Effect of proteolysis on aflatoxin B_1 (+ B_1) recovery from nondefatted peanut milk (NDPM) and partially defatted peanut milk (PDPM).

insufficient knowledge of aflatoxin-protein binding, the actual effect of groundnut protein on aflatoxin B_1 recovery is not yet known.

Discussion

Results of these experiments confirm those of earlier studies (Ciegler et al. 1966) in clearly demonstrating that *F. aurantiacum* can reduce aflatoxins in some foods. Although it is likely that a detoxification system using this bacterium would work best for liquid products, it has also been shown to work with whole groundnuts (Ciegler et al. 1966).

One of the major problems in using *F. aurantiacum* to detoxify peanut milks is that this organism is brightly pigmented orange-pink. Thus, the addition of the bacterium to the clear white milks results in a discolored product. In most cases, this would be considered an undesirable quality. Moreover, a thorough evaluation of the safety of this organism would be necessary before it could be added to foods or feeds.

The potential for using metabolic systems associated with *F. aurantiacum* offers more promise for removing aflatoxins from foods or feeds. However, much more work needs to be done on both the basic physiology of this bacterium, and the mechanism it uses to degrade aflatoxin.

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Discussion

S. Nahdi. You said that the organisms removed the aflatoxins, but do they not merely adsorb the aflatoxin molecules? If this is the case, and you change the substrate environment, could the adsorbed molecules again enter into the media?
T.O.M. Nakayama. We have not determined whether the aflatoxin is merely adsorbed or broken down. Our experiments merely show removal.
F.S. Chu. Concerning the mechanism of removal of aflatoxin by *F. aurantiacum*, two possible mechanisms might be involved: (1) If aflatoxin B_1 is metabolized to aflatoxin B_{2a}, aflatoxin B_{2a} may then form a "Schiff's base" with the amino group in proteins; or/and (2) aflatoxin B_1 may be activated by the bacterium and then react with DNA and other proteins.
I.A. Rana. You have suggested that the aflatoxin molecule breaks down at a suitable pH to two aldehyde molecules which behave as sugars and react with protein to form a Schiff's base. Is this reaction possible at room temperature? I believe heat is needed for this reaction to occur.
T.O.M. Nakayama. I believe it occurs at ambient temperature, but it would be more rapid at elevated temperatures.

H. Amra. Can this method of biological control by *F. aurantiacum* be used on a large scale?
T.O.M. Nakayama. Not at present.
B. Singh. Does this bacterium cause flavor problems or color problems? Have you thought about the practical aspects of this problem?
T.O.M. Nakayama. We did not consider the method to be a practical one, but rather a tool to investigate the basic processes involved.
T. Shantha. You said when toxin was added externally, that 70% was removed. Did you conduct the experiment on naturally occurring toxin?
T.O.M. Nakayama. No.
T. Shantha. In my experience, an added external toxin behaves somewhat differently from a naturally occuring toxin, and is less difficult to remove.
A.H. Siwela. Assuming that aflatoxin B_1 is metabolized to aflatoxin B_{2a}, and binds to protein by forming a Schiff's base, then surely the activated epoxide species must bind covalently to DNA. What is its mutagenecity on *F. aurantiacum*?
T.O.M. Nakayama. No studies on mutagencity were conducted.
V.K. Mehan. How popular is peanut milk in the USA?
T.O.M. Nakayama. Peanut milk is not a commercial product in the USA to my knowledge. There are other nondairy milks produced, but not specifically from groundnuts.

Detoxification of Groundnut Seed and Products in India

T. Shantha[1]

Abstract

The exposure of groundnut oil contaminated with aflatoxin B_1 to bright sunlight for a given period of time completely destroys the toxin. The safety and shelf life of the sunlight-exposed oil have been confirmed. Aflatoxin is present in finely suspended solids in the oil and most of it can be removed by filtration or by extraction with 10% NaCl. Aflatoxin-contaminated groundnut kernels (0.5-mm thick flakes) can be partially detoxified if exposed to sunlight for 14 h. To remove aflatoxin from groundnut cake, 50% alcohol, acetone, methanol, or 1% calcium chloride can be used. Treating the protein isolate with hydrogen peroxide, and groundnuts in the form of thin flakes or groundnut cake powder with urea and soyabean flour, with or without formaldehyde can destroy 90% of their aflatoxin content.

Résumé

Détoxification des graines d'arachide et de leurs produits : *L'exposition de l'huile contaminée par l'aflatoxine B_1 au soleil pendant une durée déterminée permet de détruire complètement la toxine. Il a été confirmé que ce traitement donne une huile saine tout en prolongeant sa durée de conservation. Une grande partie de l'aflatoxine présente dans les particules fines suspendues dans l'huile peut être enlevée par la filtration ou par l'adjonction d'une solution de NaCl à 10%. L'exposition au soleil pendant 14 heures de graines d'arachide contaminées (écailles fines de 0,5 mm) permet de les détoxifier partiellement. Le traitement par l'alcool à 50%, l'acétone, le méthanol ou le chlorure de calcium à 1% assure la détoxification des tourteaux. Le traitement des isolats de protéine par l'eau oxygénée et celui des arachides en écailles fines ou en poudre par l'urée et la farine de soja, avec ou sans formaldéhyde, suppriment 90% des aflatoxines. Les bénéfices et les inconvénients de ces méthodes sont discutés.*

Resúmene

La eliminación de toxinas de la semilla de cacahuate y otros productos en la India : *La exposición del aceite de cacahuate contaminado con aflatoxina B_1 a la luz solar intensa durante un lapso de tiempo determinado, destruye completamente la toxina. Se ha confirmado que el aceite expuesto a la acción de la luz solar es completamente inocuo y se conserva en buenas condiciones comerciales en forma prolongada. La aflatoxina se encuentra presente en forma de sólidos finamente divididos y suspendidos en el aceite, y la mayor parte de éstos puede ser*

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eliminados por filtración o mediante extracción con una solución de NACL al 10%. En los granos molidos de cacahuate (en forma de escamas de 0.5 mm de espesor) contaminados con aflatoxinas, es factible eliminar parcialmente las toxinas, mediante su exposición a la acción de la luz solar durante 14 horas. Para eliminar totalmente las aflatoxinas de la torta de cacahuate, se puede utilizar alcohol al 50%, acetona, metanol, o una solución de cloruro de calcio al 1%. El tratamiento de la proteína purificada con agua oxigenada, y de los cacahuates molidos en forma de escamas delgadas o pulverizadas con urea y harina de soya, con o sin formaldehído, puede destruir el 90% de su contenido de aflatoxinas. Se discuten las ventajas y las desventajas de estos procedimientos.

Introduction

Aflatoxin contamination of groundnuts is a serious problem in India where the average level of aflatoxin in groundnuts is about 100 $\mu g\ kg^{-1}$ (survey by Central Food Technological Research Institute (CFTRI), Mysore), and in groundnut oil about 170 $\mu g\ kg^{-1}$ (Shantha and Sreenivasamurthy 1975), levels that are not acceptable to the Indian Standards Institution (ISI) or any international agency. A general practice is to handpick healthy seeds for export and table purposes and to divert the remainder to oil mills. About 15% of the total toxin will be carried in oil and 85% will remain in the cake (Basappa and Sreenivasamurthy 1974). Depending on the quality of the raw material, the oil often contains aflatoxin above the permissible limit of 30 $\mu g\ kg^{-1}$. The extensive research work carried out by CFTRI to decontaminate groundnuts and their products is reviewed.

Methods Used to Detoxify Groundnut Oil

Photolysis (Shantha and Sreenivasamurthy 1977)

Since toxin in oil is in the form of very fine suspended particles, it is inactivated by exposure to sunlight (50 000 to 100 000 Lux) for 15 to 30 min. Ultraviolet, infrared, and visible light (Tungsten lamp 150W) could not individually detoxify oil to any significant extent in 2 h (Table 1). Sunlight can destroy 100% of the toxin in about 15 min but since the other lights are not so strong as sunlight, prolonged exposures (18 h) are necessary to significantly decrease aflatoxin content.

Detoxification by photolysis was verified by a feeding test using albino rats. Rats fed on photolyzed oil showed no abnormalities.

Exposure of oil to direct sunlight is more effective than exposure to ultraviolet light in destroying the toxin as measured by fluorescence or biological activity. Such oil retains its organoleptic quality even after being stored for 3 months in the dark. (Shantha and Sreenivasamurthy 1980).

Extraction with sodium chloride

Extraction of contaminated oil with a 10% aqueous solution of sodium chloride (1:4) at 80° C for 30 min removes 85% of the aflatoxin from the oil (Shantha and Sreenivasamurthy 1975). Any oil mill with facilities to refine crude groundnut oil can easily follow this method.

Table 1. Effect of light of different wavelengths on aflatoxin content of groundnut oil.

Source of light	Aflatoxin content ($\mu g\ g^{-1}$)					
	Oil exposed for 2 h.			Oil exposed for 18 h.		
	Before exposure	After exposure	Destruction (%)	Before exposure	After exposure	Destruction (%)
Shortwave ultraviolet	0.2	0.17	45	0.11	Not estimated	Not estimated
Longwave ultraviolet	0.17	0.10	40	0.11	0.015	87
Tungsten lamp 150W (visible)	0.17	0.13	30	0.11	0.022	82
Infrared	0.25	0.25	0	0.11	0.033	73

Filtration

Based on the principle that aflatoxin is in finely suspended form in the oil, special filter pads were developed by Basappa and Sreenivasamurthy (1979).

Use of two such filter pads to filter 100 kg of contaminated commercial groundnut oil, at a filtration rate of 12 kg h^{-1}, resulted in an average removal of 88% of the toxin.

Detoxification of Groundnut Seed and Cake

Aflatoxin in groundnut oil can easily be detoxified by sunlight as it remains in finely suspended form, but in groundnut seeds or cake it is bound to the protein molecules (Shantha and Sreenivasamurthy 1981). Sunlight cannot destroy this bound aflatoxin, so it is necessary to use alternative physical or chemical treatments to detoxify seeds and cake.

Detoxification of groundnut meal by hydrogen peroxide

This method (Fig. 1) uses hydrogen peroxide and alkali to destroy the toxin. It is the most suitable method of detoxification, because hydrogen peroxide does not leave any residual taste or smell in the product, and the safety of detoxified products can be demonstrated by feeding tests in ducklings.

Removal of aflatoxin by salt solutions

Several salt solutions are effective in removing toxin from groundnut meal. Results (Table 2) indicate that 1% calcium chloride is better than other salt solutions since it removes 80% toxin with only 6% protein loss.

Removal of aflatoxin by various solvents

Among different solvents tried at CFTRI, stirring contaminated powdered groundnut cake in 50% ethyl alcohol adjusted to pH 4.5, and then exposing it to sunlight for 6h was found to remove about 70% aflatoxin.

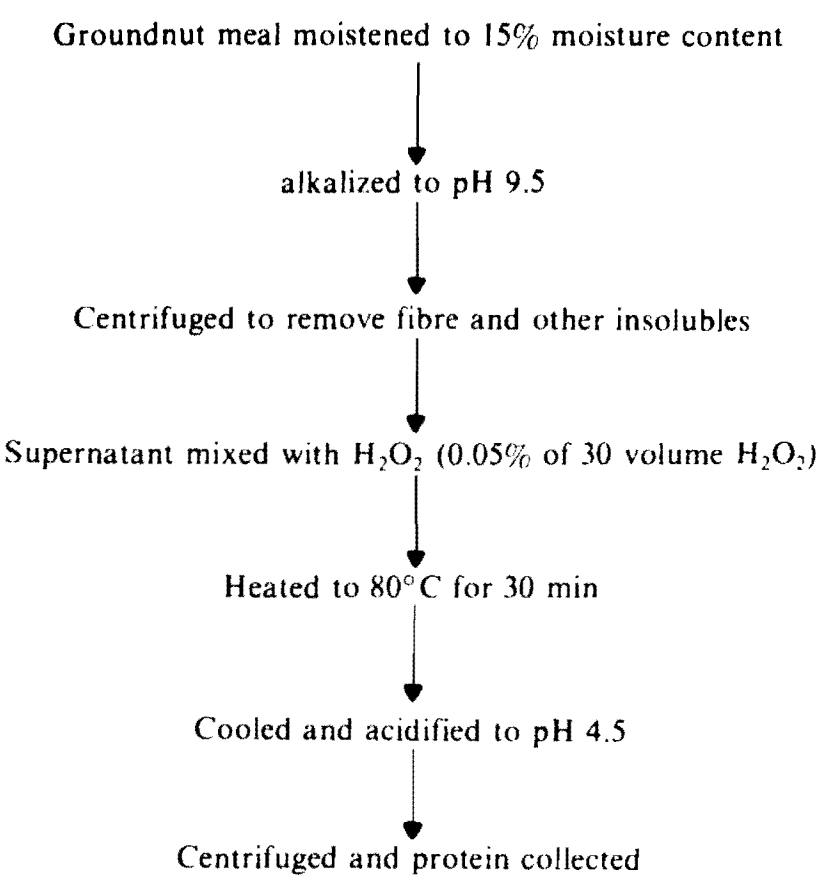

Figure 1. Stepwise method of detoxification using hydrogen peroxide.

Detoxification of groundnut flakes by sunlight and urea

When groundnut flakes 0.25- to 0.5-mm thick are spread in thin layers under sunlight of 50 000–100 000 Lux for about 14 h, 60% of aflatoxin B_1 contamination is lost. Pretreatment of the groundnut flakes with urease enzyme from soybean flour and urea in aqueous solution enhances detoxification by sunlight (Shantha et al. 1986). This method can remove 85–90% of the toxin and any residual ammonia can be removed by blowing air at 60°C through the detoxified product. Since this method is inexpensive and preliminary toxicological and nutritional tests on rats have shown it to be safe, it has potential use for the detoxification of cake used for cattle feed, even though the product is dark brown.

Table 2. Extraction of aflatoxin by different salt solutions.

Salt solution	Protein extracted (%)	Initial aflatoxin ($\mu g\ kg^{-1}$)	Residual aflatoxin ($\mu g\ kg^{-1}$)	Toxin extracted (%)
NaCl (2%)	8.0	10	5	50
NaCl (1%)	Negligible	10	10	0
$NaHCO_3$ (1%)	33.0	10	0	100
$CaCl_2$ (1%)	6.0	10	2	80
Sodium citrate (1%)	-	10	10	0
$NaHCO_3$ + NaH_2PO_4 (1%, pH 7.4)	-	10	7	30
Buffer (carbonate + bicarbonate pH 9.05)	-	10	2	80

Detoxification of groundnut cake by urea-urease and formaldehyde

The addition of formaldehyde to moisten groundnut cake, to the urea-urease treatment outlined above enhances the detoxification effect, but the method required high temperatures (121°C for 30 min), and although 95% of the toxin is removed, the available lysine and methionine contents of the product are reduced.

Preliminary feeding tests using rats indicate that the treated product is not safe if used as a sole source of protein. However, it has not been tried on cattle and since it is reported that ruminants can metabolize formaldehyde and formaldehyde-treated groundnut meal (Codifer et al. 1976). Groundnut cake treated with urea, urease, and formaldehyde could be tried as a cattle feed, provided toxicological tests were first conducted to prove its safety.

Acknowledgment

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Discussion

I.A. Rana. What is the cost-effectiveness of the urea formaldehyde method?
T. Shantha. We have only recently established this method and have yet to work out its cost effectiveness.

Session VI

Methods for Aflatoxins Analysis

Current Immunochemical Methods for Analysis of Aflatoxin in Groundnuts and Groundnut Products

F.S. Chu[1]

Abstract

With the availability of specific monoclonal and polyclonal antibodies against mycotoxins in recent years, simple, sensitive and specific radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) of mycotoxins have been developed. The sensitivities of RIA were in the range of 0.1-0.5 ng and of ELISA 2.5-25 pg assay^{-1}. Simple and quick immunoassay protocols (ELISA) for monitoring aflatoxin B_1 in groundnuts and groundnut products, that require less than 1 h to complete, have been developed and successfully tested in naturally contaminated groundnut samples at levels about 5 to 10 $\mu g\ kg^{-1}$. In addition, antibodies against mycotoxins have been used as an immunohistochemical tool to monitor mycotoxins in tissues and for the preparation of immunoaffinity columns that were then used, either for aflatoxin determination in groundnuts, or as a cleanup tool for aflatoxin analysis. Details of recent progress on the production of antibodies, antibody specificity, and the advantages and disadvantages of different immunoassays, as well as problems associated with immunochemical research on mycotoxins, with emphasis on aflatoxin, are reviewed. Emphasis is centered on the immunoassays of aflatoxin in groundnut products.

Résumé

Les méthodes immunochimiques courantes de l'analyse des aflatoxines dans les arachides et leurs produits : *La découverte récente des anticorps monoclonaux et polyclonaux spécifiques aux mycotoxines a abouti à la mise au point des tests d'une simplicité, sensibilité et spécificité adéquates, notamment le radio immuno dosage (RIA) et la méthode immunoenzymatique de dosage des anticorps (ELISA). La sensibilité du RIA varie de 0,1 à 0,5 ng et de l'ELISA de 2,5 à 25 pg par essai. Des protocoles ELISA simples demandant moins d'une heure ont été élaborés pour le dosage de l'aflatoxine B_1 dans les arachides et leurs produits. Ces essais ont permis d'analyser avec succès les échantillons ayant subi une contamination naturelle de 5-10 $\mu g\ kg^{-1}$. Les anticorps des mycotoxines sont utilisés en tant qu'un outil immunohistochimique afin de doser les mycotoxines présentes dans les tissus et d'établir les colonnes d'immunoaffinité. Celles-ci sont utiles au dosage de l'aflatoxine dans les arachides, et à la purification pour l'analyse des aflatoxines. Le progrès réalisé dans la production des anticorps, la spécificité des anticorps ainsi que les avantages et inconvénients des différents tests immunochimiques sont examinés en même temps que les problèmes liés à la recherche immunochimique sur les mycotoxines, en particulier les aflatoxines. L'accent est mis sur les méthodes immunochimiques d'évaluation des aflatoxines dans les produits arachidiers.*

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Resúmena

Métodos inmunoquímicos comunes para analizar las aflatoxinas en el cacahuate y sus derivados : *Con el reciente descubrimiento de anticuerpos monoclonales y policlonales específicos para las micotoxinas, se han desarrollado pruebas sencillas, muy sensibles y específicas, tales como las cuantificaciones tipo radioinmunológico (RIA) y el método inmunoenzimático de cuantificación de anticuerpos (ELISA). Las sensibilidades del RIA varían de 0.1 a 0.5 ng y de ELISA de 2.5 a 25 pg por prueba. Se han desarrollado sencillos y rápidos protocolos ELISA, para la cuantificación de aflatoxina B_1 en el cacahuate y sus derivados, que requieren menos de una hora para efectuarse, y que se han probado con éxito en muestras de cacahuate contaminadas bajo condiciones naturales, a niveles de 5 a 10 $\mu g\ kg^{-1}$. Los anticuerpos de las micotoxinas han sido utilizados como un procedimiento, inmunohistoquímico, para la cuantificación de micotoxinas en tejidos y para la preparación de columnas de inmunoafinidad, que posteriormente se utilizaban, ya sea para la determinación de aflatoxinas en cacahuates, o en el proceso de extracción y "lavado" en el análisis de aflatoxinas. Se examinan los avances recientes en la producción de anticuerpos, la especificidad de los anticuerpos, pruebas inmunoquímicas, así como problemas asociados con las investigaciones inmunoquímicas sobre micotoxinas, y de manera especial sobre las aflatoxinas. Se hace hincapié en los métodos inmunoquímicos para cuantificar las aflatoxinas en productos derivados del cacahuate.*

Introduction

The main objective of this paper is to describe current immunochemical methods for analysis of aflatoxin in groundnuts. I appreciate the opportunity to share our experiences in this rapidly developing research area.

Contamination of aflatoxin in groundnuts and groundnut products was first realized in the early 1960s and this led to a renewed interest in research into mycotoxins. Further studies have led us to not only recognize that groundnuts under certain conditions are prone to invasion by the aflatoxin-producing fungus *Aspergillus flavus*, but also to understand the potential hazards of the presence of aflatoxin and other mycotoxins in foods and feeds to human and animal health. Because the mycotoxin problem is difficult to avoid, the most effective measure in controlling the problem depends on a rigorous program of monitoring such toxins in food and feeds. Consequently, there is a need to develop a sensitive, specific, and simple method for toxin determination. The rapid progress in this area of research is evidenced by the vast numbers of research papers cited by the "General Referee on Mycotoxins" in the "Annual Report on Mycotoxins" which appears every year in the March issue of the Journal of the Association of Official Analytical Chemists (AOAC) (Scott 1986). This paper concentrates on immunochemical methods for detection and quantification of mycotoxins, an area of research with which we have been heavily involved for the last 14 years. Details of aflatoxin analysis by other methods and other commodities are available in several recent reviews (van Edgmond and Paulsch 1986, Cole 1986).

General Considerations for Mycotoxin Analysis

Because of the diversity of their chemical structures and physicochemical properties, approaches for mycotoxin analysis vary considerably. The analysis is further complicated by the uneven distribution of toxins in the sample as well as interference from the sample matrix, in which

different interference materials are present in different samples. Consequently, different procedures are used for different commodities even within a group of mycotoxins, and this is the case with aflatoxins. For example, the AOAC procedure for analysis of aflatoxin in groundnuts is different from those used for maize, cottonseed, and other nuts (Horwitz 1980). In general, the suspected contaminated commodity is first subjected to a rigorous sampling program (Campbell et al. 1986). The toxin is then extracted from the sample and subjected to a cleanup treatment to remove interference substances before analysis. In the actual analysis, methods for separation and quantification are included in a single step. In some instances, it is necessary to derive the toxin before separation and quantification. In addition, known standards are included in the protocol to assure the identity of the toxin present in the sample.

With so many steps involved, the analysis of aflatoxins by chemical methods is time consuming, and each step could introduce errors into the analysis. Thus, coefficients of variation of more than 30% are not uncommon (van Edgmond and Paulsch 1986). To overcome difficulties encountered with the chemical and biological methods, immunological methods have been developed for mycotoxins assays (Chu 1984, 1986a-d, Garner et al. 1985). Methods have been developed for the production of specific antibodies for the most important mycotoxins, including aflatoxin B_1 and some of its metabolites (Chu 1986d, Chu et al. 1985, Garner et al. 1985), diacetoxyscirpenol (Chu et al. 1984, Zhang et al. 1986a), deoxynivalenol (DON) and related mycotoxins (Zhang et al. 1986b, Kemp et al. 1986), kojic acid (Abdalla and Grant 1981), ochratoxin A (Chu 1986d), patulin (Mehl et al. 1986), rubratoxin B (Chu 1986d), sterigmatocystin (Chu 1986d, Morgan et al. 1986a), T-2 toxin and its major metabolites (Chu 1986d, Fan et al. 1987a, Wei et al. 1986, Zhang et al. 1986a), and zearalenone (Chu 1986d).

These antibodies have been utilized in radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) for the estimation of mycotoxins (Chu 1984, 1986d). Specific antibodies have also been used as immunohistochemical reagents for monitoring mycotoxins in animal tissues (Pestka et al. 1983, Lee et al. 1984a, b). In addition, antibodies have been conjugated to a solid phase and then used in immunoassays (Groopman et al. 1985, Sun and Chu 1977, Wu et al. 1983) and by affinity columns for purification and concentration of mycotoxins in biological fluids. As a result of these developments, a new dimension has emerged for mycotoxin analysis and for diagnosis of mycotoxicoses in humans and animals. Several kits for the analysis of aflatoxins by immunochemical methods are commercially available.

Mycotoxins are not antigenic. Hence, they must be conjugated to a protein or polypeptide carrier before immunization. Methods have been developed to conjugate mycotoxins to proteins. Although in earlier studies polyclonal antibodies produced in rabbits were used, more recently monoclonal antibodies specific for mycotoxins have been used (Fan et al. 1987c, Gendloff et al. 1987, Groopman et al. 1984, Hunter et al. 1985, Lubet et al. 1983, Sun et al. 1983, Woychik et al. 1984). Approaches used for the production of antibodies against aflatoxins are shown in Figure 1.

The effectiveness of the antibodies used in different immunological studies is dependent upon their specificity and titers. Whereas specificity provides information on the cross-reactivity of antibody with different toxin analogs (quality), the antibody titers express both the quantity and quality. Experimentally, the antibody specificity is measured by a competition binding assay with different structurally related analogs, whereas the titers are measured by the amount of a specific marker ligand bound to the antibody. The specificity of an antibody is determined primarily by the chemical structure and conformation of a mycotoxin, its derivatives, or its metabolites, that have been used in the antibody production, and the site in the mycotoxin molecule where linkage was made for conjugating to a protein carrier. The side chain groups in the whole molecules also play important roles. It is very important to determine the specificities

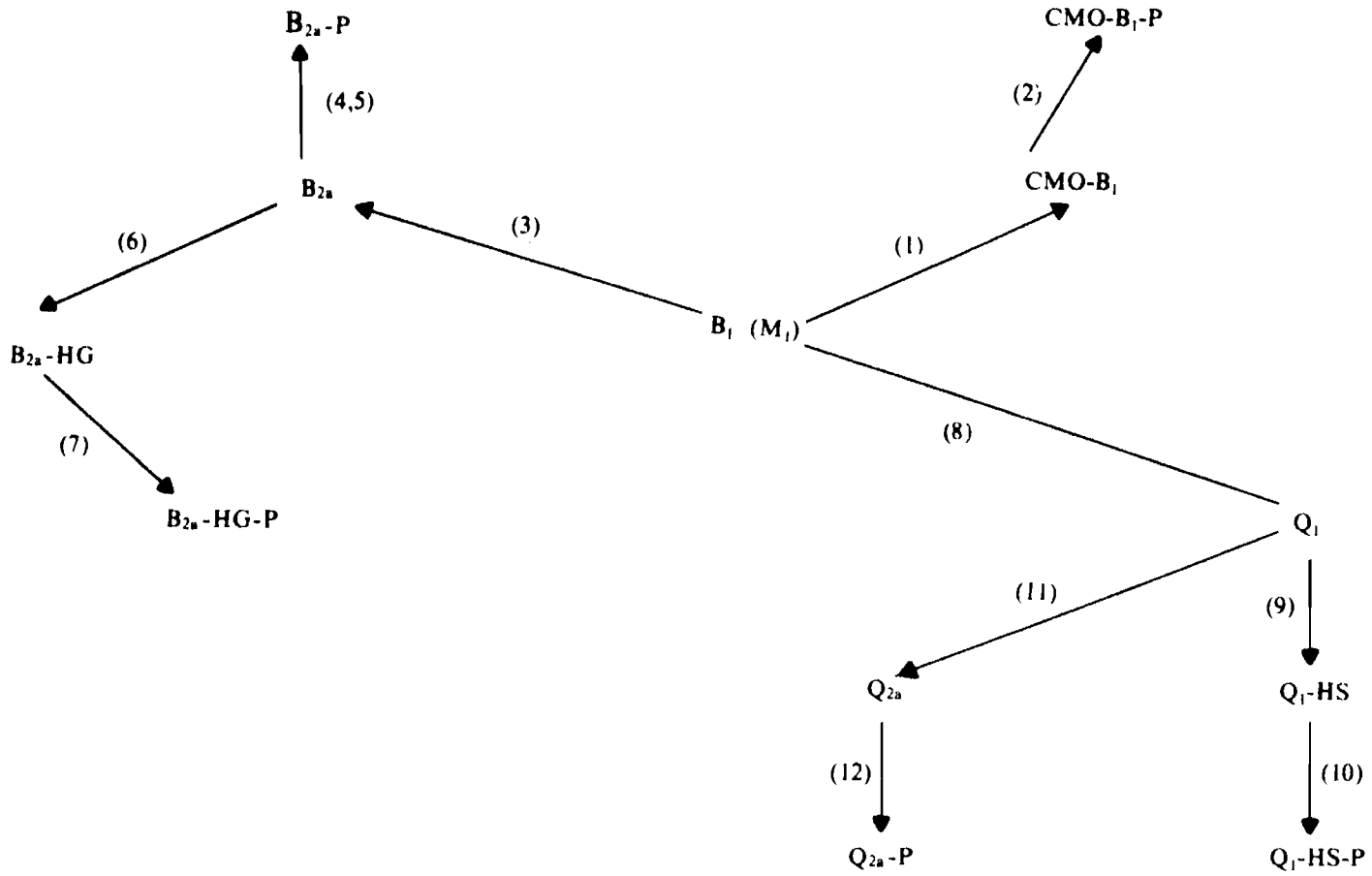

Figure 1. Approaches used in the conjugation of aflatoxin B_1 to protein (P). B_1 (1) or M_1 may be converted to its O-carboxymethyl oxime (CMO), which is then conjugated to protein (2); or may be converted to B_{2a} (3), in which the furan ring opens (4) at > pH 7.0 and subsequently forms a Schiff's base with protein. The bond is stabilized by reduction with sodium borohydride (5). Acylation of B_{2a} with glutaric anhydride (6) results in a hemiglutarate (HG) which is then conjugated to protein.B_1 may also be chemically converted to Q_1 (8), which is then either acylated to hemisuccinate (9) for conjugation (10) or converted to Q_{2a} for subsequent coupling to protein via the reductive alkylation route such as that for B_{2a}.

of particular antibody preparations prior to using them in immunoassays. For future reference, the specificity of antibodies for aflatoxins prepared by different approaches is presented in Table 1. It is apparent that when rabbits immunized with conjugates prepared by linking the carboxymethyl oxime of aflatoxin B_1 (Biermann and Terplan 1980, Chu 1986d) and aflatoxin M_1 (Chu 1986d, Woychik et al. 1984) with polypeptides, the antibodies generally recognize the dihydrofuran portion of the molecule. They can also distinguish the structural variation in the dihydrofuran portion of aflatoxin molecules. Antibodies highly specific against aflatoxin B_1 and aflatoxin M_1 were produced in this manner. These antibodies have been shown to be very useful in the monitoring analysis of aflatoxin B_1 in agricultural commodities (Chu 1986d) and aflatoxin M_1 in milk and urine (Chu 1986d, Fremy and Chu 1984, Hu et al. 1983). When conjugates prepared through the dihydrofuran portion of the aflatoxin molecule were used in the immunization, the antibodies had a specificity directed toward the cyclopentenone ring. Antibodies with similar specificity and a wider range of cross reactivities were obtained from rabbits when conjugates prepared by coupling aflatoxin B_{2a}, aflatoxin B_1 diol, and aflatoxin B_1 chloride to protein were used for immunization (Chu 1986d). These antibodies have been shown to cross-react with aflatoxin B_1-DNA adducts and other related compounds. With such a wide spectrum of antibody specificities, the aflatoxin B_{2a} antibodies were demonstrated to be useful

Table 1. Specificity of antibodies obtained from rabbits after immunization with different aflatoxin conjugates[1].

Conjugates used[2]	Aflatoxin used								
	B_1	B_2	G_1	G_2	M_1	B_{2a}	P_1	Q_1	R_0
BSA-CMO-B_1	100	11	9	1	<1	-[3]	-	6	3
	100	100	33	1	<1	-	-	-	100
PLL-CMO-B_1	100	43	15	5	-	1	<1	8	-
PLL-CMO-B_{1a}	100	19	2	<1	-	1	<1	1	-
BSA-B_{2a}	100	13	3	<1	6	8	-	-	<1
	100	25	<1	<1	1	125	-	-	1
BSA-B_1-Cl[4]	100	238	3	2	3	-	-	-	-
BSA-B_{2a}-HG	100	10	6	<1	2	11	-	-	<1
BSA-G_{2a}	2	2	8	15	2	-	-	-	-
BSA-CMO-M_1	56	<1	<1	<1	100	<1	-	-	<1
	1	<1	<1	<1	100	<1	-	-	<1
BSA-Q_1-HS	100	1	<1	1	-	-	-	3	33
BSA-Q_{2a}	<1	-	-	-	-	1	-	1	-

1. Expressed as % reactivity relative to aflatoxin B_1 except BSA-G_{2a}, BSA-CMO-M_1, and BSA-Q_{2a} antibodies which are relative to aflatoxins G_{2a}, M_1, and Q_{2a} respectively. The data were obtained from both RIAs and ELISAs (Chu 1986d Garner et al. 1985.)
2. BSA = bovine serum albumin; CMO = carboxy-methyloxime; HG = hemiglutarate; HS = hemisuccinate; and PLL = polylysine.
3. - = Not determined.
4. BSA-B_2-Cl: Aflatoxin dichloride directly conjugated to BSA.

immunohistochemical reagents for monitoring aflatoxin B_1 in liver nuclei. Antibodies against aflatoxin Q_1 also had a wide spectrum of specificities against different aflatoxins (Chu 1986d).

Immunochemical Analysis of Aflatoxin in Groundnut

Although a number of immunochemical methods have been used for the analysis of biological substances of small molecular weights, only three methods; RIA, ELISA, and affinity binding assay, have been developed for the analysis of mycotoxins in different foods and feed. All these methods have been tested for the analysis of aflatoxin in groundnuts. The first two methods are based on the competition between the unlabeled aflatoxin in the sample and the labeled aflatoxin in the assay system for the specific binding sites of antibody molecules. In the RIA, a radioactive aflatoxin is used. Thus, the specific activity of radioactive ligands plays an essential role in the sensitivity of RIA. In the ELISA, either the toxin-enzyme conjugate, or toxin-polypeptide is used. The affinity binding assay involves the use of an antibody column that specifically traps the aflatoxin in the column (Sun and Chu 1977, Groopman et al. 1985, Wu et al. 1983). The toxin can then be eluted from the column for subsequent analysis. This method can only be used for aflatoxins and mycotoxins that fluoresce with high intensity.

Radioimmunoassay of mycotoxins

The RIA procedure involves simultaneous incubation of a specific antibody with a solution of unknown sample or known standard, and a constant amount of labeled toxin. After separation

of the free and bound toxin, the radioactivity in each fraction is then determined. The toxin concentration of the unknown sample is determined by comparing the results to a standard curve established by plotting the ratio of radioactivities in the bound fraction and free fraction vs log concentration of unlabeled standard toxin. RIA can detect even 0.25–0.50 μg of purified mycotoxin per assay in a standard preparation. In the presence of a sample matrix, the lower limits for mycotoxin detection in food or feed samples is about 2–5 $\mu g\ kg^{-1}$ of purified mycotoxin in a standard preparation. The sensitivity of RIA can be improved by partial purification of the toxins (Chu 1986d, Lee and Chu 1981, Qian et al. 1984, Xu et al. 1986). RIA can only be used in a laboratory which has facilities for determining radioactive ligands.

Enzyme-linked immunosorbent assays

Two types of ELISA have been used for the analysis of mycotoxins; both are heterogenous competitive assays which involve the separation of free (unreacted) toxin in one phase (generally liquid) from the bound toxin in another (solid-phase). One type, direct ELISA, involves the use of mycotoxin/aflatoxin-enzyme conjugate and the other system, indirect ELISA, involves the use of a protein-mycotoxin/aflatoxin conjugate and secondary antibody such as goat anti-rabbit IgG to which an enzyme has been conjugated. Although horseradish peroxidase (HRP) is the most commonly used as enzyme for conjugation, other enzymes such as alkaline phosphatase and beta-galactosidase have also been used (Chu 1986d).

Direct competitive ELISA. In this assay, specific antibodies are first coated to a solid phase, e.g., polystyrene tubes (Biermann and Terplan 1980), nylon beads, and Terisaki plates (Pestka and Chu 1984); most commonly a microtiter plate (Chu 1986d) is used. The sample solution or standard toxin is generally incubated (0.5 to 2 h) simultaneously with enzyme conjugate or separately incubated in two steps. After appropriate washings, the amount of enzyme bound to the plate is determined by incubation with a substrate solution for an appropriate time. The resulting color is then measured spectrophotometrically or by visual comparison with standards.

Several protocols have been reported (Chu 1986a-d) for the analysis of aflatoxin in groundnuts and related products by the direct competitive ELISA in the microplate format. The protocols developed in earlier studies took a rather long time to complete, and had large standard deviations and coefficients of variation for each sample (Chu 1986d). We have recently developed a new ELISA protocol for quantitive analysis of aflatoxin B_1 in groundnuts and maize (Chu et al. 1987) using a microplate coated with anti-aflatoxin antibody. In this assay, aflatoxin-contaminated samples were first extracted with 70% methanol in water containing 1% dimethylformamide (1 g of groundnuts in 5 mL of extraction solvent), diluted with assay buffer (sodium-phosphate buffer, pH 7.2, 0.01 $molL^{-1}$ with 0.15 $molL^{-1}$ of NaCl) to a final concentration of 3.5% methanol and then directly subjected to the following ELISA procedure: (a) 50 μL of aflatoxin standard or diluted sample was added to each well followed by 50 μL of aflatoxin-HRP conjugate, and plates were then incubated at room temperature (25-28° C) for either 30 or 10 min (for screening test); (b) the wells were washed with 300 μL of assay buffer 3 to 5 times; (c) 100 μL of enzyme substrate was added, followed by incubation at room temperature for 10 min; and (d) 100 μL of stopping reagent was added and absorbance measured at 490 nm. Thus, the whole ELISA took less than one hour for quantitative analysis and less than 30 min for screening tests. In this assay, aflatoxin in the sample and toxin-enzyme conjugate competes for the same binding site with the antibody coated to the solid phase. Since the toxin-enzyme (El-Nakib et al. 1981) and antibody concentrations are constant, the color intensity that results from the enzyme

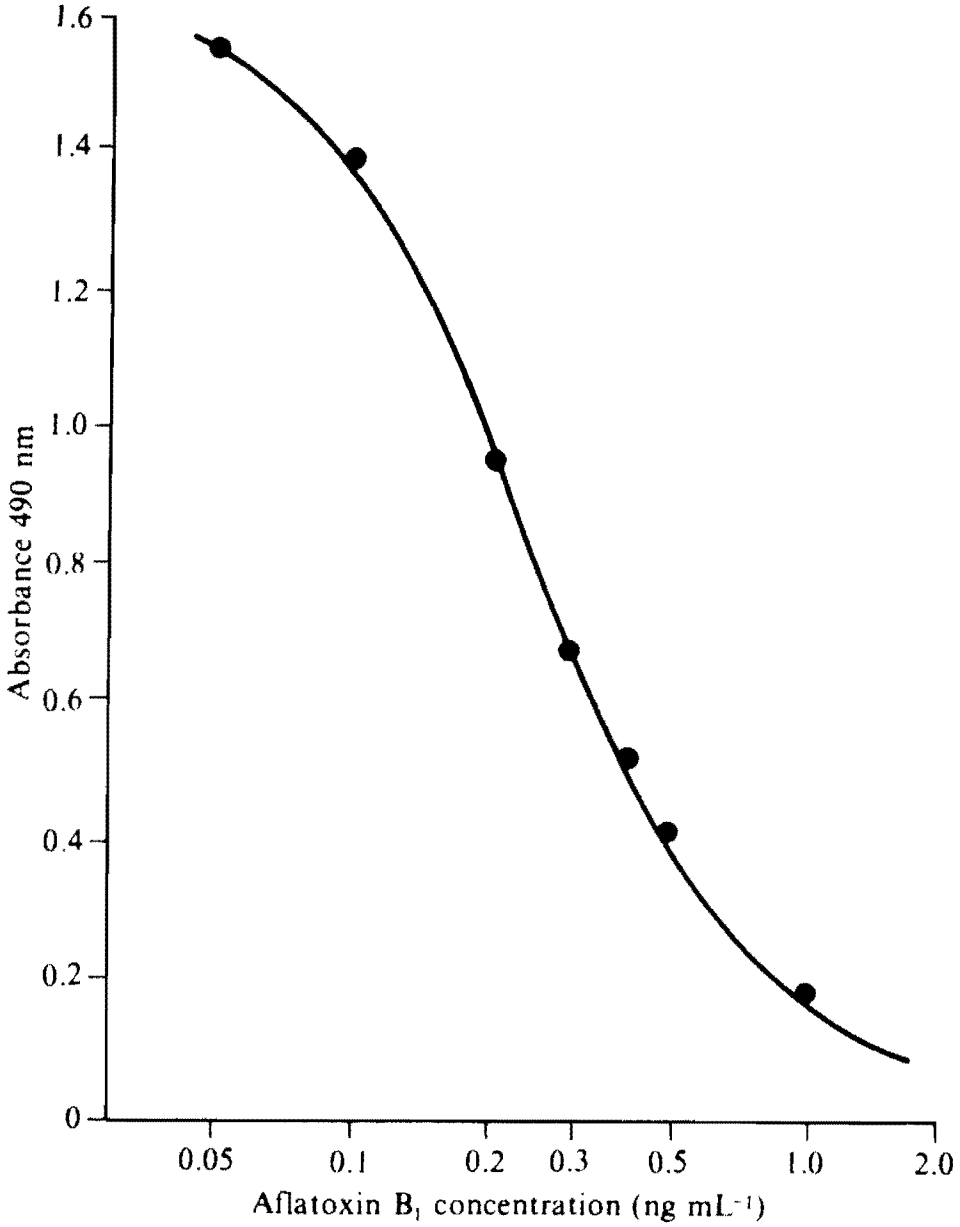

Figure 2. ELISA standard curve for aflatoxin B_1.

reaction is inversely proportional to the toxin concentration in the tested sample. A typical standard curve for this assay is shown in Figure 2. The analytical recovery for 5-100 $\mu g\ kg^{-1}$ aflatoxin B_1 added to maize meal was 90% and for peanut butter, 95.4%. The interwell and interassay coefficient of variation was 10% or less at the 20 $\mu g\ kg^{-1}$ level and above. Excellent agreement for aflatoxin levels in more than 30 naturally contaminated maize, mixed feed, and groundnut samples were found between the ELISA data, and the data obtained from different independent laboratories using TLC or other analytical methods.

In a more recent study, we evaluated this protocol against a collaborative study on a new TLC method for raw groundnut meals and peanut butter. Again, we found good analytical recovery for aflatoxin added to the samples at levels above 10 $\mu g\ kg^{-1}$. However, we also found that the coefficient of variation decreased to less than 10% at the 10 $\mu g\ kg^{-1}$ level when an additional extraction step of sample extracts with hexane before ELISA was incorporated into the protocol. A similar protocol with longer incubation time for the analysis of aflatoxin B_1 in groundnut and related products was described by Ram et al. (1986) who included a defatting procedure for the peanut butter samples before ELISA. To overcome the interference problem,

these investigators used aflatoxin-free sample extracts in the preparation of standards (Ram et al. 1986). These new protocols have been used in the analysis of naturally occurring aflatoxin in groundnut products (Chu et al. 1987, Ram et al. 1986, Zhu et al. 1987).

The sensitivity, analytical time, and assay range of different ELISA protocols for analysis for aflatoxin in groundnuts are compared in Table 2. In general, direct ELISA is approximately 10-100 times more sensitive than RIA when purified aflatoxin B_1 is used. Quantities as low as 2.5–5 $\mu g\ kg^{-1}$ of pure mycotoxin can be measured. However, when different commodities containing various toxin concentrations were tested, the lower limit for detection was comparable to, or slightly better than, that obtained by RIA. A major advantage of ELISA is that it does not use radioactive ligand. Since toxin purification is not necessary, many samples can be analyzed within a relatively short period. Like RIA, the sensitivity of ELISA can be improved by partial purification of the toxin (Fremy and Chu 1984, Hu et al. 1983, Martlbauer and Terplan 1985, Ueno 1985). Amounts of aflatoxin B_1 as low as 1 $\mu g\ kg^{-1}$ in peanut butter can be determined (Ueno 1985).

Table 2. Comparison of different ELISA protocols for analysis of aflatoxin B_1 in groundnuts and groundnut products.

Protocol	Extraction solvents	Cleanup	Incubation time (h)[1]	Standard range (pg well^{-1})[2]	Detection limits ($\mu g\ kg^{-1}$)	Samples tested
D-1[3]	BF	N	1a + 0.5s	25 - 1000	3–5	PB
D-2	55% MeOH[4] hexane	N	0.5b + 1a + 0.25s	12.5 - 300	5–10	P, PB
D-3	Chloroform + water	Y	1b + 1a + 1aa + 0.25s	10 - 1000	1	PB
D-4	70% MeOH	N	0.5a + 0.17	5 - 50	5–10	P, PB
I-1	BF	N	0.5b + 0.8a + 0.8aa + 0.6s	20 - 1000	5	PB
I-2	50% MeCN	N	3a + 2aa + 0.25s	0.2 - 10	0.25	PB

1. The letters following the incubation time indicate different steps of incubation: b = blocking step; a = first antigen-antibody incubation; aa = second antibody incubation; s = substrate incubation time.
2. Linear range of standard curve.
3. Direct competitive ELISA: D-1 El-Nakib et al. 1981; D-2 Ram et al. 1986; D-3 Ueno 1985; D-4 Chu et al. 1987. Indirect competitive ELISA: I-1 Fan and Chu 1984; I-2 Morgan et al. 1986.
4. MeOH = methanol; MeCN = acetonitrile.

Indirect ELISA. In the indirect ELISA, instead of using a mycotoxin- enzyme conjugate, a mycotoxin-protein (or polypeptide) conjugate is first prepared and then coated to the microplate before assay. The plate is then incubated with specific rabbit antibody in the presence or absence of the homologous mycotoxin. The amount of antibody bound to the plate coated with mycotoxin-protein conjugate is then determined by reaction with commercially available goat anti-rabbit IgG-enzyme complex and by subsequent reaction with the substrate. Both HRP and alkaline phosphatase conjugated to the goat anti-rabbit IgG have been used. Thus, toxin in the samples and toxin in the solid-phase competes for the same binding site with the specific antibody in the solution. Indirect ELISA has also been used for the analysis of a number of mycotoxins, utilizing different proteins (or polypeptides) as carriers for the conjugation of toxin (Chu 1986d, Fan and Chu 1984, Fan et al. 1987b, Liu et al. 1985, Morgan et al. 1986b). Indirect ELISA has been used for the analysis of aflatoxin in groundnut products (Table 2). The sensitivity of the indirect ELISA is comparable to that of the direct ELISA with the advantage

that much less antibody is required. In addition, it is not necessary to prepare a toxin-enzyme conjugate. The disadvantage of the method is that an additional incubation step is necessary for the assay and it thus requires more time (1-2 h). Morgan et al. (1986b) used an indirect ELISA for the analysis of 18 peanut butter samples obtained from retail stores and found that the aflatoxin levels ranged from less than 1 $\mu g\ kg^{-1}$ in 8 samples to as high as 775 $\mu g\ kg^{-1}$ in one sample. Most of these samples (12 of the 18) were below 10 $\mu g\ kg^{-1}$. Treatment of samples with Sep-Pak® and utilization of the biotin-avidin system resulted in higher sensitivity of indirect ELISA for the estimation of aflatoxin M_1 (Cerny et al. 1983). Because only small amounts of antibody are needed for the indirect ELISA, this method has been used extensively for monitoring the antibody titers of hybridoma culture fluids for the screening of monoclonal antibody-producing cells (Gendloff et al. 1987, Groopman et al. 1984, Hunter et al. 1985, Woychik et al. 1984) in addition to toxin analysis.

Rapid immunoscreen tests for aflatoxin in groundnuts

Several rapid immunoscreen tests were developed for analysis of aflatoxin in groundnuts. A modification of the direct competitive ELISA protocol in microplate format by shortening the first step incubation to 10 min, as a quick screening test for analysis of aflatoxin in groundnuts is described above. Another approach was to immobilize the antibody on a paper disk mounted in a plastic card instead of coating to the microtiter plate well (Immunoassay Quick-Card test). Thus, the reaction is carried out on the wetted paper disk. The principle of the reaction in this assay format is similar to that of direct ELISA. In practice, sample extracts (a few drops) are first applied to the test spot (paper disk) in the plastic card, then aflatoxin-enzyme conjugate is applied, and finally the substrate solution is added. The absence of color, generally blue, at the spot indicates the sample contains aflatoxin as compared with a negative control spot where a bright blue color appears. Details of this method have been demonstrated by Dr Goto in the present workshop (see pages 177–182). Cole et al. (1987) have found this method a convenient way to screen for aflatoxin in farm-stock groundnuts.

Affinity column immunoassays

The application of affinity column chromatography for immunoassay of mycotoxins was reported by Sun and Chu (1977). Subsequent studies by Wu et al. (1983) led to use of the affinity column as a tool for preconcentration and cleanup of samples of mycotoxins before RIA or HPLC analysis (Groopman et al. 1985). Recent advances in instrumentation have led to the use of an affinity immunoassay for aflatoxin. Aflatoxin extracted from the sample is subjected to a disposable affinity column containing anti-aflatoxin antibody Sepharose® gel. After washing, aflatoxin is removed from the column with methanol and its fluorescence determined. Thus, the affinity column merely serves as a separation and concentration tool for the analysis. This method cannot be used for mycotoxins such as trichothecenes which do not have high fluorescence or chromophores.

Conclusions

It is apparent from the data given in this paper that in the last few years, rapid progress has been made in the area of immunoassays for mycotoxins and that these are gaining acceptance as analytical tools. Good and reliable immunoassay protocols for aflatoxin in groundnuts and

groundnut products have been established. These methods are simple, sensitive, and specific. Several immunoassay kits for aflatoxins and other mycotoxins are currently available. Problems of extraction or release of aflatoxins from crude groundnut seed extract need further research. Nevertheless, we are now at least one step ahead, and the chances are good that more efficient and reliable immunoassays will soon be developed.

Acknowledgments

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Methods for the Analysis of Aflatoxins in Groundnut and Other Agricultural Commodities

T. Goto[1] and M. Manabe[2]

Abstract

Methods for aflatoxin analysis using thin layer chromatography (TLC) and fluorescence detection were developed in the 1960s and are still widely used. In the late 1970s, several applications of high performance liquid chromatography (HPLC) were developed and as they were generally more sensitive than the TLC methods, they are now popular for aflatoxin analysis when a high degree of accuracy is required. For some test purposes convenience and rapidity of analysis are more important than accuracy, and the bright greenish yellow fluorescence (BGYF) and minicolumn methods were evolved with this in mind. Recently several enzyme-linked immunosorbent assay (ELISA) systems kits for aflatoxin analysis have been developed and some of them found suitable for the kind of testing now carried out using the BGYF and minicolumn methods. Gas chromatography can also be used for aflatoxin analysis under certain conditions. From the range of aflatoxin analysis methods now available it should be possible to choose methods suitable for specific purposes.

Résumé

Méthodes d'analyse des aflatoxines dans les arachides et d'autres produits agricoles : *Les méthodes d'analyse des aflatoxines par la chromatographie en couche mince (TLC) et la détection par fluorescence, mises au point dans les années 60 sont encore largement utilisées. Plusieurs applications de la chromatographie liquide haute performance (HPLC) élaborées vers la fin des années 70, se sont avérées plus sensibles que les méthodes TLC. Elles sont donc retenues pour les analyses des aflatoxines demandant une grande précision. La méthode de la réaction vert-jaune de fluorescence (BGYF) et celle des minicolonnes sont proposées pour les analyses où la simplicité et la rapidité sont plus importantes que la précision. Les auteurs ont également examiné plusieurs systèmes ELISA parmi les différentes méthodes immunochimiques rapides développées récemment, et dont certaines sont adaptées au genre d'essais effectués actuellement avec les méthodes BGYF et de minicolonnes. La chromatographie en phase gazeuse est également utilisée sous certaines conditions. Les chercheurs disposent donc de nombreuses méthodes d'analyse pour choisir un test approprié aux besoins spécifiques.*

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Resúmene

Métodos de analizar las aflatoxinas en el cacahuate y otros productos agrícolas : *Los métodos para analizar las aflatoxinas a base de la cromatografía de capa delgada (TLC) y de detección por fluorescencia, se desarrollaron durante la década de 1960 y aún se utilizan extensamente. A fines de los años 70, se desarrollaron varios procedimientos a base de cromatografía líquida de alto rendimiento (HPLC) y como éstos generalmente son más sensibles que los métodos TLC, actualmente son muy populares para el análisis de aflatoxinas, cuando se requiere un alto grado de exactitud. Para algunos propósitos analíticos, la simplicidad y la rapidez del análisis son más importantes que la exactitud, y teniendo esto presente se desarrolló el método que utiliza la reacción de la fluorescencia amarillo-verdoso brillante (FAVB) y el método de la minicolumna. Más recientemente, se han desarrollado algunos equipos portátiles para el uso del método de cuantificación tipo radioinmunológico (RIA) y varios métodos inmunoenzimáticos de cuantificación de anticuerpos (ELISA), de análisis de aflatoxinas, y algunos de estos han resultado adecuados para el tipo de pruebas que actualmente se efectúan con los métodos FAVB y de las minicolumnas. La cromatografía en fase gaseosa también puede usarse para el análisis de aflatoxinas bajo ciertas condiciones. Entre el gran número de métodos de análisis de aflatoxinas actualmente disponibles, debe ser factible escoger métodos que sean los más apropiados para un propósito específico.*

Introduction

In 1960 in the United Kingdom, more than 100 000 young turkeys and ducks died in the course of a few months. Since the causal agent was unknown, and the affected animals exhibited similar symptoms, the disease was labeled 'Turkey "X" disease' (Blount 1961). In 1961 a toxic compound was isolated from moldy Brazilian groundnut meal (Sargeant et al. 1961) used in turkey feed. Since the compound was produced by *Aspergillus flavus* Link ex Fries it was named aflatoxin. It is now known that aflatoxin is not one compound but a group of more than fifteen toxins (Table 1, Fig. 1). Aflatoxins B_1, B_2, G_1, and G_2 have often been detected in groundnut, maize and other agricultural commodities (WHO 1979), and aflatoxin M_1 has been detected in milk and dairy products from animals fed aflatoxin B_1 contaminated feed (de Iongh et al. 1964).

Many studies have been carried out on aflatoxins due to concern about their toxic effects on humans and animals, especially their very strong mutagenicity (Wong and Heish 1976) and carcinogenicity (WHO 1979). The development of methods of analysis for aflatoxins was necessary for these studies and there is still a very strong demand for the development of new and improved techniques (Hesseltine 1986).

Methods for Aflatoxin Analysis

Thin layer chromatography (TLC) had been used as the only practical method for the analysis of aflatoxins and even now it is the method most commonly used, followed by high performance liquid chromatography (HPLC). Manabe et al. (1967) developed a method to separate the four major aflatoxins (B_1, B_2, G_1, and G_2) by column liquid chromatography (LC) but a practical

Table 1. Major physico-chemical properties of aflatoxins.

Property	Aflatoxins				
	B_1	B_2	G_1	G_2	M_1
Chemical formula	$C_{17}H_{12}O_6$	$C_{17}H_{14}O_6$	$C_{17}H_{12}O_7$	$C_{17}H_{14}O_7$	$C_{17}H_{12}O_7$
Molecular weight	312	314	328	330	328
Melting point (°C)	268-269 (D)[1]	287-289 (D)	244-249 (D)	230	299 (D)
Sorbent from	Chloroform	Chloroform + pentane	Chloroform + methanol	Ethyl acetate	Methanol
Amax nm (E) in ethanol	223 (25 600) 265 (13 400) 362 (21 800)	220 (20 500) 265 (12 700) 363 (24 000)	243 (11 500) 257 (9 900) 264 (10 000) 362 (16 100)	217 (28 000) 245 (12 900) 265 (11 200) 365 (19 300)	226 (23 100) 265 (11 600) 357 (19 000)
Fluorescence	425 nm	425 nm	450 nm	425 nm	425 nm

1. D = Decomposition.
Source: Cole and Cox 1981.

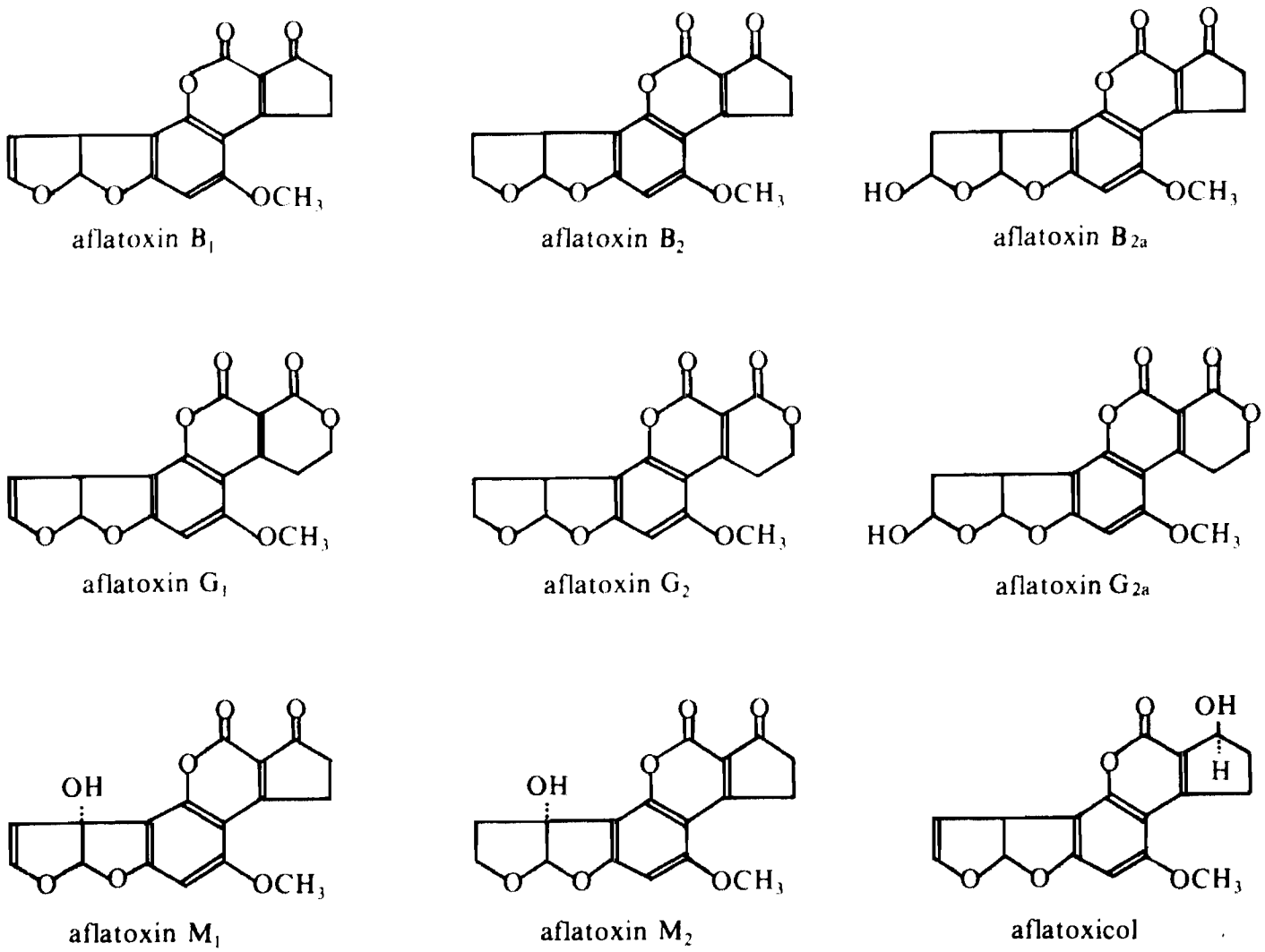


Figure 1. Molecular structure of aflatoxins.

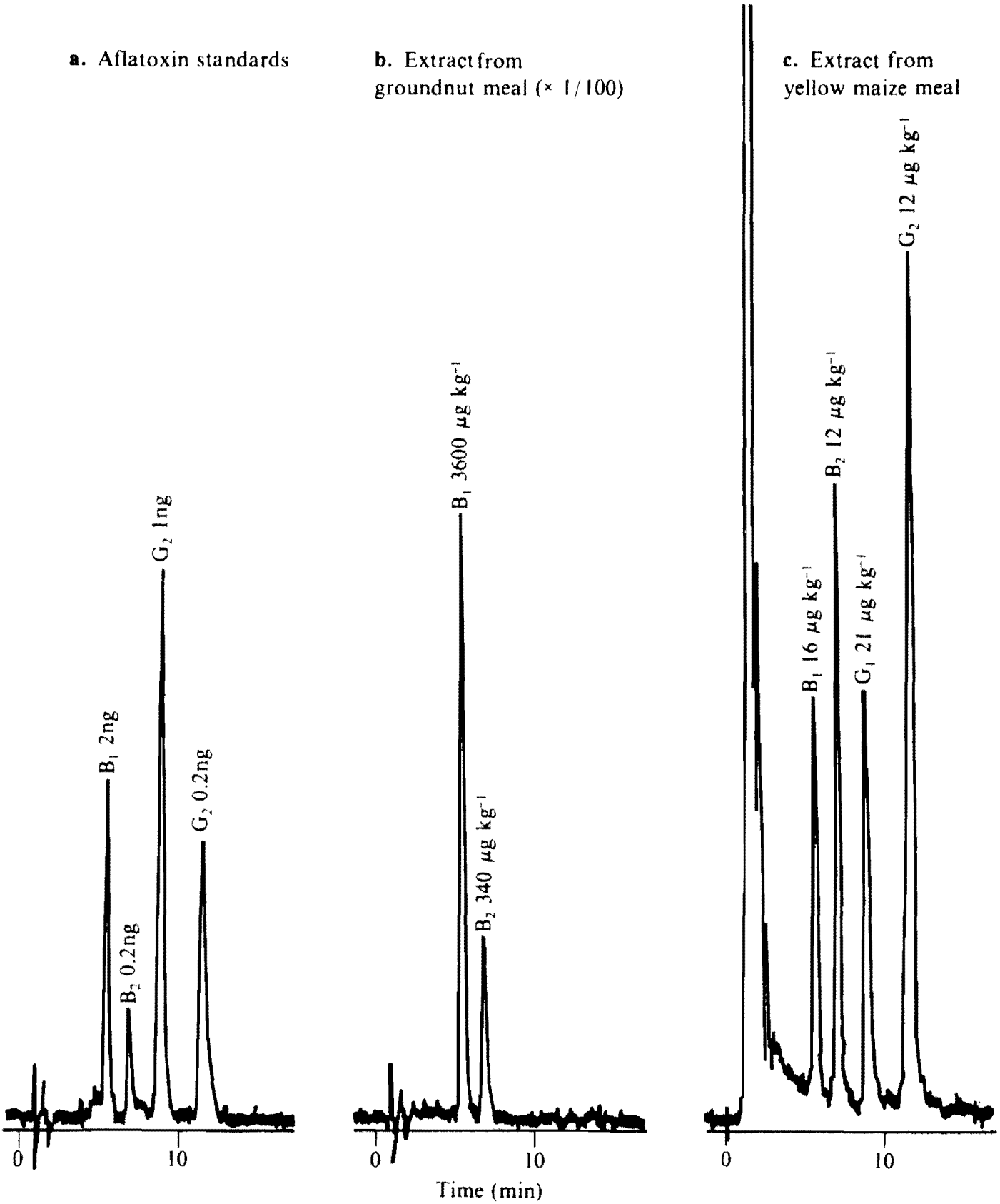
method for the analysis of aflatoxins by LC was not developed until the late 1970s. Thereafter a large number of HPLC-based methods have been developed. In contrast to these two chromatographic methods, gas chromatography (GC), one of the most popular chromatographic methods, has not been used for the analysis of aflatoxins except to confirm the compound by gas chromatography/mass spectrometry (GC/MS). While the accuracy and sensitivity of the TLC and HPLC methods was recognized, other methods for analysis were developed because of their simplicity. These included the minicolumn methods (Holaday 1981) and the BGYF (Bright Greenish Yellow Fluorescence) method (Shotwell and Hesseltine 1981) which have been widely used. Some enzyme-linked immunosorbent assay (ELISA) methods have recently been applied for the analysis of aflatoxins. As some of these methods are very sensitive and easy to use, they may eventually replace some of the chemical methods presently in use for the analysis of aflatoxins.

Analysis of Aflatoxins by TLC and HPLC

At least in the first decade of aflatoxin research, TLC was the only practical method of analysis and even now is the most commonly used technique. For the analysis of aflatoxins in groundnut, the CB or BF methods, both approved by the Association of Official Analytical Chemists (AOAC), or the water slurry method (Whitaker et al. 1980) were commonly used for the extraction and purification of samples. Commercially available precoated silica gel plates are now used instead of "homemade" plates to separate and determine aflatoxins, because they are convenient and safe. Aflatoxins are detected under long-wave (ca. 365 nm) ultraviolet (UV) light under which they exhibit a blue to blue-green fluorescence. TLC methods have been considered as semiquantitive, but the diffusion of sophisticated densitometer and sample application devices has significantly improved the accuracy of TLC analysis.

Nowadays HPLC systems are widely used in analytical laboratories. In the 1986 IARC-WHO aflatoxin check sample survey, 58 out of 227 results for the analysis of aflatoxin B_1 in groundnut meal were obtained by HPLC methods. In the history of HPLC, spectrometers with microflow cells (UVD) (365 nm or 254 nm absorption) were first used to detect aflatoxins, but these methods were less sensitive than TLC methods. Recently most of the HPLC methods employ fluorospectrometric detectors with microflow cells (FLD) to detect aflatoxins, and UVD is used for very limited and specific purposes. There are few problems in the separation of aflatoxins either in normal phase column or reverse phase column, but quenching is still the major problem in aflatoxin analysis by FLD-HPLC.

Three methods are now being widely used to analyse aflatoxins by HPLC. The first was developed by Panalaks and Scott (1977) and employs a silica gel packed flow cell for FLD to enhance fluorescence and improve the sensitivity of the analysis. In the second method, developed by Manabe et al. (1978) a normal phase silica gel column is used and a special system to reduce quenching in the mobile phase has been devised (Figure 2). The advantage of this method is that it does not require any special equipment or treatment of the samples, and samples prepared for TLC analysis can also be used for HPLC analysis. The third method depends on the derivatization of aflatoxins. As aflatoxins (especially aflatoxin B_1) are usually very strongly quenched, several methods have been developed to convert aflatoxin B_1 to other compounds that are not quenched under certain analytical conditions. This method includes several variations and each has its advantages and disadvantages. In this group, the method to convert aflatoxin B_1 to aflatoxin B_{2a} (Pons et al. 1972) before analysis, and the post-column enhancement method using bromine or iodine are often used. As the postcolumn derivatization



Flow rate, 1 mL min^{-1}; column temperature, 40° C
Column : Develosil 60-5® (4 mm i.d. × 10 cm)
Excitation 365 nm; Emission 425 nm

Figure 2. Chromatograms of aflatoxins obtained by HPLC mobile phase, toluene-ethylacetate-formic acid-methanol (89:7:2:2).

by bromine or iodine is very sensitive and relatively easy to operate, it may in future become one of the standard detection methods (Gilbert and Shepherd 1985, Kok et al. 1986).

BGYF and Minicolumn Methods

These methods were developed for simple and rapid analysis of aflatoxin and both have problems relating to the sensitivity and accuracy of the analysis. In the BGYF method, as a fungal metabolite other than aflatoxin is used for the detection, sometimes the correlation between the concentration of aflatoxin and the amount of BGYF causes problems. In the minicolumn method, the low sensitivity and the presence of unknown interfering materials make it difficult to detect aflatoxins.

For these reasons, most of the analyses that use these methods will probably be replaced by other methods such as ELISA in the near future.

Immunoassay Methods

It had been very difficult to produce antibodies for small molecular weight compounds such as aflatoxins but recent advances in biochemistry and organic chemistry opened the way to the production of antibodies for aflatoxins (Chu and Ueno 1977) and other mycotoxins (Chu 1984). Once methods for production of antibodies were developed, the application of immunological assays for aflatoxin analysis made rapid progress.

A radio immunoassay (RIA) method was first applied to the analysis of aflatoxin (Chu and Ueno 1977), but now ELISA has become more popular (Pestka et al. 1980). Further, advances in biotechnology led to the development of the monoclonal method for the production of antibodies. After the mass-production procedures of either polyclonal or monoclonal antibodies for aflatoxins were developed, several ELISA-applied aflatoxin assay kits were produced on a commercial basis. We have evaluated three different types of ELISA-applied aflatoxin assay kits for their characteristics, accuracy etc. (Table 2) (Goto and Manabe 1988). The first kit uses a standard 96-well microtiter plate. At least two companies (in Japan and UK) have developed similar kits capable of simultaneous analysis of a large number of samples (at least 20) in 2 to 3 h excluding sample preparation and extraction time. The sensitivity of these kits is higher than all other methods. The second kit was developed in the USA, it consists of a small card (like a credit card) and three small vials containing the necessary reagents. The kit can be used anywhere, even in the field, and takes only 5 min to detect aflatoxins. Although this kit enables the detection of aflatoxins in a sample it does not allow quantitative determination. The third kit was developed in the UK and uses a small column packed with antibody-bounded gel and Florisil® chips for detection. Aflatoxins are analyzed semiquantitatively without any special instruments except for a dark box with a long wavelength UV lamp. A single test takes about 15 min, and this method can be used outside the laboratory. Several other kits are available on the market, and some of them can be used independantly of a well-equipped laboratory.

These ELISA-applied analytical methods are suitable for both simple analysis and the mass sample analyses required for food quality control; they are likely to become widely used in the near future.

Table 2. Comparison of performance of Enzyme-Linked Immunosorbent Assay (ELISA) kits for aflatoxin analysis

Test/character/component	Kit		
	EIA KIT Aflatoxin B_1 (Japan)	E-Z Screen (USA)	Aflatest 10 (UK)
Antibody	Monoclonal	Polyclonal	Monoclonal
Specificity	Aflatoxin B_1	Aflatoxin B_1 and G_1	Aflatoxin B_1 and B_2
Sensitivity	10 pg	170 pg	2–10 ng
Detection	492 nm absorption	Blue-gray, color change	Fluorescence on Florisil ® chip
Quantification	0.2-100 $\mu g\ kg^{-1}$	Difficult	Semiquantitative 2-100 $\mu g\ kg^{-1}$
Extraction (% ethanol)	50-55	80	60
Aflatoxin B_1	16.5[1]	+[2]	B< <G[3]
Aflatoxin B_2	1.5	-	R
Aflatoxin G_1	11.5	+	>B
Aflatoxin G_2	0.6	-	<B
Aflatoxin in groundnut[4]	11.0	+	B<
Aflatoxin B_1 in maize[5]	6.3	+	<B

1. Amount as aflatoxin B_1.
2. Sensitivity set at 10 $\mu g\ kg^{-1}$.
3. B = 10 $\mu g\ kg^{-1}$ standard; G = 25 $\mu g\ kg^{-1}$ standard; R = 50 $\mu g\ kg^{-1}$ standard.
4. Aflatoxin $B_1+B_2+G_1+G_2$ (10+1+5+1 $\mu g\ kg^{-1}$).
5. B_1 15 $\mu g\ kg^{-1}$.

Gas Chromatography

GC has often been used and plays a major role in the analysis of various mycotoxins. However, in the case of the analyses of aflatoxins, GC has rarely been used as an analytical method because the TLC and HPLC methods were sufficiently sensitive. Moreover, aflatoxins have to be converted into volatile compounds before GC analysis. Thus GC was used only with a mass spectrometer (MS) as a detector to confirm the compound. Friedli (1981) showed that aflatoxin B_1 could be analyzed by GC/MS without chemical derivitization, and later Rosen et al. (1984) also showed that aflatoxins B_1 and B_2 in groundnuts can be detected by GC/MS without their derivatization. Recently Goto et al. (in press) succeeded in analyzing aflatoxins B_1, B_2, G_1, and G_2 using GC equipped with a flame ionization detector (FID), one of the most popular detectors for GC (Fig. 3). This system consists of a Shimadzu GC15A® gas chromatograph with a capillary or column injector, FID, and a fused silica capillary column having a chemically bonded SE-54 equivalent liquid phase. The minimum quantitation limit of aflatoxins by this method was 1 ng for aflatoxins B_1 and B_2, and 2 ng for aflatoxins G_1 and G_2. These findings suggest that aflatoxins in various agricultural commodities can be analyzed by GC depending on the development of sample preparation methods.

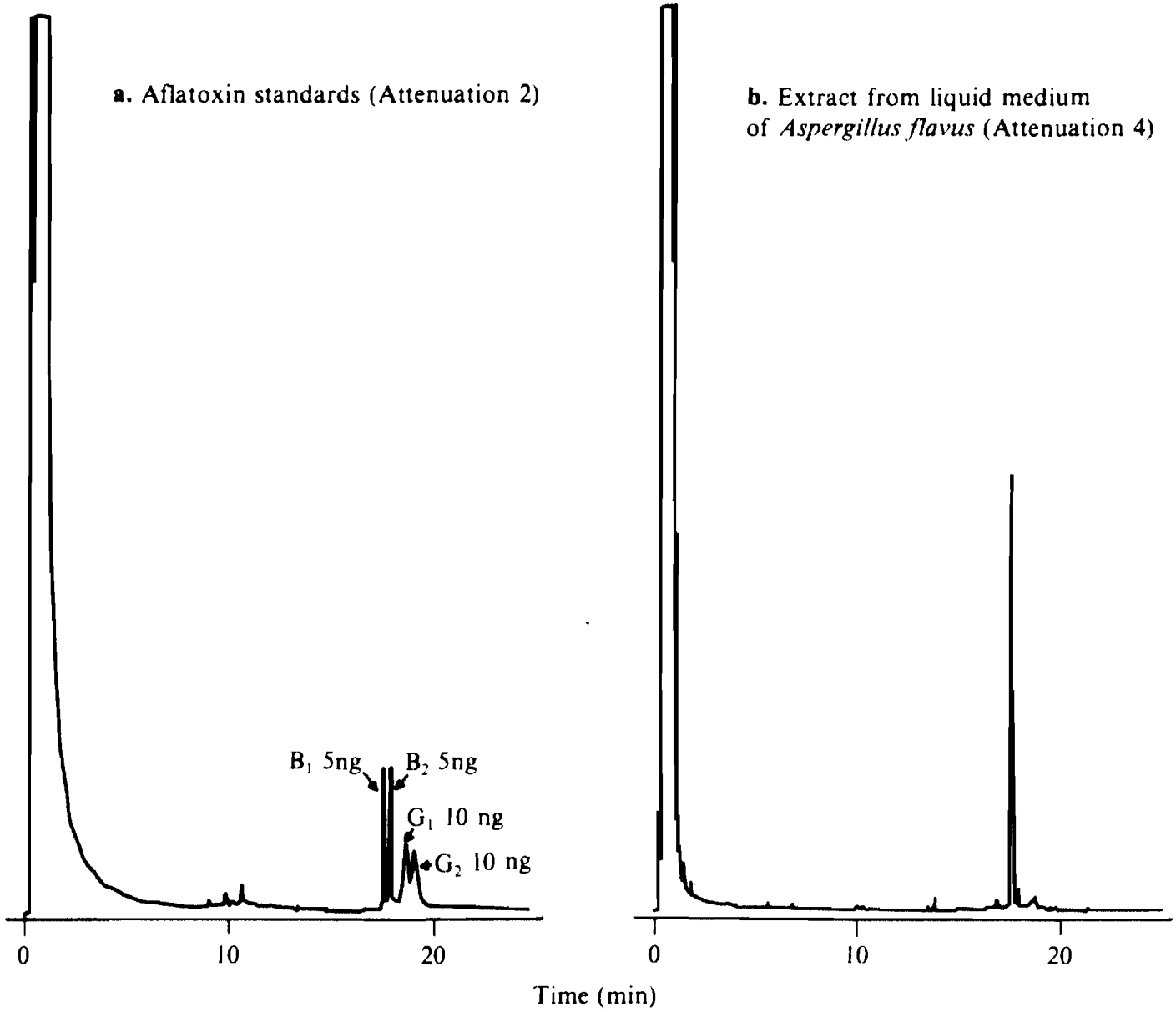


Figure 3. Chromatograms of aflatoxins obtained by gas chromatography with flame ionization detector (GC-FID)

Conclusions

Conventional chemical methods for the analysis of aflatoxins such as TLC and HPLC have been well standardized, while biochemical methods such as ELISA are making rapid progress. Some parts of the chemical analysis will be replaced by biochemical methods. Irrespective of the method chosen, effective sample preparation, including sampling, extraction, and purification, is necessary and is increasingly important. To measure the amount of aflatoxin in nuts and cereals, it should be noted that aflatoxin contamination of such agricultural commodities varies from kernel to kernel, and sometimes even within the same bag differences in the amount of aflatoxins detected ranges from zero to several thousand $\mu g\ kg^{-1}$, hence sampling and sample preparation are very important procedures (Campbell et al. 1985). In the case of the ELISA kits available in the market, methanol (50 to 80%) is recommended as an extraction solvent because it can be used for both extraction and final sample preparation. However, depending on the

extraction solvent used and the properties of the samples, recovery rates of aflatoxin vary considerably, so extraction procedures need to be carefully evaluated (Whitaker and Dickens 1986).

At present, several methods for the analysis of aflatoxins have been reported, and in most cases one or more can be used for the purpose of a specific analysis. There are still strong demands to develop new and improved methods of analysis (Hesseltine 1986). Recent improvements in instrumentation and in biochemical techniques make possible the development of new methods. Regardless of the analytical method selected careful attention should be paid to sample preparation to ensure the accuracy of results.

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Enzyme-Linked Immunosorbent Assay (ELISA) for Aflatoxin B_1 Estimation in Groundnuts

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Abstract

The commercially available hapten, afla B_1-oxime-bovine serum albumin, was used to produce an antiserum in rabbits. The same hapten was coupled with alkaline phosphatase (hapten-BSA-ALP) and used in the competitive direct enzyme-linked immunosorbent assay (ELISA) for the detection of aflatoxin B_1. Aflatoxin B_1 was extracted in methanol from naturally contaminated or 'spiked' groundnut seed samples.

Wells of a polystyrene microtitre plate were coated with the antiserum, the plates were washed in PBS-Tween, aflatoxin B_1 standards or groundnut sample extracts, and hapten-BSA-ALP conjugate were added and the plates incubated. The plates were again washed, and the amount of conjugate bound to the antibody was determined after addition of the substrate, p-nitrophenylphosphate.

The hapten-BSA-ALP conjugate has advantages in stability, simplicity of preparation, and high specificity, over the conventional toxin-enzyme conjugate in direct competitive ELISA. The assay method is more rapid and less expensive than the physico-chemical methods of aflatoxin analysis and it can detect levels of aflatoxin B_1 as low as 50 picograms.

Résumé

Détermination de l'aflatoxine B_1 dans les arachides par la méthode ELISA : *Le sérum albumine afla B_1-oximebovine, commercialisé sous le nom de Hapten, a servi à l'élaboration d'un antisérum chez des lapins. Ce produit est associé à la phosphatase alkaline enzymatique (Hapten-ALP) et utilisé dans l'essai ELISA adéquat pour la détection de l'aflatoxine B_1. Cette mycotoxine a été extraite avec le méthanol à partir des échantillons de graines d'arachide ayant subi une contamination naturelle ou celles artificiellement marquées.*

Les parois d'une plaque de microtitrage en polystyrène sont enduites d'antisérum et les plaques sont ensuite lavées au produit PBS-Tween; l'aflatoxine B_1 étalon ou les extraits des échantillons d'arachide ainsi que le conjugué Hapten-ALP y sont ajoutés avant incubation. Les plaques sont à nouveau lavées et la quantité de conjugué liée à l'anticorps est déterminée en y ajoutant le substrat p-nitrophénylphosphate.

Les avantages de Hapten-ALP par rapport aux conjugués toxine-enzyme classiques pour le

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test ELISA sont sa stabilité, sa simplicité et sa forte spécificité. La méthode est plus rapide et moins coûteuse que les procédés physico-chimiques de dosage des aflatoxines et possède un seuil de détection de l'aflatoxine B_1 inférieur à 50 picograms.

Resúmene

Determinación de la aflatoxina B_1 en el cacahuate por el método ELISA : *Se utilizó albúmina de suero bovino-afla B_1-oxime, conocido comercialmente con el nombre de "Hapten", para la producción de un antisuero en conejos. Este mismo "Hapten" fue ligado con fosfatosa alcalina (Hapten-BSA-ALP) y usado en el método directamente competitivo inmunoabsorbente vinculado a enzimas de cuantificación de anticuerpos, ELISA, para la detección de aflatoxina B_1. La aflatoxina B_1 fue extraída con metanol de muestras de semilla de cacahuate contaminadas, ya sea en forma natural o artificialmente, con esa micotoxina.*

Las depresiones de una placa de polistireno empleada para microtitulaciones se recubrieron con una capa delgada del antisuero; las placas se lavaron con el producto PBS-Tween; se agregaron soluciones calibradas de aflatoxinas B_1 o extractos de las muestras de cacahuates y, a continuación, el conjugado Hapten-BSA-ALP; luego se incubaron las placas. Se lavaron nuevamente las placas, y la cantidad de conjugado ligado al anticuerpo, se determinó después de la adición del substrato, p-nitrofenilfosfato.

El conjugado Hapten-BSA-ALP tiene marcadas ventajas de estabilidad, simplicidad y alta especificidad, sobre el uso del conjugado toxina-enzima convencional, en el método competitivo directo ELISA. El método analítico primeramente citado es más rápido y menos costoso que los métodos físico-químicos de análisis de aflatoxinas y puede detectar niveles de aflatoxina B_1 hasta del orden de 50 picograms.

Introduction

The thin layer chromatography (TLC) systems developed in the 1960s and 1970s are still the most commonly used methods for detection and estimation of aflatoxins in groundnut and several other agricultural commodities. These methods are expensive and time consuming and so efforts have been made to develop more rapid and less expensive methods for aflatoxin analysis (Pestka et al. 1981, Morgan et al. 1986, and El-Nakib et al. 1981).

Several enzyme-linked immunosorbent assay (ELISA) procedures have been reported for the estimation of aflatoxin B_1 in groundnut and groundnut products (El-Nakib et al. 1981, Fan and Chu 1984, Morgan et al. 1986). These assays have advantages over conventional analytical procedures using TLC and high pressure liquid chromotography (HPLC) in terms of speed, ease of sample preparation and use, and are potentially cheaper for aflatoxin analysis. The major application of ELISA procedures at present is analysis of aflatoxin B_1 in such agricultural commodities as maize, groundnut, and groundnut products (El-Nakib et al. 1981, Fan and Chu 1984, Morgan et al. 1986). A few direct and indirect ELISA procedures have been developed for analysis of aflatoxin B_1 in groundnut, groundnut meal, and peanut butter. All direct competitive ELISA procedures necessitate the use of aflatoxin-horse radish peroxidase (HRP) conjugate. Two problems are encountered in preparation of the conjugate: (1) instability of the toxin-

enzyme conjugate, and (2) variations in the amounts of toxin conjugated to the enzyme (Fan and Chu 1984). Both these factors affect the sensitivity of ELISA. To overcome these factors, we have used alkaline phosphatase (ALP) enzyme in place of HRP and have coupled it directly with the commercially available afla B_1-oxime-BSA. Using this afla B_1-oxime-BSA-ALP conjugate, we have developed a simple, rapid, specific, and comparatively inexpensive direct competitive ELISA for analysis of aflatoxin B_1 in groundnuts.

Materials and Methods

Chemicals and reagents

Aflatoxin B_1, bovine serum albumin (BSA, RIA grade), afla B_1-oxime-BSA, alkaline phosphatase (Type VII-NT), p-nitrophenyl phosphate disodium, glutaraldehyde (RIA grade), and Tween 20 ® were purchased from Sigma Chemical Co., St. Louis, MO, USA. Complete and incomplete Freund's adjuvants were obtained from Difco Laboratories, Detroit, MI, USA. Polystyrene microtitre plates were obtained from Dynatech Lab, Virginia, USA. All other organic solvents and inorganic chemicals used were of the highest analytical grade.

Production of antiserum against aflatoxin B_1

Antiserum against aflatoxin B_1 was produced by immunizing rabbits with afla B_1-oxime-BSA, using the methods of Chu and Ueno (1977) and El-Nakib et al. (1981). Antiserum titre was determined by the indirect competitive ELISA procedure described by Morgan et al. (1986).

Preparation of afla B_1-oxime-BSA-ALP conjugate

Afla B_1-oxime-BSA was conjugated to ALP through the glutaraldehyde bridge using the method of Avrameas et al. (1978). Several ratios of ALP and afla B_1-oxime-BSA were tried in initial experiments and the most suitable ratio was 1 mg of afla B_1-oxime-BSA to 4 mg of ALP. Afla B_1-oxime-BSA was dissolved in phosphate buffered saline (PBS, pH 7.4, 1 mg mL^{-1}) and mixed in proportions of 1:2, 1:4, 1:6, and 1:8 with ALP. The mixtures were dialyzed with 0.06% glutaraldehyde in PBS at room temperature for 2 h for conjugation. Excess glutaraldehyde was removed by dialyzing in PBS at 4°C for 3 h with three changes of PBS. The resultant afla B_1-oxime-BSA-ALP conjugate was stored at 4°C.

Sample preparation and extraction

Groundnut seeds (cv J 11) were obtained from the 1986 rainy-season crop grown at ICRISAT Center. Healthy, mature, finely ground seeds (500 g) were divided into several lots of 5- and 10-g samples and these were 'spiked' with aflatoxin B_1 standard to give concentrations of 10, 20, 40, and 50 $\mu g\ kg^{-1}$. Samples were spiked by directly adding a measured volume of aflatoxin B_1 standard solution in methanol and then mixing thoroughly. These samples were used to test the recovery of the toxin by the ELISA procedure as described below. Some nonspiked samples were used to determine naturally occurring aflatoxin B_1. Naturally contaminated seeds were also tested for aflatoxin B_1 levels using TLC and ELISA procedures. For ELISA, aflatoxin B_1 was

extracted from the spiked or naturally contaminated samples with 55% methanol (5 mL g^{-1}) in a Waring blender for 3 min. The extract was filtered through Whatman No.1 paper, the filtrate concentrated by flash drying, and then diluted in PBS to avoid methanol interference (to have below 11% methanol in each assay). The Pons' method of extraction (Pons et al. 1966) was used in the detection and estimation of aflatoxin B_1 by TLC.

Aflatoxin B_1 standard

Various concentrations of standard aflatoxin B_1 ranging from 100 ng to 50 pg (in two-fold serial dilutions) in PBS containing 11% methanol were used in ELISA.

Direct Competitive ELISA Procedure

In the first step, 200 μL of crude antiserum (1:5000 in 0.05 M carbonate buffer, pH 9.6) were incubated in each well of polystyrene microtitre plates for 2 h at 37°C. The plates were then washed three times in PBS-Tween. Next, 100 μL of various dilutions of aflatoxin B_1 standard or sample extracts were added to each well, followed by 100 μL of afla B_1-oxime-BSA-ALP conjugate (diluted in PBS-Tween containing 1% BSA, 1:4000). The plates were incubated at 37°C for 2 h. The wells were then washed with PBS-Tween, 200 μL of p-nitrophenyl phosphate (enzyme specific substrate) added, and the plates incubated at room temperature for 30 min. The reaction was stopped by the addition of 50 μL of 3 mol sodium hydroxide, and the absorbance at 405 nm was measured using a micro-ELISA reader.

Results and Discussion

The relationship between different concentrations of standard aflatoxin B_1 and absorbance values in the ELISA procedure is shown in Figure 1. All points are means of two replicates. There was no strictly linear relationship across the different concentrations of aflatoxin B_1 tested. This is probably due to reactions between different proportions of toxin and toxin-BSA-ALP conjugate that influence the amount of the toxin bound to the antibody adsorbed to the well surface. Only at optimal concentrations can a linear relationship be expected. A linear relationship can be obtained experimentally by utilizing a particular range of concentrations of the toxin and using a predetermined toxin-BSA-ALP conjugate dilution. The linear regression line (standard curve of aflatoxin B_1) is shown in Figure 2. This linear regression equation was used to calculate the toxin concentrations from the sample extracts.

Recovery of Aflatoxin B_1 from Spiked Groundnut Samples

The recovery of the toxin was 62–86% for 5-g samples and 70–107% for 10-g samples (Table 1). The recovery of the toxin was significantly lower when higher levels (50 μg kg^{-1}) of the toxin added than when lower levels (10 or 20 μg kg^{-1}) were added in the case of 5-g samples, while the recovery increased from 70 to 107% when 10-g samples were used. Similar results have been reported by other workers, particularly while using larger samples (Fremy and Chu 1984, Fan and Chu 1984, and El-Nakib et al. 1981). Dilution of the sample extracts would have avoided this problem. However, in both sample sizes, recovery of the toxin ranged from 70 to 96% from samples 'spiked' with concentrations of the toxin at 10 or 20 μg kg^{-1}. These results are supported

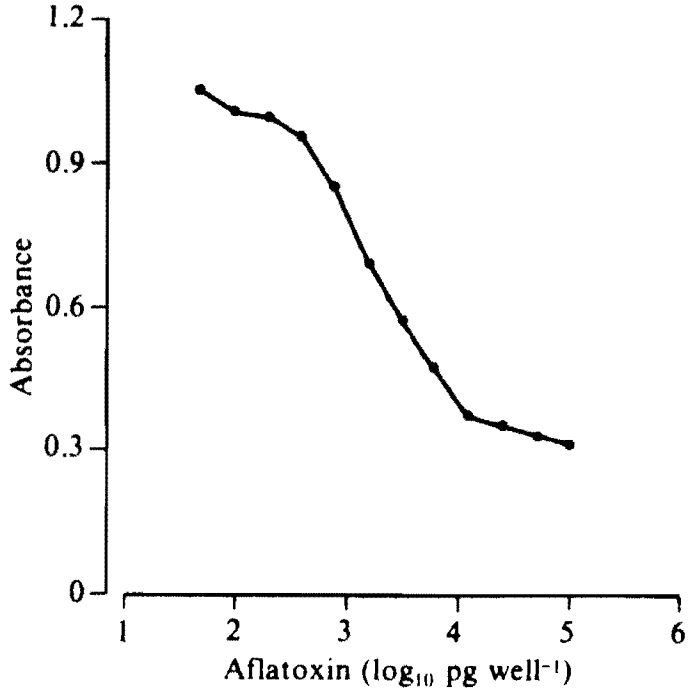
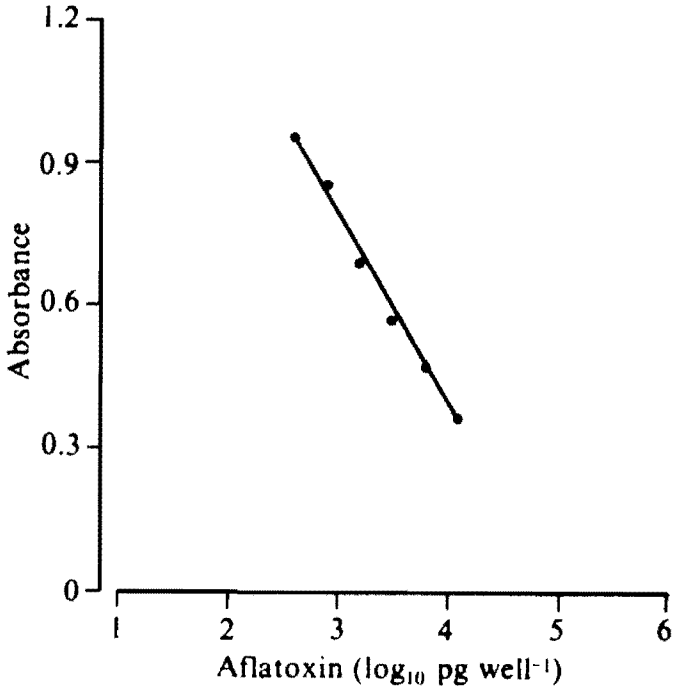


Figure 1. Relationship between aflatoxin B_1 concentration and intensity of alkaline phosphatase (ALP) reaction measured as absorbance.



$y = 1.97 - 0.39x$ ($r = 0.99$, $P < 0.01$)

Figure 2. Linear regression line (standard curve) of aflatoxin B_1.

Table 1. Recovery of aflatoxin B_1 by ELISA from 5-g and 10-g groundnut samples spiked with different concentrations of the toxin, ICRISAT Center, 1987.

Toxin added ($\mu g\ kg^{-1}$)	Toxin recovered			
	($\mu g\ kg^{-1}$)	Recovery (%)	SE	CV (%)
5-g samples				
10	8.6	86	± 3.9	21
20	16.4	82	± 2.9	9
50	31.0	62	± 3.9	5
10-g samples				
10	7.0	70	± 6.3	20
20	19.2	96	±31.3	30
40	43.0	107	±44.8	18

by the findings of the other studies (Fan and Chu 1984, and Fremy and Chu 1984, and Hu et al. 1984). Aflatoxin was not detected in samples that had not been 'spiked'.

Comparison of ELISA and TLC Procedures

The ELISA procedure estimated markedly more aflatoxin B_1 in the samples than the TLC procedure (Table 2). The levels of toxin estimated by this ELISA procedure indicated that it is possible to detect high levels of toxin in naturally contaminated samples, provided appropriate

Table 2. Comparison of TLC and ELISA procedures for estimation of aflatoxin B_1 in naturally contaminated groundnut samples, ICRISAT Center, 1987.

	Aflatoxin B_1 ($\mu g\ kg^{-1}$)			
	TLC method		ELISA	
Sample	Mean	Range	Mean	Range
1	3.9	3.6–4.0	6.5	5.4–8.2
SE	±0.20		±0.84	
CV (%)	5		22	
2	3.9	3.6–4.0	6.0	4.9–7.0
SE	±0.20		±0.61	
CV (%)	5		18	

dilutions of the sample extracts are used. The procedure can detect aflatoxin at levels as low as 5 $\mu g\ kg^{-1}$ with a simple extraction procedure. The ELISA procedure described in this paper can be used to screen large numbers of groundnut samples for aflatoxin B_1 content in either naturally or artificially contaminated seeds. Thus, it is possible to screen large numbers of genotypes simply and inexpensively for their ability to support aflatoxin production when seeds are artificially inoculated with aflatoxigenic isolates of *Aspergillus flavus*. This ELISA procedure has an advantage over the TLC procedure in terms of cost and time for aflatoxin analysis.

El-Nakib et al. (1981) described a direct competitive ELISA procedure for aflatoxin B_1 in groundnut and other agricultural commodities that is more sensitive (detecting down to 3 $\mu g\ kg^{-1}$) than the presently described procedure. However, they used the BF extraction for their ELISA procedure which also differed in its use of toxin-HRP conjugate prepared by the conversion of aflatoxin B_1 to afla B_1-oxime and subsequently coupled with the HRP. In the presently described ELISA procedure, the commercially available afla B_1-oxime-BSA has been used to produce antiserum as well as for conjugation with the enzyme-ALP. In this case, the preparation of aflatoxin B_1-oxime-BSA-ALP conjugate is relatively simple and the conjugate is stable. No loss in activity of the toxin-enzyme conjugate was detected when the conjugate was stored at 4°C for 6 months. Other workers (Fan and Chu 1984, and Morgan et al. 1986) have described indirect competitive ELISA procedures for estimation of aflatoxin B_1. These methods take longer than the direct competitive ELISA procedure because additional incubation steps are required. The ELISA procedure described in this paper is relatively simple and economical, and only requires a simple extraction procedure, and low concentrations of antiserum and the toxin-enzyme conjugate. It can detect aflatoxin B_1 in samples at a level of 50 pg per assay.

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Aflatoxin Analytical Methods for Groundnuts

D.M. Wilson[1]

Abstract

Aflatoxin determination in groundnuts can be approached in several ways. Groundnuts are often contaminated with aflatoxins B_1 and B_2, less often with aflatoxins B_1, B_2, G_1, and G_2 so it is important to have analytical values that represent the total aflatoxin content. Some countries are only interested in B_1 content and others are interested in the total aflatoxin content. It is essential to safely handle all experimental materials associated with aflatoxin analyses or the aflatoxigenic fungi. Visual screening of suspect groundnut lots, based on the presence of conidial heads of the Aspergillus flavus *group, is not a chemical test and may allow aflatoxin-contaminated lots into commerce. Minicolumn screening techniques can be useful but they should always be used in conjunction with a quantitative method. Several thin layer chromatographic (TLC) and high performance liquid chromatographic (HPLC) methods are suitable for quantification and are in general use. The newer immunochemical methods such as the enzyme-linked immunosorbent assay(ELISA) or affinity column methods are being rapidly developed. ELISA methods are available for screening as well as quantification, but these methods are temperature-sensitive and they should only be used with proper controls. The affinity column method is less temperature-sensitive and can be used for either screening or quantification. The chemical and immunochemical methods are reliable if care is taken and personnel are well trained. All analytical laboratories should stress safety and include suitable analytical validation procedures.*

Résumé

Méthodes d'analyse des aflatoxines dans les arachides : *Il y a plusieurs approches au dosage des aflatoxines dans les arachides. Les arachides sont souvent contaminées par les deux aflatoxines B_1 et B_2 et moins souvent par l'ensemble B_1, B_2, G_1 et G_2; il convient donc de déterminer les valeurs analytiques qui représentent la teneur totale en aflatoxines. On considère soit la teneur totale, soit uniquement la teneur en B_1, selon le pays. On ne peut trop souligner l'importance des précautions à prendre dans la manipulation du matériel expérimental et des champignons producteurs d'aflatoxines.*

*Le contrôle visuel des lots suspects à cause de la présence des conidies du groupe d'*Aspergillus flavus, *n'est pas un essai chimique et n'empêchera pas la diffusion de certains lots contaminés. Les méthodes de criblage par minicolonnes, quoique utiles, doivent être accompagnées d'analyses quantitatives. Plusieurs méthodes de dosage des aflatoxines par la chromatographie en couche mince (TLC) et la chromatographie liquide haute performance (HPLC) sont d'usage commun. D'autres nouvelles méthodes immunochimiques telles que la méthode immunoenzymatique de*

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dosage des anticorps (ELISA) et celle de colonnes d'affinité sont de plus en plus perfectionnées. Les tests ELISA permettent à la fois la détection et le dosage. Cependant, ils sont sensibles à la température et nécessitent une maîtrise adéquate des conditions. La méthode des colonnes d'affinité est moins sensible à la température et peut être utilisée soit pour le dosage, soit pour la détection. Les méthodes chimiques et immunochimiques sont valables à condition que toutes les précautions soient prises et que le personnel soit bien formé. Les précautions et les analyses de confirmation appropriées sont d'importance capitale dans un laboratoire d'analyse.

Resúmena

Métodos para analizar las aflatoxinas en el cacahuate : *La determinación de aflatoxinas en el cacahuate puede llevarse a cabo de diversas maneras. Los cacahuates frecuentemente están contaminados con las aflatoxinas B_1 y B_2 y menos frecuentemente con la combinación de B_1, B_2, G_1, y G_2, de manera que es importante la obtención de valores analíticos que representan el contenido total de aflatoxinas. Algunos países se interesan por el contenido total de aflatoxinas. Es esencial manejar con las precauciones debidas todos los materiales experimentales asociados con los análisis de aflatoxinas o los hongos aflatoxigénicos.*

La selección y depuración, en forma visual, de lotes de cacahuates dudosos, que se basan en la detección de conidióforos y conidios del grupo de hongos Aspergillus flavus, *no es tan confiable como una prueba química y puede ocasionar que lotes de semillas contaminadas con aflatoxinas entren en el comercio. Las técnicas de depuración por medio del uso de minicolumna pueden ser útiles, pero siempre deben usarse combinadas con algún método de análisis cuantitativo. Varios métodos analíticos a base de cromatografía de capa delgada (TLC) y de cromatografía líquida de alto rendimiento (HPLC) son satisfactorios para la cuantificación de aflatoxinas, y se utilizan de manera general. Los métodos inmunoquímicos más recientes, tales como el método inmunoabsorbente vinculado a enzimas de cuantificación de anticuerpos (ELISA) o métodos de columnas de afinidad, se están perfeccionando rápidamente. Los métodos ELISA pueden usarse para fines de depuración, así como para cuantificaciones de aflatoxinas, pero son sensibles al efecto de las temperaturas y solamente deberán usarse cuando se dispone de un control apropiado de este factor. El método de columnas de afinidad es menos afectado por la temperatura, y puede usarse ya sea para fines de depuración de lotes de materiales o de cuantificación de las aflatoxinas. Los métodos químicos e inmunológicos son confiables, si se usan con los cuidados necesarios por personal bien capacitado.*

Todos los laboratorios analíticos deben enfatizar la adopción de medidas de seguridad, e incluir procedimientos adecuados para verificar la exactitud de los resultados analíticos obtenidos.

Introduction

The study of aflatoxin contamination of foods and feeds is important because aflatoxins are toxic and carcinogenic to humans and animals. This paper reviews the sampling plans and analytical methods for aflatoxins in groundnuts and groundnut products, and also refers to the safety aspects of such methods.

Safety

Chemical safety

Guidelines for mycotoxin safety precautions are given by the Association of Official Analytical Chemists (AOAC) in Official Methods of Analysis Chapter 26 (1984). The mycotoxin analysis publication from the International Agency for Research on Cancer (IARC) also has a good discussion of safety precautions (Stoloff et al. 1982). The safety guidelines discussed in these books are appropriate for both crude and pure aflatoxin preparations. The chemicals should only be handled with gloves and used only in properly ventilated hoods or glove boxes.

Biological safety

Spores and other viable propagules of *Aspergillus flavus, A. parasiticus*, and other fungi can cause three types of disease in humans: allergy, poisoning, and infection (Hill et al. 1985). *A. flavus* infection in humans is uncommon but possible. Airborne spores and dust containing propagules of the *A. flavus* group may cause allergies in some people and the inhaled particles may contain aflatoxins (Shotwell et al. 1981). Two thin layer chromatography (TLC) methods have been developed to measure aflatoxins in maize and grain dust (Ehrlich and Lee 1984, Shotwell et al. 1981).

Hill and co-workers (1984) found between 10^4 and 10^9 viable fungal propagules per m^3 of air containing maize dust; air containing groundnut dust is probably equivalent. The majority of the *A. flavus* propagules in air samples were deposited on the stages of the Andersen sampler corresponding to the trachea, primary bronchi, and secondary bronchi in the human respiratory system (Hill et al. 1984). *A. flavus* spores and propagules in dust associated with inoculation, shelling, grinding, and extraction procedures are sufficiently hazardous to require safe handling procedures including gloves, masks, protective clothing, and efficient dust collection mechanisms.

Sampling

Sampling is the most important contributor to the variability of analyses for aflatoxin in agricultural commodities, particularly groundnuts, because of the nonhomogenous nature of aflatoxin distribution. The first consideration in any experimental or regulatory protocol should be the sampling method. Protocols have been published on sampling techniques (Dickens and Whitaker 1986). Schuller et al. (1976) published an excellent review of sampling plans and collaboratively studied methods for aflatoxin analysis.

Aflatoxin contamination in groundnuts generally is more variable in single fields, single test plots, or single lots than aflatoxin contamination in maize and some other crops. Therefore a 22-kg sample is needed for groundnut whereas a 4.54-kg sample is usually sufficient for maize, especially when several analytical samples are averaged to approximate the true mean (Whitaker and Dickens 1983). In groundnut lots composed of mixed loads from different sources, a larger initial sample should be taken. In the United States three 22-kg samples are taken from each groundnut lot (Dickens and Whitaker 1986). The total sample should be ground so that it passes through a 0.85-mm sieve, thoroughly mixed or divided, and properly subsampled before analytical samples are taken. Sampling protocols for test plots must be part of the experimental design and should be tailored to meet the experimental objectives.

Aflatoxin Standards

Criteria for aflatoxin standards (Rodricks 1973) and procedures for checking the concentration and purity of aflatoxin standards can be found in AOAC Official Methods of Analysis (1984). The use of calibrated standards in all analytical laboratories is essential. Prepared standards are available from several commercial companies at reasonable prices and analytical laboratories should, if possible, routinely use these standards. Velasco (1981) found that cyclohexane, heptane, and toluene could be substituted for benzene in standards if the solutions were not exposed to light. Analysts should take solvent composition into consideration when standards are prepared for high performance liquid chromatography (HPLC) as it has been found that solvent composition affects aflatoxin fluorescence (Chang-Yen et al. 1984).

Presumptive and Screening Methods

Some applications require only presumptive or screening tests while others require the quantification of only B_1 or several of the aflatoxins. Groundnuts at the buying point are visually inspected in the United States for evidence of *A. flavus* conidial heads and if present the suspect lots are not allowed into commerce for human consumption (Dickens and Whitaker 1986). This visual examination is not a chemical test and may result in family acceptances or rejections. The other commonly used screening technique is the application of one of several minicolumn procedures to detect aflatoxin contamination above a predetermined level (Holaday 1981, Romer et al. 1979). Shannon and Shotwell (1979) conducted a collaborative study of two minicolumn methods and found that a combination method using the Holaday extraction and the Velasco minicolumn was the most satisfactory method. Minicolumn techniques should not be used for quantitative purposes where accurate quantitative data are required. Madhyastha and Bhat (1984) recently developed a minicolumn confirmation method for aflatoxins. These workers confirmed the identity of aflatoxins on the developed minicolumn by applying 20% H_2SO_4, 20% HCl, or trifluoroacetic acid (TFA) in 20% HNO_3. All acids changed the fluorescence from blue to yellow, with the TFA in 20% HNO_3 having the lowest detection limit.

Quantitative Methods

Many of the methods adopted by scientific groups and government agencies are based on TLC detection and quantification procedures that have been evaluated in collaborative studies. The HPLC methods are not more often recommended only because very few collaborative studies have been conducted on them as yet. The AOAC (1984) recommends the contamination branch (CB) and the best foods (BF) methods for aflatoxin analysis in groundnuts. Nesheim (1979) and Shotwell (1983) have also reviewed methods on aflatoxin analysis.

Thin layer chromatography (TLC) methods

The CB method (AOAC 1984) is the standard by which other methods are judged. Details can be found in Stoloff et al. (1982). Shotwell and Goulden (1977) compared the AOAC BF groundnut method and the AOAC cottonseed method with the CB method for analysis of aflatoxins in maize. The BF method uses a methanol+water (55+45) extraction solution, while the cottonseed method uses an acetone+water (85+15) extraction solution. Neither of these solvents extracted

aflatoxins from maize as efficiently as did the chloroform+water (250+15) extraction of the CB method. The BF method is suitable for groundnuts with aflatoxin contents below 50$\mu g\ kg^{-1}$. Lee and Catalano (1981) developed a scaled-down cleanup column as a solvent-saving modification of the CB method. Laboratories which use fluorodensitometry for quantitative measurements need to be careful to avoid fading of aflatoxin spots on TLC plates; fading could be delayed by covering the layer on the TLC plate with another glass plate (Nesheim 1971).

The CB method is an excellent TLC method, but it has two major disadvantages: (1) it is expensive because it uses large amounts of solvents which create a disposal problem, and (2) the major solvent used is chloroform which may be a hazard to workers. Dantzman and Stoloff (1972) developed a modified screening method in which they omitted the column chromatography step of the CB method and directly spotted the residual oil from maize extracted with $CHCl_3$-water. Spilman (1985) modified this screening method for maize by adding benzene+acetonitrile (98+2) to the residual oil and measuring the volume to obtain quantitative TLC results. Groundnuts would have to be defatted with hexane before the $CHCl_3$-water extraction for these screening methods.

Kamimura et al. (1985) recently described a simple rapid HPTLC method which compared favorably with the CB method. Davis et al. (1981) used a novel approach by devising a method using the fluorescence of the iodine derivative of aflatoxin B_1 for quantification and TLC confirmation. Josefesson and Moller (1977) developed a multimycotoxin screening method for detection of aflatoxins, ochratoxin, patulin, sterigmatocystin, and zearalenone, while Seitz and Mohr (1976) and Thomas et al. (1975) developed methods for aflatoxin and zearalenone determination.

The AOAC aflatoxin confirmation method is based on the TFA reaction with B_1, G_1, or M_1 (Przybylski 1975). The TFA procedure or direct acetylation (Cauderay 1979) can be carried out on a TLC plate before development. Trucksess et al. (1984) recently published a rapid TLC method using a disposable silica-gel column for cleanup and confirmation by gas chromatography/mass spectroscopy. No matter which TLC method is used the aflatoxin identified needs to be confirmed. A review of confirmation methods has been written by Nesheim and Brumley (1981).

High performance liquid chromatography (HPLC) methods

Aflatoxin analysis using HPLC for separation and detection is quite similar to TLC because similar sampling and extraction procedures are used. The major advantages of HPLC over TLC are speed, automation, improved accuracy, and precision. Both normal-phase and reverse-phase HPLC separations have been developed for aflatoxin analyses. Early experimental work by Seitz (1975) and Garner (1975) on HPLC separations revealed that aflatoxins could be separated on normal-phase columns and detected with either a UV detector or a fluorescence detector. Seitz (1975) noted that the fluorescence detector had limited usefulness for aflatoxin B_1 and B_2 with normal phase separations.

Panalaks and Scott (1977) developed a silica-gel packed flow cell for fluorometric detection of B_1, B_2, G_1, and G_2 with normal phase aflatoxin separations. A silica-gel packed cell was used by Pons (1979) and Thean et al. (1980) in two different HPLC methods for determination of aflatoxins. The major disadvantage of the packed cell is lack of stability. The cell needs to be repacked often and the detector signal weakens with time. The advantages of a packed cell method are that no derivative is necessary for detection and the mobile phase can be recycled.

Reverse-phase HPLC separations of aflatoxins are more widely used than normal-phase

HPLC separations. However, the fluorescence intensities of B_1 and G_1 are diminished in reverse-phase solvent mixtures so the derivatives B and G are generally prepared before injection. Analysts should be aware that derivatives B and G are not stable in methanol, which should be used with caution, especially in the injection solvent. Acetonitrile-water mixtures do not degrade B and G rapidly and are preferred to methanol-water mobile phases.

Several reverse-phase methods have been published (Cohen and Lapointe 1981, Stubblefield and Shotwell 1977) including three with comparisons to the CB method (DeVries and Chang 1982, Hutchins and Hagler 1983, Tarter et al. 1984). Stubblefield and Shotwell (1977) found that M_1 and M_2 as well as B_1, B_2, G_1, and G_2 could be resolved and detected with a UV detector at 350 nm using reverse-phase chromatography. The methods developed by Hutchins and Hagler (1983), DeVries and Chang (1982) and Tarter et al. (1984) all use TFA derivatization and apparently compare favorably with other methods. Diebold et al. (1979) used laser fluorometry to detect aflatoxin B after reverse-phase chromatography.

Davis and Diener (1980) found that the iodine derivative of B_1 could be used for confirmation and developed a reverse-phase method with fluorescence detection for this derivative. This work led to the development of a postcolumn iodination method to enhance B_1 and G_1 fluorescence after reverse-phase chromatography. Shepard and Gilbert (1984) investigated the conditions needed for the postcolumn iodination reaction to enhance fluorescence of aflatoxins B_1 and G_1.

Immunochemical methods

Aflatoxin B_1 in groundnuts can be determined using solid-phase radio-immunoassay (RIA). (Langone and van Vunakis 1976, Sun and Chu 1977), monoclonal affinity column immunoassay (Groopman et al. 1984), or enzyme-linked immunosorbent assay (ELISA) techniques (Chu and Ueno 1977, El-Nakib et al. 1981, Lawellin et al. 1977, Pestka et al. 1980). ELISA or affinity column techniques are more suited to field use than RIA techniques and will probably be extensively developed and utilized. The major advantages of the ELISA and affinity column methods include speed, ease of sample preparation, ease of use, and a potentially low cost per analysis. The disadvantages include different antibody specificities for B_1 and cross reactivity with other aflatoxins. ELISA procedures are qualitative or semi-quantitative at best and are temperature sensitive. The major application for ELISA procedures at present is screening for aflatoxin B_1 below a predetermined concentration. The color developed by the enzyme-mediated reaction gives an indication of the amount of B_1 present. More development is needed before immunochemical techniques will be generally useful for applications where quantification is critical. Methods also need to be developed that will distinguish between B_1, B_2, G_1, and G_2 individually or collectively.

Selection of Analytical Approach

Regulatory and experimental applications of methods for aflatoxin analysis do not always need to be the same. Regulatory applications need to be quantitative and legally acceptable, but acceptable methods may vary within a country or between countries. However, it is important to use validated methods for regulatory applications.

Aflatoxin analysis in experimental work must be tailored to the objectives and method selection should be a part of the experimental design. Inexpensive minicolumn data may be sufficient for some experimental purposes whereas quantitative data on B_1, B_2, G_1, and G_2 may be required for other purposes. Costs and data requirements can sometimes seem to be at odds

when quantitative data are necessary. The TLC and immunochemical methods may not always be cheaper than HPLC in the long run because HPLC requires a single large initial investment, and TLC and ELISA both use expensive disposable plates. HPLC is possibly more suitable for large analytical laboratories while TLC is more suitable for laboratories with only a few samples to be analyzed. With further development, immunochemical methods will probably become more versatile and suited to a wider variety of applications.

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Session VII

Research on Aflatoxin Contamination of Groundnut: General

Aflatoxin Research in the Peanut-CRSP: An Overview

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Abstract

The global problem of aflatoxin is being pursued by the Peanut Collaborative Research Support Program (Peanut-CRSP) through: (1) development of cultivars resistant to invasion by aflatoxin-producing fungi; (2) cultural practices to minimize insect damage which facilitates fungal invasion; (3) detoxification of contaminated nuts and their products; and (4) separation of contaminated nuts. The dimensions of the problem appear to indicate that a substantial portion of the crop must be sorted out to eliminate aflatoxin. Progress in each of these areas gives promise of the elimination of aflatoxin from food-grade groundnuts.

Résumé

Recherches sur les aflatoxines dans le cadre du programme "Peanut CRSP" : *Le problème des aflatoxines est étudié dans sa totalité dans le cadre du Peanut Collaborative Research Support Program (Peanut CRSP). Ce programme comprend plusieurs volets : (1) la création des cultivars résistants à l'invasion des champignons aflatoxinogènes; (2) la mise au point des techniques culturales permettant de minimiser les dégâts par les insectes qui favorisent l'invasion par les champignons; (3) la détoxification des arachides contaminées et de leurs produits; et enfin, (4) le triage des arachides contaminées. Compte tenu de l'importance de ce problème, il faudrait éliminer une grande partie des récoltes afin d'en enlever les aflatoxines. Les progrès réalisés dans chacun de ces domaines permettront d'obtenir des arachides de bouche exemptes d'aflatoxine.*

Resúmene

Investigaciones sobre las aflatoxinas en el Programa de Apoyo Conjunto a la Investigación del Cacahuate (CRSP): Una revisión general : *EL problema de las aflatoxinas se está estudiando en forma integral por el personal del Programa de Apoyo Conjunto a la Investigación del Cacahuate (Peanut Collaborative Research Support Program o "Peanut CRSP"), mediante trabajos sobre: (1) el desarrollo de cultivares resistentes a la invasión de hongos aflatoxígenos; (2) prácticas de manejo del cultivo que reducen los daños por insectos que facilitan las invasiones fungosas; (3) la eliminación de toxinas de los cacahuates contaminados y los productos derivados de los mismos; y, (4) la separación de los cacahuates contaminados. La magnitud del problema parece indicar*

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que una parte considerable de la cosecha deberá seleccionarse y desecharse para lograr la eliminación de las aflatoxinas. Los avances logrados en cada uno de los rubros mencionados permiten abrigar esperanzas de lograr la obtención de cacahuates para el consumo humano prácticamente libres de aflatoxinas.

Introduction

One of the constraints to increasing production/utilization of groundnuts listed by the Peanut Collaborative Research Support Program (CRSP) is the control of mycotoxins, especially aflatoxin, in the final product.

Efforts to overcome this problem have been pursued in several projects in the Peanut CRSP such as the Texas A&M University project with l'Institut de technologie alimentaire in Senegal, the University of Georgia project in Thailand and the Philippines, and Alabama A&M University's project in Sudan and the Caribbean. Other Peanut CRSP efforts have been made in the insect management project in Burkina Faso with the University of Georgia, and the University of Florida's efforts in the Caribbean project.

Breeding

Breeders have been looking for cultivars that are resistant to invasion by *Aspergillus* spp. These efforts are pursued by screening groundnut seeds or pods against *Aspergillus* spp in the laboratory and selecting those that show resistance for further trials. A completely resistant genotype has yet to be found. Although some promising leads have been uncovered, the goal of a resistant cultivar still eludes us.

Insect Management

The role of insects such as termites in Burkina Faso and the lesser cornstalk borer, *Elasmopalpus lignosellus* in Georgia as facilitators for *Aspergillus* invasion has been directly correlated with the presence of aflatoxin in groundnuts. Cultural practices such as prompt harvesting at the end of the rainy season to minimize insect damage have resulted in a lower incidence of aflatoxin.

The harvesting, drying, and storage phases of groundnut production are distinctively risky for the development of aflatoxin. Studies by the Alabama-Sudan group indicate a generally low level of aflatoxin in groundnuts at the farmer's level in Sudan (Singh 1986). A recent Peanut CRSP effort has been initiated in the Caribbean to study problems of drying and storing groundnuts.

Removal/Destruction

Once aflatoxin is formed in the groundnuts, there are several options one can take. One is to ignore it, although most persons would not find that option acceptable. Another is to remove or destroy the aflatoxin, and studies on removal from groundnut oil by adsorption with inorganic clays, such as aluminum silicates, have been pursued by the Texas group. Removal from peanut

"milk" by a bacterium, *Flavobacterium aurantiacum*, has been studied by the Georgia Food Technology group (Hao et al., see pages 141–151). The question of whether the aflatoxin is simply removed or destroyed has yet to be answered.

Chemical detoxification has been studied by the Florida group who chlorinated aflatoxin and identified the resulting products. Although the aflatoxin was eliminated, the method is not proposed for use because of health concerns. Studies using other chemical agents such as bisulfite and ammonia have been proposed, as has photochemical degradation.

The third option for controlling mycotoxins is to remove or sort out the contaminated nuts. The Food Science Project in Thailand and the Philippines with the University of Georgia, and the Texas A&M project have taken this approach. The Texas group has used dielectric characteristics to distinguish sound from moldy nuts, but the necessary precision has yet to be achieved.

The task of separation or removal is affected by the nature of the distribution of the contaminated nuts. This has been studied by Whitaker and Wiser (1969) at North Carolina State University, USA, who showed that in samples averaging an aflatoxin content of 100 $\mu g\ kg^{-1}$, the toxicity resided in less than 0.5% of the nuts. While this is a statistical abstraction, it indicates that a small fraction of the nuts contain a high level of aflatoxin. The problems of sampling, which are exacerbated by such a distribution, make it very difficult to study the effect of variables in a statistically sound manner.

Studies by Blankenship et al. (1984) at the National Peanut Research Laboratory of the United States Department of Agriculture, Dawson, Georgia, USA, have shown a direct relationship between pod damage, pod strength, and aflatoxin levels, and an inverse relationship between pod size, seed density, pod terminal velocity, and aflatoxin levels. The commercial methods of separation are based on these characteristics to isolate contaminated nuts from a stream containing a mixture of contaminated and aflatoxin-free nuts.

The Georgia-Philippines-Thailand group used visual sorting preceded by blanching and steaming. The rationale was that a method utilizing "appropriate technology" was needed. The ultimate product was the nut, therefore testae and embryos were removed. The goal was to detect the presence of mold rather than aflatoxins, inasmuch as there might possibly be other mycotoxins in the nuts. Steaming was used for several reasons, firstly to inactivate lipoxygenase, and secondly to enhance any chromogenic reactions resulting from the presence of molds.

Other considerations were to limit the destruction of antioxidants by providing an anaerobic atmosphere that also minimized oxidative browning reactions. Using this method, a batch of groundnuts averaging an aflatoxin content of 120 $\mu g\ kg^{-1}$ were treated and sorted into acceptable and questionable groups. They were then processed into peanut butter and analyzed. The acceptable lot had no detectable aflatoxin whereas the rejects contained aflatoxin of more than 800 $\mu g\ kg^{-1}$. The rejects amounted to about 13% of the total.

Variations of this procedure are seen in such practices as dry roasting, blanching, and inspection for confectionery nuts; or blanching, oil roasting, and inspection for roasted nuts. When carefully done, tests have shown little or no aflatoxin in the nuts.

On the other hand, the processing of peanut butter is such as to mask any imperfections present in the nuts, and it is entirely possible that the aflatoxin levels in the 800 $\mu g\ kg^{-1}$ range seen in some samples are a result of utilizing reject nuts. A similar situation exists when nuts are chopped and mixed with other condiments for use as decorative toppings or as a base for cooking.

Discussion

The questions that arise are:

1. If the aflatoxin is concentrated in a small percentage of kernels, is it necessary to remove perhaps 10 times as many?
2. Is the rate of infection by aflatoxin-producing fungi usually in the range of perhaps 1-10% of all fungi infecting groundnuts?

The removal of nuts that have been attacked by mold thus results in 10 times more nuts being rejected than if methods for detecting and removing individual aflatoxin-contaminated nuts could be perfected. But during the process of removing mold-damaged nuts, other mycotoxins are eliminated. The amount of rejects depends on the initial condition of the sample, but adds up to a sizable portion, and thus methods for using reject nuts would be a further incentive for separation.

Studies by the Texas group on the incidence of *Aspergillus* spp in groundnut seed from Senegal gave average seed infection levels of 11% for *A. flavus* and 36% for *A. niger*. However, considerable variation was noted between samples; and effects of location, environment, variety, etc. were also noted. It was concluded that most seed had *A. flavus* spores on their surfaces when sown. Thus, it appears reasonable at this time to accept the premise that aflatoxin-producing fungi usually infest groundnuts at a level of 1-10% of the total fungal population, but have a potential for 100% infestation.

The potential for improvement is in reducing the total fungal infection rate by use of resistant cultivars, improved cultural practices, protective handling during harvesting, drying, and storage, and better sorting methods.

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Discussion

J.I. Pitt. Regarding the comment by Dr Nakayama on *A. flavus* invasion of groundnuts being very variable (1-22%) in the USA, I have found variation in Australia (5-88%) to depend greatly upon drought stress and cultural conditions.

T.O.M. Nakayama. I agree.

K.K. Shreshtha. In Nepal, we don't have very good technology for detection and analysis for aflatoxin. You metioned sorting, inspection, rapid drying and good storage conditons. Are these appropriate technologies for developing countries?

T.O.M. Nakayama. I certainly think so.

K.K. Shreshtha. Is there any other method you would like to suggest?

T.O.M. Nakayama. Technology is a pretty loose term. We chose the method we did because we didn't need the color sorter. Secondly we addressed the number one constraint which is inadequate food supply.

T. Shantha. 20 to 25% is a considerable amount of produce to reject. What do you do with the rejects? Can one afford to reject so much?

T.O.M. Nakayama. This is a serious problem. Once a nut has been removed from the food chain because of possible mycotoxin contamination, it should not return to the human food chain, but should be used for other purposes where it can do no harm.

Queensland Department of Primary Industries' Involvement with Aflatoxin in Groundnuts in Australia and Indonesia

K.J. Middleton[1]

Abstract

Rainfed groundnut production in Queensland, Australia is often severely affected by aflatoxin contamination. The Queensland Department of Primary Industries (QDPI) provides extension and research services to groundnut producers, and has addressed this problem in a variety of ways since becoming aware of it.

Extension activities have attempted to improve producers' understanding of the causes of aflatoxin formation in groundnuts and of the management methods available at the farm level and at the shelling plant.

Scientific support has been made available to: assist the groundnut industry establish its own quality-control facilities; help define some of the local factors important in aflatoxin development; conduct an Australian site for the International Groundnut Aspergillus flavus *Nursery; and to collaborate with industry, the Commonwealth Scientific and Industrial Research Organization (CSIRO), and the Australian National University (ANU) in aflatoxin research activities.*

The Australian Centre for International Agricultural Research (ACIAR)-funded groundnut project in Indonesia conducted in collaboration by scientists from QDPI and the Agency for Agricultural Research and Development (AARD) may, in future, consider the inclusion of research on production aspects of aflatoxin contamination.

Résumé

Participation du Queensland Department of Primary Industries aux travaux sur les aflatoxines dans les arachides en Australie et en Indonésie : *Les arachides cultivées pendant la saison des pluies au Queensland en Australie sont souvent fortement contaminées par les aflatoxines. Le Queensland Department of Primary Industries (QDPI), ayant pris connaissance du problème, a adopté plusieurs approches en vue de maîtriser la contamination et offre des services de recherche et d'encadrement aux producteurs.*

Les services d'encadrement cherchent à sensibiliser les producteurs en ce qui concerne les causes provoquant la formation des aflatoxines et aux techniques de lutte utilisables au champ et dans les unités de décorticage.

La recherche assure son appui pour : l'établissement des contrôles de qualité au niveau industriel, la détermination des facteurs locaux qui favorisent le développement des aflatoxines et

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la conduite de l'essai International Groundnut Aspergillus flavus *Nursery. Les services scientifiques coopèrent avec le secteur industriel, le Commonwealth Scientific and Industrial Research Organization (CSIRO) ainsi que l'Australian National University (ANU) dans le cadre de la recherche sur les aflatoxines.*

Le projet sur l'arachide financé par l'Australian Centre for International Agricultural Research (ACIAR), est situé en Indonésie et mené en collaboration avec les chercheurs du QDPI et ceux de l'Agency for Agricultural Research Development (AARD). Il devrait également étudier l'effet de la contamination par les aflatoxines au niveau de la production.

Resúmene

Participación del Departmento de Industrias Primarias de Queensland en los trabajos sobre las aflatoxinas en el cacahuate, en Australia e Indonesia : *La producción de cacahuates bajo condiciones de secano en Queensland, Australia, frecuentemente es contaminada en forma severa con aflatoxinas. El Departmento de Industrias Primarias de Queensland (QDPI), que proporciona servicios de asistencia técnica y de investigación a los productores de cacahuates, se ha dedicado a este problema en diversas formas desde que lo detectó.*

Los servicios de asistencia técnica han tratado de sensibilizar a los productores acerca de las causas de la formación de las aflatoxinas en los cacahuates, y sobre las prácticas de manejo del cultivo que pueden usarse para evitar su formación, tanto en los predios rurales, como en las plantas de descascaramiento.

Se ha proporcionado apoyo científico para: auxiliar a la industria cacahuatera en el establecimiento de controles de calidad a nivel industrial; ayudar en la identificación de factores locales que son importantes en la formación de las aflatoxinas; establecer en Australia el Vivero Internacional de Aspergillus flavus *en Cacahuate y colaborar con la industria, la Organización para la Investigación Científica e Industrial del Commonwealth (CSIRO), así como con el personal de la Universidad Nacional Australiana, en trabajos de investigación sobre aflatoxinas.*

El proyecto sobre cacahuates financiado por el Centro Australiano para la Investigación Agrícola Internacional (ACIAR), situado en Indonesia, que se realiza con la colaboración de los científicos del QDPI y de la Agencia para el Desarrollo y la Investigación Agrícolas (AARD), podrá, en el futuro, considerar incluir en sus trabajos investigaciones sobre los aspectos industriales de la contaminación con aflatoxinas.

Australia

Almost all of the groundnuts grown in Australia are produced in the state of Queensland. There are two main centers of production. The Atherton Tableland in north Queensland is favored by adequate rainfall, produces high yields (2 t ha^{-1}), and has few problems with aflatoxin. South Burnett and Central Burnett in south Queensland form a much larger area, but due mainly to less-reliable rainfall, yields there are generally lower (1 t ha^{-1}), and aflatoxin occurrence is more common.

The Australian groundnut industry is entirely mechanized, with all production intended for confectionery and table purposes. A small quantity of oil is produced from groundnuts unfit for these purposes.

The Queensland Department of Primary Industries (QDPI) is the principal agency servicing

primary producers in Queensland with applied research, extension, and regulatory functions. The QDPI has about 2950 staff, many of whom are located in country centers. Research is carried out at 45 laboratories and research stations strategically located throughout the state. Extension services are widely offered with extension officers disseminating up-to-date knowledge to producers in their areas.

The role of the QDPI in assisting the groundnut industry to address the aflatoxin problem has been one of providing various aspects of scientific support.

Until 1977, there was no requirement in Australia to test foods for aflatoxin. At that time, the Peanut Marketing Board (PMB) was conducting random checks of the graded product as a means of gathering data on the incidence of aflatoxin in the Queensland crop, and of meeting export requirements. The QDPI provided the PMB with technical support, initially conducting the aflatoxin analyses, and later assisting the PMB to establish its own aflatoxin test laboratory. This has since been developed, largely independently of the QDPI, to recognized world standards.

In 1977, a year when all south Queensland production areas experienced severe drought, aflatoxin contamination was widespread and severe. As a consequence, health authorities recommended the implementation of maximum tolerable levels of 15 $\mu g\ kg^{-1}$ total aflatoxin contamination in groundnuts and groundnut products. The industry was then confronted with the long-term need for information on techniques to deal with the contaminated crop in store. Producer representatives, a production specialist employed by the industry and a QDPI plant pathologist acting as scientific adviser, undertook a fact-finding mission to the USA, a country with similar production technology to Australia. Among other things, they were able to confirm that:

- aflatoxin contamination may occur preharvest under drought conditions;
- aflatoxin can be rapidly detected in samples taken from individual deliveries at the buying point;
- frequently, not all kernels in a contaminated lot will contain aflatoxin;
- careful sorting can often separate aflatoxin-contaminated kernels from noncontaminated kernels;
- blanching kernels (testa removal) can assist the sorting process; and
- in cases where aflatoxin production commenced before digging, careful drying and storage (on-farm and in processing plants) can prevent further production of aflatoxin during these procedures.

As a result of these findings the aflatoxin problem in the Australian groundnut industry has been satisfactorily managed, despite some further serious occurrences, by:

- being aware of the close link between drought and aflatoxin;
- careful harvesting, drying, and storage, especially when local conditions indicate potential for contamination;
- monitoring individual deliveries, and segregating contaminated lots from clean ones;
- careful sorting and grading of produce from the contaminated portion of the crop;
- using dry roasting and blanching as a means of improving sorting efficiency; and
- rigorous testing, using sophisticated procedures, at all stages of processing from shelling to dispatch to user.

The costs of this approach have been significant, but at no time have Australian consumers been denied groundnuts or groundnut products because of aflatoxin contamination. Similarly, at no time have producers' incomes been dramatically slashed because of aflatoxin contamination in droughted crops. The QDPI has contributed significantly to the efforts to improve growers' awareness and understanding of the aflatoxin problem.

The QDPI conducted a research project from 1979 to 1981 to verify the role of those factors believed to make important contributions to the recurrent problem facing the Australian groundnut industry. Graham (1982a,b,c) formed the following conclusions:

- The bulk of the fungal contamination and toxin production occurs preharvest.
- Soil populations of the *Aspergillus flavus* group fungi are frequently adequate for infection, particularly in soils with low water-holding capacity or during drought.
- At pod moisture contents from 20% to 75% intact pods were rarely invaded by *A. flavus*, while broken pods and insect-damaged pods were frequently invaded by the fungus. This suggests that intact pods provide some barrier to invasion. This statement is significant as the Red Spanish cultivar grown on soils prone to moisture limitations, frequently splits its shell during the process of pod filling (maturation).
- Drought stress during early stages of crop growth does not lead to aflatoxin contamination, whereas stress in the last 30 days before harvest can lead to high levels of contamination.
- Aflatoxin contamination induced by drought stress is not amenable to control through manipulation of plant population and/or special arrangement, within agronomically acceptable ranges of these factors.
- When aflatoxin is not present at digging (lifting), curing in a warm humid environment is unlikely to lead to aflatoxin contamination although other spoilage fungi may damage the crop.
- The adjustment of harvest date to prevent contamination incurs a yield penalty of greater value than the cost of recovering an edible commodity from the crop produce.

The QDPI has conducted an Australian site of the ICRISAT International Groundnut *Aspergillus flavus* Nursery (IGAFN) to complement data supplied from other countries. The QDPI was assisted with this trial by the PMB who conducted aflatoxin analyses on kernels at harvest, and after inoculation with a known aflatoxin-producing strain of *A. flavus* which was supplied by CSIRO. High levels of *Aspergillus niger* infection were recorded on seeds after harvest. Some of the materials grown for the IGAFN have been used by scientists at the Australian National University who are working with ICRISAT scientists to understand the mode of *A. flavus* resistance offered by groundnut germplasm.

The QDPI has assisted the Queensland groundnut industry to manage its aflatoxin problem through scientific assistance and advice, a significant extension commitment, a limited research role, and by coordinating and advising others involved in either commercial management, or detailed pathogen or host investigations, thereby maintaining a balance between studies on the host, the pathogen, and the environmental factors affecting them.

Indonesia

Since 1985, scientists from the QDPI and the Food Crops Institutes of the Agency for Agricultural Research and Development (AARD) in Indonesia have conducted a collaborative "Peanut Improvement in Indonesia" project funded by the Australian Centre for International Agricultural Research (ACIAR). A conscious decision was taken during planning discussions not to include aflatoxin in this project, which is funded until October 1988. It can be anticipated, however, that aflatoxin would be considered for inclusion in any continuation of the current work if funding of a follow-up project were recommended.

QDPI mycotoxicological expertise has been made available to assist Indonesian scientists associated with Indonesia's intensive animal industries to develop the facilities necessary to address the aflatoxin problems facing those industries.

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Discussion

A.H. Siwela. You said that no oil extraction is done using rejected groundnuts, what do you do with the groundnuts? If oil is extracted, what do you do with the contaminated cake?
K.J. Middleton. Groundnuts are not deliberately produced with oil extraction in mind, but contaminated groundnuts are used for oil extraction: oil is further refined. Contaminated cake is used for stockfeed, but aflatoxin content of formulated stockfeed is also regulated.
R.E. Pettit. Mention was made that groundnuts are sorted in Australia. What procedure is used to sort these groundnuts?
K.J. Middleton. Electronic color sorting, often following blanching and/or light roasting.
D. Caley. Can you confirm that Queensland has only three receiving points for buying groundnuts?
K.J. Middleton. Yes. One in north Queensland, two in south Queensland.
D. Caley. If you only buy groundnuts at three locations, are delivery distances very long?
K.J. Middleton. Delivery distances of 50–60 km are common. The maximum is 600 km. All deliveries are in bulk, usually by 10-t semi-trailers.
D. Caley. How many staff are employed in research and extension?
K.J. Middleton. QDPI has a staff total of 2950 for all functions. Staff associated full or part-time with the groundnut industry would be Research 15, Extension 6, and Regulations 3.

Groundnut Aflatoxin Problems in Indonesia

M. Machmud[1]

Abstract

Aflatoxin research in Indonesia was initiated in 1969. Of the marketable groundnuts tested 60-80% were contaminated with aflatoxin at levels from 40 to 4100 μg kg^{-1} seeds; retail groundnuts being the most highly contaminated.

Processing raw groundnut seeds into other products, such as peanut butter and fermented groundnut presscake significantly reduced aflatoxin contamination. Clinical studies suggested a positive correlation between aflatoxin ingestion and human hepatic cancer.

More research is needed on the role of preharvest fungal infection on postharvest aflatoxin contamination, the control of storage contamination, and on fungi × groundnut × environment interactions favoring aflatoxin production.

Résumé

Problème des aflatoxines dans l'arachide en Indonésie : *La recherche sur les aflatoxines a débuté en 1969 en Indonésie. Entre 60 et 80% de la production commerciale des arachides était alors contaminée à des niveaux allant de 40 à 4100 μg kg^{-1} de graines. Les arachides faisant l'objet du commerce de détail étaient les plus fortement contaminées.*

Le traitement des graines d'arachide crues pour la fabrication des produits tels que le beurre d'arachide et les tourteaux fermentés réduit sensiblement la contamination. Les études cliniques suggèrent une corrélation positive entre l'ingestion des aflatoxines et le cancer du foie chez l'homme.

Les recherches plus poussées sont nécessaires sur le rôle de l'infection par les champignons avant la récolte dans la contamination des arachides par les aflatoxines après la récolte, sur la maîtrise de la contamination des arachides stockées, ainsi que sur les interactions champignons × arachides × milieu qui favorisent l'élaboration des aflatoxines.

Resúmene

Problemas de las aflatoxinas en el cacahuate en Indonesia : *Las investigaciones sobre las aflatoxinas se iniciaron en Indonesia en 1969. Sesenta a ochenta por ciento de la producción comercial de cacahuates estaba contaminada con aflatoxinas, con niveles de 40 hasta 4100 μg kg^{-1} de semilla; los cacahuates en el comercio al menudeo fueron los más altamente contaminados.*

El procesamiento de las semillas crudas, para su conversión en otros productos, tales como mantequilla de cacahuate y torta de cacahuate fermentado, redujo significativamente la contami-

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nación con aflatoxinas. Estudios clínicos sugirieron la existencia de una correlación positiva entre la ingestión de aflatoxinas y la incidencia de cáncer hepático en los humanos.

Se necesitan más investigaciones sobre los efectos de la infección fungosa antes de la cosecha en la contaminación con aflatoxinas después de la cosecha; el control de la contaminación durante el almacenaje; y sobre las interacciones hongo x cacahuate x medio ambiente, que favorecen la producción de aflatoxinas.

Introduction

Groundnut is a major food legume in Indonesia and ranks second after soybean, annual production being approximately 0.5 million t of unshelled nuts from 550 000 ha, with yields ranging from 0.5 to 1.5 t ha^{-1} (Bell et al. 1986). Groundnuts produced in Indonesia are used almost exclusively for human consumption.

The humid tropical conditions of Indonesia and its agricultural practices favor rapid growth and widespread infestation of crop plants, including groundnut by *Aspergillus flavus*,the mold responsible for aflatoxin production (Husaini et al. 1974). The aflatoxin hazards to man and animal have been known for a long time in Indonesia (Pang and Husaini 1972, Pang et al. 1974), but specific attention and research on groundnut aflatoxin was initiated only in April 1969 by the Nutrition Research Institute, Bogor, in conjunction with the first Five-Year Development Program of the Government of Indonesia. Studies have been done in three phases, (1) a survey of *Aspergillus flavus* invasion and aflatoxin contamination in groundnuts, (2) studies on prevention of aflatoxin production in groundnuts, and (3) assessments of the relationship between the aflatoxin content of diets and human primary hepatic cancer (Husaini et al. 1974). Recently other institutions such as the Institute for Veterinary Research (Blaney, B.J. personal communication) and the Southeast Asian Regional Center for Tropical Biology (SEAMEO-BIOTROP) have initiated research on aflatoxin (Dharmaputra and Rahayu 1985).

Groundnut Production, Marketing, and Consumption

Most groundnuts in Indonesia are grown by small-scale farmers. The crops are usually grown in upland conditions or in lowlands after rice, either as a monocrop or intercropped with maize or cassava. The major groundnut-growing areas in Indonesia are located in 8 of the 27 provinces of the country, namely Bali, Central Java, East Java, West Java, Lampung, North Sumatra, South Sulawesi, and West Nusatenggara (Muhilal and Nurjadi 1977, Machmud and Middleton 1987) with about 70% of the area in Java (Figure 1). The annual harvested area, production, and yields of groundnuts from 1969 to 1985 are shown in Table 1.

Farmers sell their groundnuts either as fresh pods, dry pods, or dry nuts to "village buyers", after retaining some for their own consumption. The village buyers sell the groundnuts to collectors or directly to industrial factories. The time taken for the groundnut to reach the consumers after harvest ranges from 40 to 110 days (Muhilal and Nurjadi 1977). Figure 2 shows channels of groundnut marketing in Java.

Although Indonesia exports some of its groundnuts, most of the produce is used domestically, primarily for human consumption as raw nuts or in other food products (Muhilal and Nurjadi 1977). The foods made of groundnuts can be divided into two categories, those in which the nuts are still recognizable, such as fried groundnuts, made from selected, good quality nuts, and those

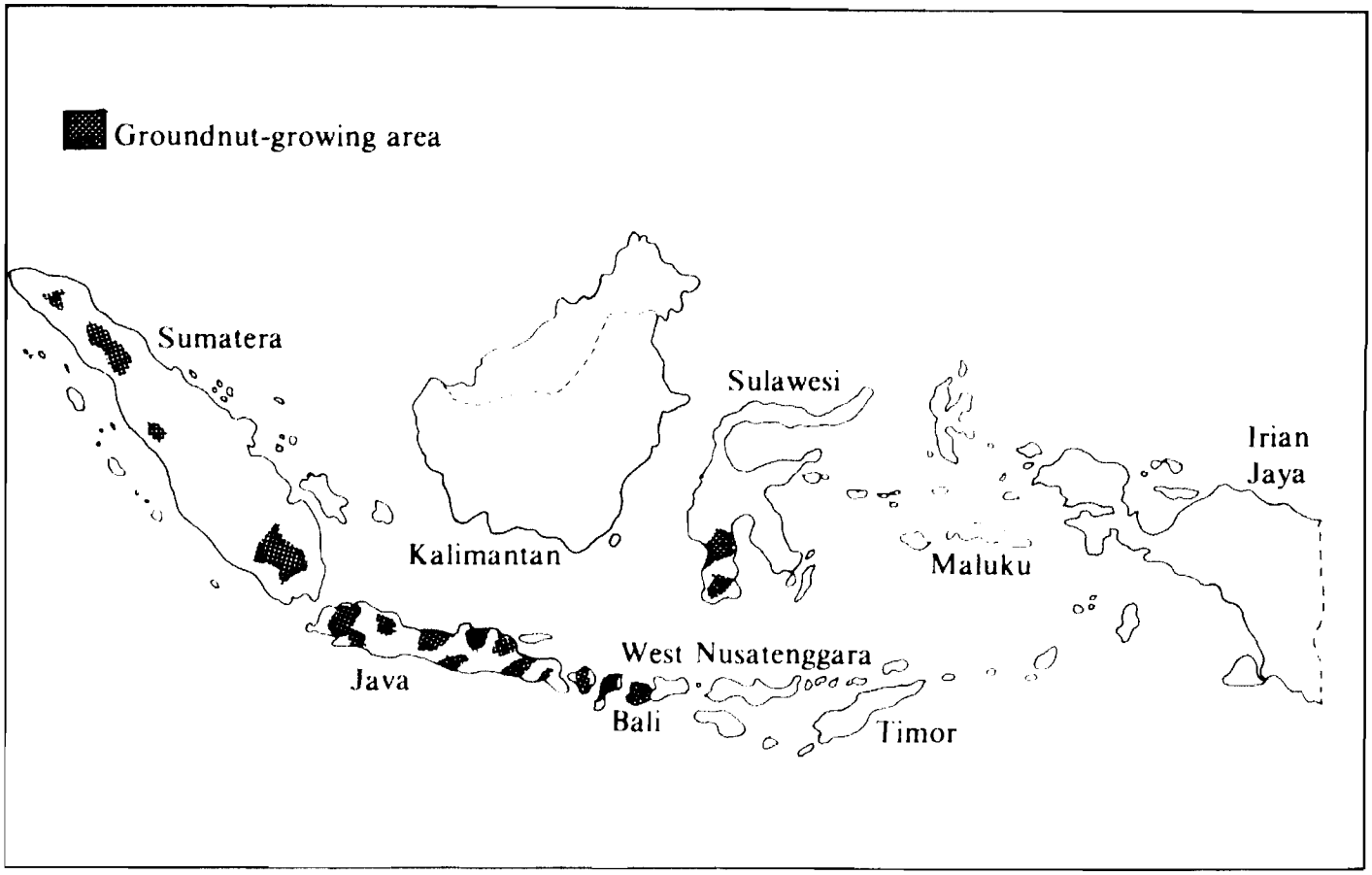

Figure 1. Major groundnut-growing areas (shaded) in Indonesia.

in which the nuts are ground into powder or pastes, such as peanut butter, that are made from the lower-grade nuts and are likely to be contaminated by aflatoxin. There is also a delicacy called *oncom* made of groundnut presscake (*bungkil*), a waste product of groundnuts that have been pressed to extract the oil. The presscake is fermented by molds, *Rhizopus oligosporus* (for black *oncom*) and *Monilia sitophila* (for red *oncom*). This food is particularly popular in West Java (Hermana 1973). Table 2 shows some of the groundnut and groundnut products commonly consumed by Indonesians, and their aflatoxin content.

Table 1. Annual groundnut harvested area, production, and yield ha^{-1} in Indonesia, 1969-1985.

Year	Harvest area ('000 ha)	Production ('000 t)	Yield (kg ha^{-1})	Year	Harvest area ('000 ha)	Production ('000 t)	Yield (kg ha^{-1})
1968	395	287	730	1977	507	409	810
1969	372	267	720	1978	506	446	880
1970	380	281	740	1979	473	424	900
1971	376	284	750	1980	506	470	930
1972	352	282	800	1981	519	475	920
1973	416	290	700	1982	461	437	950
1974	411	307	750	1983	480	460	960
1975	474	380	800	1984	576	535	990
1976	414	341	820	1985	490	493	1000

Source: Directorate General of Food Crops 1986.

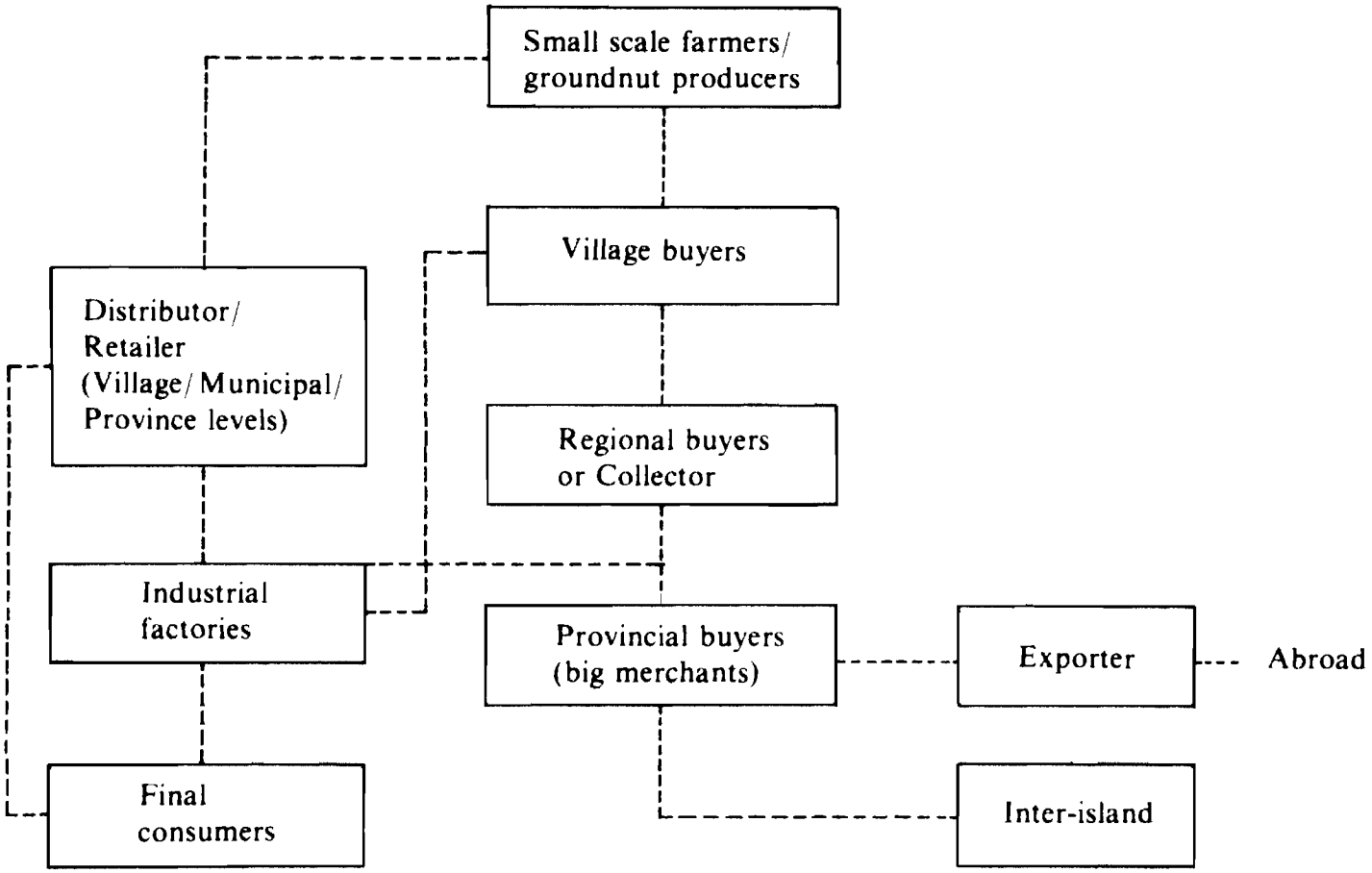

Figure 2. Channels of groundnut marketing in Java.
Source: Survey of Agricultural Economics, Department of Agriculture, 1972, *in* Muhilal and Nurjadi 1977.

Table 2. Aflatoxin contents of groundnuts and groundnut products in Indonesia.

Item	Samples (no)	Aflatoxin content[1] ($\mu g\ kg^{-1}$)	
		B_1	G_1
Groundnut (raw)	20	180	353
Groundnut oil	20	61	82
Groundnut presscake (*bungkil*)	20	126	174
Fermented presscake (*oncom*)	39	67	120
Fried groundnut	4	ND[2]	ND
Boiled groundnut	4	80	ND
Flour-coated fried groundnut	3	ND	3
Groundnut sauce	-	83	49
Peanut butter	3	13	ND
Sweetened groundnut cake	2	170	83
Fried *oncom* (red or black)	16	41	83

1. Average value.
2. ND = not detectable.
Source: compiled from Muhilal, Karjadi, and Prawiranegara (1971).

Studies revealed that the groundnut crop could be infested by aflatoxin-producing fungi while it is still in the field (Roedjito 1971, Machmud and Middleton 1987). Thus, the source of aflatoxin contamination may be in the fields. Since Indonesian farmers usually harvest, process, and dry their groundnuts manually, groundnut quality is not well controlled and varies considerably among farmers. Surveys indicated that most groundnuts sold in the market were invaded by fungi capable of producing aflatoxin, although the level of infestation varied from market to market. Muhilal, Karyadi, and Prawiranegara (1971) reported that 60–80% of groundnut market samples were invaded by fungi and contained from 40 to 4100 $\mu g\ kg^{-1}$ aflatoxin. Interestingly, groundnut samples obtained from research institutions and distributors contained virtually no aflatoxin (Table 3), while those obtained from the retailers contained considerable quantities of aflatoxin. Thus, aflatoxin contamination is more likely to occur while the groundnuts are in the retailers' stores or shops.

Table 3. Aflatoxin contents of groundnuts in Indonesia.

Groundnut source	Samples (no)	Aflatoxin content[1] ($\mu g\ kg^{-1}$)	
		B_1	G_1
Central Research Institute for Agriculture	5	ND[2]	ND
Distributor	5	ND	ND
Subdistributor	5	8	ND
Retailer	12	615	67

1. Average value from the samples analyzed.
2. ND = not detectable.
Source: compiled from Muhilal, Karjadi, and Prawiranegara 1971.

Groundnut products may also be contaminated with aflatoxin, Muhilal Karyadi, and Prawiranegara (1971) reported that almost all the groundnuts and groundnut products sampled from markets, stores, and food manufacturers contained aflatoxin from trace amounts to more than 1 000 $\mu g\ kg^{-1}$, except in fried groundnuts in which aflatoxin was not detectable (Table 3). Compared to the maximum permissible aflatoxin contamination in food products designated by the United States and Canadian Food and Drug Administrations, the aflatoxin contents of Indonesian products are, on average, hazardous to human health.

A study was also undertaken to find ways to control aflatoxin production in groundnuts. The study was designed to distinguish between the effects of early or late harvesting, drying, and storage on aflatoxin contamination in Gadjah variety. The results suggest that the most appropriate time to harvest this variety is between 100–110 days after sowing, otherwise aflatoxin contamination is likely to occur. Groundnuts should be stored in dry, closed containers (Muhilal and Nurjadi 1977).

Aflatoxin contamination was first detected in home-stored, good quality groundnuts 10 weeks after storage. The level increased rapidly to over 500 $\mu g\ kg^{-1}$. The moisture content of the groundnuts usually increases from about 8% when first stored to 11.4% after 10 weeks (Husaini et al. 1974). In another study of home-stored groundnuts aflatoxin was detected after 16 weeks of storage and an increase was noted after 28 weeks (Table 4). Most of the aflatoxins contained

Table 4. Effect of harvest and storage on aflatoxin content of groundnuts (cv Gajah) in Indonesia.

	Aflatoxin content ($\mu g\ kg^{-1}$)			
	B_1		G_1	
Weeks after harvest[1]	100 DAS[2]	110 DAS	100 DAS	110 DAS
0	ND[3]	ND	ND	ND
12	ND	ND	ND	ND
16	ND	ND	0.7	1.4
20	6.7	0.8	1.6	2.6
24	6.7	1.6	2.2	3.9
28	27.2	12.7	2.8	4.8

1. During storage the moisture contents of the stored seed varied between 6% and 8%.
2. DAS = days after sowing.
3. ND = not detected.

Source: Husaini et al., personal communication.

in the groundnuts and groundnut products were aflatoxins B_1 and G_1 which have been reported as potential liver carcinogens. (Pang et al. 1974, Muhilal and Nurjadi 1977). The incidence of liver cancer in humans and animals in Indonesia is high (Muhilal and Nurjadi 1977). The association of aflatoxin with human and animal diseases has been recognized for some time (Blaney 1987). We studied the influence of aflatoxin on human liver cancer, using 81 patients, 80 suffering from primary liver cancer, and one who was healthy. The patients included 66 males and 15 females ranging in age from 7 to 75 years. Aflatoxin was detected in the liver tissues of 58% of the patients suffering from primary liver cancer. The types and amounts of aflatoxin found in the liver specimens were B_1, G_1, and M_1 at levels from trace to $> 400\ \mu g\ kg^{-1}$. The foods commonly consumed by the contaminated patients included *oncom*, fried groundnuts, groundnut sauce, groundnut cakes, soy sauce, salt fish, and various kinds of traditional medicine made from herbs (*jamu*). These foods usually contain aflatoxin B_1, G_1 or both, the aflatoxin B_1 content ranging from 8 to 1190 $\mu g\ kg^{-1}$ and aflatoxin G_1 from 4 to 690 $\mu g\ kg^{-1}$ (Muhilal and Nurjadi 1977).

Recently, more institutions, plant pathologists, and veterinarians have become interested in aflatoxin problems in Indonesia. Dharmaputra and Rahayu (1985) surved storage fungi, and reported that at least four *Aspergillus* species and one *Penicillium* species infest groundnuts during storage.

Prospects for Future Research

Research on groundnut aflatoxin in Indonesia has so far concentrated on the toxin and its effect on man and animals, rather than on the causal fungus and factors influencing aflatoxin production. Research in the following areas would help to reduce aflatoxin hazards.

1. Surveys on the distribution and incidence in groundnut seed of the fungi involved in preharvest aflatoxin contamination.
2. Studies on the relationship among the fungi, the groundnut hosts, and environmental factors in relation to aflatoxin production.
3. Further studies on the effect of aflatoxin on man and animals.

4. Studies on practical methods that could be adopted to reduce aflatoxin production in groundnuts in the field and during storage.

Coordinated national research programs involving scientists from different disciplines, and cooperative activities with international scientists and institutions would enhance Indonesia's capability to solve its groundnut aflatoxin problems.

Conclusions and Suggestions

Groundnut aflatoxin is an important problem in Indonesia. Research was initiated in 1969, and considerable results have been achieved to date but further work is still needed. A national coordinated research program with an integrated approach and cooperative work among domestic and international scientists is necessary to enhance our problem-solving capability. A working group composed of scientists from different countries and disciplines who are interested in groundnut aflatoxin problems should be regularly convened to report research progress and to reevaluate future problems. To solve these problems internationally, it is necessary to organize an interdisciplinary working group of scientists from different countries who are interested in the groundnut aflatoxin problem. Such a group should meet regularly to evaluate the problems and to report research progress.

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Discussion

D. Caley. I note that the concentration of aflatoxin G_1 exceeded that of B_1 in two instances in your results. This is contrary to a common belief that B_1 always exceeds G_1.

J.I. Pitt. *Aspergillus parasiticus* isolates mostly produce both B_1 and G_1 aflatoxins, but the ratio varies widely. Some *A. parasiticus* isolates produce more G_1 than B_1, and a few make G_1 almost exclusively, therefore B_1 does not invariably exceed G_1.

Field Studies on *Aspergillus flavus* and Aflatoxins in Australian Groundnuts

J.I. Pitt[1]

Abstract

Aflatoxins have been a serious problem in Australian groundnuts in the past decade. With the aid of government and industrial funding, the Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Food Research has carried out an ongoing project for most of this period, with emphasis on studies under commercial field conditions.

Research has been primarily concerned with understanding the variables that influence the invasion of groundnuts by Aspergillus flavus *and* A. parasiticus, *and the subsequent production of aflatoxins. Factors studied include: levels of* A. flavus *in soils, environmental factors; farm management practices affecting* A. flavus *invasion; and the influence of drying and storage procedures on aflatoxin development.*

Most groundnut soils in Kingaroy have been found to contain detectable levels of A. flavus, *while surrounding virgin soils usually do not. Levels of* A. flavus *in groundnut soils vary widely, from less than 10^2 spores g^{-1} to as high as 10^5 spores g^{-1}: high levels are much more likely to lead to invasion. Some fields contained consistently high levels over several years. The* A. flavus/ A. parasiticus *ratio also varies widely from farm to farm, and may influence invasion and toxin production.*

Investigations have shown that invasion of groundnuts by A. flavus *takes place before groundnuts are harvested. Invasion will not occur subsequently, neither will aflatoxin be produced, even under the least effective drying procedures. In all but exceptionally dry seasons, little aflatoxin is produced while groundnuts are in the ground, i.e., most aflatoxin is produced postharvest. Under the most favorable conditions, groundnuts require 6 to 10 days to dry in the field after harvest, a period sufficiently long for aflatoxin to reach unacceptable levels. Field drying cannot be sufficiently rapid, even in dry seasons, to ensure aflatoxin-free nuts at intake to shellers.*

The perceived importance of preharvest invasion as the necessary condition for the production of unacceptable aflatoxin levels has led to attempts to predict aflatoxin levels at shelling intake from A. flavus *levels at harvest. Success rates have been encouraging but are not yet of practical utility.*

Résumé

Etudes au champ sur *Aspergillus flavus* et sur les aflatoxines dans les cultures d'arachide en Australie : *Au cours des dix dernières années, les aflatoxines ont posé un problème très grave chez les cultures d'arachide en Australie. Pendant la majeure partie de cette période, la division de*

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la recherche sur les cultures vivrières de la Commonwealth Scientific and Industrial Research Organization (CSIRO) a mené un projet axé sur les cultures commerciales, grâce à l'appui financier de l'Etat et des entreprises privées.

Ces recherches portent essentiellement sur une appréhension des variables qui influencent l'invasion des arachides par Aspergillus flavus *et* A. parasiticus *ainsi que la production ultérieure des aflatoxines. Les éléments étudiés sont : les niveaux d'* A. flavus *dans le sol, les facteurs écologiques, les pratiques culturales qui influencent l'infection par* A. flavus, *l'influence des méthodes de séchage et de stockage sur le développement des aflatoxines.*

A. flavus *est présent à des niveaux détectables dans les sols cultivés en arachide de Kingaroy, tandis que le champignon est absent dans les sols non cultivés aux alentours. Le taux d'*A. flavus *dans les premiers varie considérablement de moins de* $10^2 g^{-1}$ *jusqu'à* $10^5 g^{-1}$. *Le risque d'invasion est d'autant plus grand que le taux est plus élevé. On constate des taux systématiquement élevés dans certains champs. La variation du rapport* A. flavus/A. parasiticus *d'un champ à l'autre pourrait influencer l'invasion et la production des toxines.*

D'après nos observations, l'invasion des arachides par A. flavus *a lieu avant la récolte. Même en cas d'un séchage inefficace, il n'y a pas d'invasion par le champignon après la récolte, donc aucune production d'aflatoxines. La production d'aflatoxines est négligeable lorsque les arachides sont encore en terre sauf pendant des saisons très sèches. Les aflatoxines sont donc produites après la récolte. Sous conditions favorables, les arachides mettent 6 à 10 jours à sécher au champ après la récolte; cette période est suffisamment longue pour la production d'aflatoxines qui atteignent des niveaux inacceptables. Le séchage au champ n'est jamais assez rapide, même en saison sèche, pour garantir la non production d'aflatoxines avant le décorticage.*

Etant donné que l'invasion par A. flavus *avant la récolte est responsable des taux de contamination inacceptables, ce paramètre est retenu pour la prévision du niveau au décorticage. Cette méthode a donné des résultats satisfaisants, mais elle nécessite d'autres perfectionnements avant d'être d'utilité pratique.*

Resúmene

Estudios de campo sobre *Aspergillus flavus* y las aflatoxinas en el cacahuate en Australia : *En la década pasada, las aflatoxinas han constituido un grave problema en las siembras del cacahuate en Australia. Durante la mayor parte de ese período, la División de Investigaciones sobre los Alimentos de la Organización para la Investigación Científica e Industrial del Commonwealth (CSIRO) ha desarrollado un proyecto, que actualmente continúa en marcha, con el apoyo financiero del gobierno y las empresas industriales, con especial atención en los estudios realizados bajo las condiciones existentes en las siembras de campos comerciales.*

Estas investigaciones primordialmente han sido enfocados hacia el conocimiento de las variables que influyen en la invasión de los cacahuates por Aspergillus flavus *y* A. parasiticus, *y la producción subsecuente de aflatoxinas. Los factores estudiados incluyen: niveles de infestación con* A. flavus *en los suelos; factores del medio ambiente; prácticas de manejo del cultivo en los predios rurales, que afectan las invasiones de* A. flavus; *y el efecto de los procedimientos empleados en el secado y almacenamiento de la cosecha, sobre la formación de aflatoxinas.*

Se encontró que la mayoría de los suelos dedicados a la producción de cacahuates en la región de Kingaroy, contienen niveles detectables de A. flavus, *mientras que en los suelos vírgenes colindantes, generalmente este hongo estaba ausente. Los niveles de infestación con* A. flavus *en los suelos cacahuateros varió marcadamente, desde menos de* $10^2 g^{-1}$ *hasta niveles tan altos como* $10^5 g^{-1}$; *los altos niveles aumentan el riesgo de invasión fungosa en el cultivo. Algunos campos tuvieron altos niveles de infestación a través de varios años. La relación* A. flavus/A. parasiticus *también varía considerablemente de predio a predio, y puede influir en la invasión fungosa y la producción de aflatoxinas.*

Nuestras investigaciones han demostrado que la invasión de los cacahuates por A. flavus *ocurre antes de la cosecha. La invasión no ocurre subsecuentemente, ni tampoco se producen aflatoxinas, incluso cuando se usan los procedimientos menos eficaces de secado. En todos los ciclos agrícolas, excepto aquellos con severas sequías prolongadas, pocas aflatoxinas se producían mientras los cacahuates permanecen dentro del suelo, es decir, la mayoría de las aflatoxinas se producen en el período post-cosecha. Bajo las condiciones más favorables, los cacahuates requieren de 6 a 10 días para secarse en el campo después de haberse cosechado el cultivo, lo cual es un período de suficiente duración para que los niveles de aflatoxinas lleguen a niveles inaceptables. El secado en el campo, aun en la temporada de estiaje, no puede realizarse con suficiente rapidez, que permita asegurar que los granos se encuentren libres de aflatoxinas, al llevarse las vainas a las plantas descascasadoras.*

La importancia plenamente demostrada de la invasión precosecha, como condición necesaria para la producción de niveles inaceptables de aflatoxinas, ha originado intentos de predecir los niveles de aflatoxinas que pueden esperarse al entregarse la cosecha a las plantas descascaradoras, a partir de los niveles de A. flavus *presentes en el cultivo en el momento de la cosecha. Este método ya ha dado resultados prometedores, pero requiere de perfeccionamiento debido a lo cual todavía no es de utilidad práctica.*

Introduction

In Australia, the groundnut industry is concentrated in the northeastern state, Queensland, with a few exceptions. In Queensland, the traditional area for groundnut production is the Burnett region, northwest of Brisbane, centred on the town of Kingaroy (Graham 1982). A second major area is the Atherton Tableland, west of Cairns in the far north of the state. Groundnuts in Australia are grown primarily on volcanic soils of high water-holding capacity and nutrient status. The availability of such soils is limited, so groundnuts are also grown on poorer quality soils in the Burnett area and elsewhere. Most groundnuts are grown under rainfed conditions. Moisture deficits are common in the Burnett region, where rainfall is often sporadic, but the major growing areas in the Atherton region have reliable rainfall.

Studies on aflatoxins at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Division of Food Research began in 1979, after it had become apparent that aflatoxins were a major problem for the Australian groundnut industry. The studies described in this paper have all been carried out in the field, on commercial groundnut farms in the Burnett region.

During the first 3 years of the study (seasons 1980-1982), investigations were confined to the postharvest period. In the following 3 years, studies were carried out to investigate *Aspergillus flavus* invasion and aflatoxin formation from the time of flowering right through pod development, harvesting and drying, to delivery to the shellers.

Aims of the Project

The aims of this project evolved as the work progressed. At first, the major aims were to develop media and methods for rapid and reliable detection of *A. flavus* and *A. parasiticus* , to use such methods to determine levels of these fungi in soils, and to develop some understanding of factors influencing the growth of *A. flavus* and the development of aflatoxins in groundnuts postharvest. After it became clear that *A. flavus* invasion was almost exclusively a preharvest pheno-

menon, attention focused on the causes of preharvest invasion and the factors controlling it. Later studies were oriented more towards developing an early warning system for the industry, to enable prediction of good or bad seasons at or before harvest.

Basic Concepts

The primary factor influencing the production of aflatoxins in foods is the ability of the fungus to grow. In laboratory culture, *A. flavus* grows well between 10 and 37° C at high moisture levels, and between about 25 and 35° C when relative humidity (RH) is reduced to 80%. Below 80% RH growth is very slow (Ayerst 1969, Pitt and Hocking 1977). Aflatoxin production can occur over most of the growth range (Northolt et al. 1976, 1977; Diener and Davis 1977), but is greater at higher humidities.

Groundnuts are in equilibrium with very high humidities when harvested, usually in excess of 99% (our unpublished observations). Thus, prime concern for prevention of aflatoxin production is rapid drying of nuts after harvest. Safe moisture contents for prevention of *A. flavus* growth and aflatoxin production in Australian groundnuts are near 12% (wet weight basis). The safe moisture content for prevention of spoilage is about 8%.

Growth of *A. flavus* in living substrates, such as developing groundnuts, has been less well studied than growth in laboratory culture. Indeed it has only been accepted quite recently that *A. flavus* will invade living tissue, as a pathogen or commensal, rather than invading only postharvest (Lillehoj et al. 1976, Klich et al. 1984). Little is known about aflatoxin production under such conditions.

When *A. flavus* invades groundnuts, a positive correlation appears to exist between drought stress and aflatoxin formation (McDonald and Harkness 1967, Pettit et al. 1971, Dickens et al. 1973, Diener and Davis 1977), but results have not always been clear (Wilson and Stansell 1983).

Drying Times for Groundnuts in the Field

It is current practice in the Burnett region to allow groundnuts to dry on the plants in the field for periods ranging from several days to 2 or more weeks before threshing. The nuts are then placed in bins where further drying will be slow unless mechanical air flow or dehydration is used. Our data show that, even in a year like 1980, when drought resulted in most nuts being partially dry when lifted, and low humidities throughout the drying phase resulted in optimum drying conditions, nuts required 6-10 days to dry to a safe moisture content (around 12%). This time interval provided ample time for *A. flavus* to grow and produce high levels of aflatoxin. In the 1981 season, nuts harvested before the onset of rain required 16 to 20 days to dry to a safe moisture level.

During the 1982 season, farm management practices were studied on 27 farms. Groundnuts were allowed to dry in the field for 2 weeks or more before threshing on 70% of the farms. The longest drying period noted was 4 weeks. Kernel moisture content at the time of harvest ranged from 30% to 46%. As in 1980, 6 to 10 days in the field were required on most farms to reduce moisture levels sufficiently to prevent growth of *A. flavus* . On three farms, 12 days were needed to reach this point.

In the 1983 season, heavy rains occurred in the region after harvest. Most farms received between 90 and 100 mm of rain between harvest and threshing, which dramatically affected the moisture content of nuts during postharvest field drying. For instance, in one field where kernel

moisture had fallen from 50% to 25% over 10 days, it rose in the next week to over 40%. On the 12 farms studied, drying to a moisture level where *A. flavus* could not grow required an average of 31 days.

In seasons in which *A. flavus* invasion is high, the use of forced ventilation or mechanical dehydration appears to be essential to restrict aflatoxin levels, even when field drying conditions appear to be very favorable.

Development of an Enumeration Medium for *A. flavus* and *A. parasiticus*

To permit the reliable detection of *A. flavus* and *A. parasiticus* in the presence of bacteria and other soil fungi, a new medium was needed, selective against bacteria and spreading fungi, selective for *A. flavus* and *A. parasiticus* , and enabling their ready recognition. This was developed in our laboratory: Aspergillus Flavus and Parasiticus Agar (AFPA: Pitt et al., 1983) is based on Aspergillus Differential Medium (Bothast and Fennell, 1974), and is commercially available. On AFPA, *A. flavus* and *A. parasiticus* grow rapidly, and produce brilliant orange yellow colony reverse colors after incubation for only 42 to 48 h at 30°C. Aside from good selectivity, AFPA has the further advantage that no special skill is needed to interpret results, and false positives or negatives are rare.

By shaking a 10-g soil sample in 100 mL of 0.1% peptone water, dilution plating 0.1 mL aliquots onto 20 petri dishes containing AFPA, and counting total *A. flavus* colonies on the 20 plates, an effective selective medium, AFPA (Pitt et al., 1983), was used for the enumeration of *A. flavus* and *A. parasiticus* from soils. It is possible to detect 5 propagules of *A. flavus* per gram of soil or nuts. This level is much lower than can be detected by other media or techniques and, at the limit of 1 colony per 20 petri dishes.

Levels of *Aspergillus flavus* in Groundnut and Other Soils

In 1980 and 1981, about 300 samples of soil from the Kingaroy and Atherton districts were examined for *A. flavus* populations, using AFPA. Most were taken from land in which groundnuts had been grown recently, or from virgin forest soils in areas adjacent to groundnut farms. Nearly 90% of the samples from land in which groundnuts had been grown contained readily detected levels of *A. flavus* , usually 100 to 5000 spores g^{-1} of soil. The spores appeared to be uniformly dispersed, rarely varying by more than a factor of 10 over the 8-12 sites sampled in a single 1-ha field.

In contrast, virgin soils and pasture land contained much lower numbers of *A. flavus* spores. Of 30 virgin soil samples taken, only three were found to contain detectable levels of *A. flavus*. One of these, containing 100 spores g^{-1}, was from a site in the drainage path of a groundnut field. The other two, containing 5 spores g^{-1}, were from within 30 m of two sites in a groundnut field which each contained 800 spores g^{-1}.

Because the levels found in virgin forest soils are so low, it is not certain whether *A. flavus* occurs naturally in undisturbed forest soils. It is clear, however, that cultivation of groundnuts results in greatly increased levels in soils. Further studies will be needed to determine whether this is specifically due to groundnut cropping, and whether *A. flavus* levels are materially affected by crop rotation.

Some farms in the Burnett region have shown consistently low soil levels of *A. flavus* over several years, while others have been consistently high, and some quite erratic. The reasons

remain elusive, but soil moisture certainly plays a role. Levels of viable *A. flavus* in soils drop rapidly during wet weather, and rebuild slowly during dry periods. Decreased levels in wet soil may be attributed to the action of competing bacteria and fungi. Increases in dry times may occur directly in the soil, in the absence of competitors which are inhibited by the dryness; but may also be due to growth of the fungus in nuts detached from plants during harvest and left in the fields. Further studies on this aspect are needed.

Levels of *Aspergillus flavus* in Groundnuts

In the first 5 years of this study, intact kernels were used to assess levels of kernel invasion at all stages of development, and postharvest. Fifty carefully hand-shelled kernels were surface sterilized for 1–2 min in chlorine solution (10% household bleach; Andrews 1986) and then plated directly onto AFPA plates (10 kernels per plate). After incubation for 2–3 days at 30°C, the percentage invasion was assessed from numbers of orange-yellow reverse colors at the point of medium contact with the kernels. Levels of invasion ranged from 0–100% of kernels, and varied with farm, groundnut variety, and season.

This method of assessing *A. flavus* levels in groundnuts is effective when invasion rates are high, but practical limits on sample size make it relatively imprecise. In later studies, sample size was increased by the following technique. Samples (1 kg) of nuts in shell, usually collected at harvest, were surface sterilized by immersion in a boiling water bath for 15 sec, then cooled (2 min) in sterile (boiled) cold water. The nuts, still unshelled, were then chopped (in approximately 350-g batches) in a Bamix® food processor for 30–35 sec, a procedure which produced finely divided, friable samples. Batches were recombined and mixed. Subsamples (approximately 2 g) were transferred aseptically to each of 5 petri dishes containing AFPA. The petri dish lids were replaced, and the dishes reweighed. Samples were spread with sterile glass spreaders, plates incubated at 30°C for 2–3 days, and *A. flavus* colonies enumerated, with results expressed as colonies g^{-1} of sample. Such a large-scale technique was difficult to keep entirely sterile: background contamination was assessed over a large number of samples as 1–2 colonies $sample^{-1}$ (i.e., 0.1 colonies g^{-1} of sample). Low levels of invasion were established as less than 1 colony g^{-1} and high invasion as greater than 10 colonies g^{-1}, as higher numbers were difficult to count accurately against the background of groundnut meal. Counts obtained in this manner showed much lower variability between duplicate samples than direct plating of kernels, and were used as a basis for experimental prediction of aflatoxin levels in dried nuts.

Development Stage and Invasion

Griffin and Garren (1976) and more recent work has suggested that *A. flavus* may be able to invade groundnuts through the flowers, as is the case in cotton (Klich et al. 1984), travel down the pegs, and be present in the groundnuts from the time of setting onwards. On the other hand, it is well recognized (Hill et al. 1983) that invasion can occur during growth and maturation of the nuts, and is also possible after pulling and during drying. To attempt to establish the most important invasion times under Australian conditions, the invasion of groundnuts by *A. flavus* was studied from the time of flowering until nuts were fully dried. Branches were cut from plants in fields under study, transported to the laboratory, and cut into appropriate pieces. Flowers, pegs, and developing nuts were surface sterilized with chlorine solutions (10% household bleach) for 1–2 min (Andrews 1986), and then incubated on AFPA for 2 days at 30°C. In the 1983 and

1984 seasons, 171 samples of flowers and pegs, 219 of kernels before harvest, and 150 of kernels after harvest were examined. Altogether, more than 16000 flowers and pegs and 19000 kernels were sterilized, individually cultured, and examined for the presence of *A. flavus* in this way.

As mentioned above, the 1983 season was very wet postharvest, but conditions before harvest had been excessively dry. A maximum of 17% of flowers in any one sample were invaded before harvest, with the average being 3%. Pegs were much more frequently affected, the maximum invasion being 71%, and the average 8%. This invasion appears to have no effect on the health of the peg, or the plant. In that dry season, high levels of invasion of flowers and pegs appeared to lead to high levels of kernel invasion. Percentage infection of the 132 preharvest kernel samples examined ranged as follows: no infection (40% samples), 1–9% infection (43% samples), 10-19% (10% samples), and 20% or more infection (7% samples). The maximum level of kernel infection observed was 47%.

On most farms studied in 1983, the initially dry season was followed by substantial rain before harvest, and farmers chose to allow a second crop to develop before harvesting. Thus, the crop harvested on most farms was not the crop studied preharvest. Invasion of kernels in this second crop was much lower than in the first crop. Of 88 samples examined, only 13 had greater than 5% invasion by *A. flavus*.

The wet season had compensations, as an unequivocal answer was obtained to the question of whether or not postharvest invasion by *A. flavus* occurs. As noted above, prolonged postharvest rain resulted in nuts being left in the field for long periods, 31 days being the average time required to reach a safe moisture content on the 12 farms studied. Only 10 farms actually threshed their crop; nuts left partially dried in the field for long periods were markedly discolored because of invasion by soil fungi. However, fields free of *A. flavus* invasion at harvest showed no subsequent invasion, or aflatoxin formation, despite the worst field drying conditions. The 1983 season therefore provided conclusive evidence that, under Australian conditions, groundnuts are not invaded by *A. flavus* during postharvest field drying. In 1984, it was possible to monitor a crop for *A. flavus* invasion from flowering through harvest. Although individual data mean little, because of the small samples involved, the data taken overall provide a valuable picture (Table 1).

Invasion levels in 1984 were low by comparison with those of 1983. Flower invasion was 0–4% on eight farms and up to 20% for individual samples on two farms. Peg invasion was up to 8% on eight farms, but over 20% for some samples on two farms. In general, levels of peg invasion were found to be higher than levels of flower invasion. Much more sampling would be needed to show whether pegs can be invaded only through the flowers or directly through intact aerial surfaces of the peg itself. Flower and peg anatomy suggest that flowers may be the only route for peg invasion, and these data are not inconsistent with that viewpoint.

With reference to the question of whether peg invasion can lead to kernel invasion, the answer in 1984 appeared to be negative (Table 1). With the exception of farm T, invasion levels in immature kernels were always less than, or equal to, invasion levels in pegs. On farms E and N, some peg samples showed high levels of invasion, but immature groundnuts did not. Observed invasion rates in immature groundnuts were very low on the farms studied; farm T was perhaps an exception.

These data differ from those obtained in 1983, and emphasize the difficulty of obtaining definitive answers in field studies such as this.

In general, *A. flavus* invasion levels increased as nuts matured (Table 1). If 10% or lower invasion levels are relatively unreliable (a reasonable assumption with the techniques used) only farm T shows significant invasion of immature groundnuts. However, seven of the 12 farms had some samples with significant invasion of maturing groundnuts. Invasion levels in groundnuts

Table 1. *Aspergillus flavus* invasion of flowers, pegs, preharvest, and postharvest groundnuts, and aflatoxin levels postharvest, Burnett region, Queensland, Australia, 1984.

	Invasion by *A. flavus* (%)[1]					
Farm	Flowers	Pegs	Immature kernels	Mature kernels	Postharvest kernels	Aflatoxins ($\mu g\ kg^{-1}$)
A	0	0–2	0	0–12	0–16	0–46
C	0	0	0	0–14	0–22	0–125
E	0–4	0–26	0–2	0–2	0–2	0
F	0	0–2	0–2	0–8	0–4	0
H	0–4	0–4	0–6	20–48	24–88	0–208
I	0–6	0–8	0–4	0	0–6	0
L	0–20	0–8	0–6	0–2	–[2]	–
N	0–10	7–40	0–2	2–16	0–6	0
P	0–4	0–4	0	0–12	0–16	0
Q	0–20	0–17	0	4–13	2–6	0
R	0–2	0–3	0	0–19	16–20	170–540
T	0–4	0–11	3–16	0–4	0–8	0

1. Ranges generally of 4 samples for flowers and nuts, and 4 to 6 samples for pegs.
2. – = No data.

during drying and on-farm storage show a high correlation with those for maturing nuts preharvest, lending support to the conclusion drawn earlier in this paper that fungal invasion only occurs preharvest.

Aflatoxin Formation

Aflatoxins were assayed by standard thin layer chromatography techniques. Sampling systems simultaneously used for mycological studies often meant that sample sizes for aflatoxin assays were relatively small (0.5–1 kg), and hence, aflatoxin levels observed showed considerable variability. Nevertheless valuable information has been obtained.

Given that aflatoxin can only form after *A. flavus* invasion, the crucial question remains: does aflatoxin form before or after harvest? Evidence from our studies indicates the latter. In normal seasons, little aflatoxin has been detected in nuts at harvest, but has built up subsequently (Table 2). In very dry seasons, aflatoxin levels at harvest have sometimes been unacceptably high (> 15 $\mu g\ kg^{-1}$), but an increase in unacceptable levels has still occurred during drying.

Prediction of Aflatoxin in Dried Nuts from *A. flavus* Levels Preharvest

One aim of this study was to ascertain whether knowledge of invasion levels of *A. flavus* in flowers, pegs, or developing groundnuts preharvest could provide a basis for predicting aflatoxin occurrence in groundnuts delivered to shellers. If so, a relatively simple monitoring system could be established to provide an early warning of a bad season to the industry. Such a system could be operated at a central location by one or two microbiologists. It would allow ameliorat-

Table 2. Aflatoxin content ($\mu g\ kg^{-1}$) in groundnut samples taken after harvest, Burnett region, Queensland, Australia, 1980 and 1981.

Variety	Farm	At harvest	Partially dried[1]	Fully dried[1]
Virginia Bunch	A	90	480	1680
	C	<1	45	500
	E	<1	<1	<1
	F	<1	<1	400
	G	<1	<1	<1
	J	<1	<1	250
	K	219	3660	190
	M	2	<1	170
	N	<1	<1	<1
	P	<1	<1	20
	S	<1	<1	660
Red Spanish	B	<1	<1	140
	H	10	10	20
	I	115	<1	45

1. Partially dried sampled taken 6–10 days after harvest; fully dried, 16–22 days after harvest.

ing measures, such as careful and rapid drying, to reduce the aflatoxin levels at intake and also permit slower, cheaper field drying without excessive aflatoxin levels in seasons where invasion was low.

Data from the 1983 season suggested that flower and peg invasion might be a useful predictor, but data from 1984 did not. Studies therefore concentrated on prediction of aflatoxin levels at sheller intake from *A. flavus* levels in nuts at the time of harvest.

Together with data on *A. flavus* invasion (Table 1), Table 2 provides a summary of 1984 data on levels of aflatoxin contamination postharvest. It is evident that 4 of the 5 farms with $> 10\%$ invasion by *A. flavus* in postharvest nut samples also showed aflatoxin in some samples. Of those farms with $< 10\%$ invasion, no aflatoxin was detected in any sample. Correlations within individual samples were not so good, but as each test sample for *A. flavus* consisted of only 50 kernels, the overall correlations were encouraging.

Correlations between preharvest *A. flavus* and postharvest aflatoxin, while still encouraging, were not quite so good. Farms N and Q both showed levels of invasion ranging up to 12 or 16% preharvest without such high levels being found postharvest, and without aflatoxin being detected. However the correlations among the other farms were good.

Preliminary studies in 1985 demonstrated the feasibility of a larger-scale *A. flavus* enumeration technique using 1-kg samples of nuts in shell. During the 1986 season, a full-scale trial was carried out. Farms were sampled at the time of harvest. Duplicate 1-kg samples of nuts in shell were processed as outlined earlier, and *A. flavus* levels enumerated. On the basis of these results, acceptable or unacceptable levels of aflatoxins in dried nuts from the same farms were predicted. Samples for *A. flavus* assay were obtained at harvest from 61 farms, and dried samples of nuts for aflatoxin assay received from 48 of them at delivery to the sheller. Data from the first 20 farms to deliver dried samples are given in Table 3.

Table 3. Correlations between *Aspergillus flavus* invasion at harvest and aflatoxin in dried nuts from the first 20 farms with complete data, Burnett region, Queensland, Australia, 1986.

Farm	*A. flavus* invasion[1]	Aflatoxin contents ($\mu g\ kg^{-1}$)[2]	Prediction based on invasion levels[3]		
1	+	0	-	+	+
3	+	25	+	-	-
4	+++	65	+	+	+
5	++	0	-	-	+
7	++	1690	+	+	-
8	+	40	+	-	-
9	++	0	-	-	+
11	+	0	-	+	+
12	++	0	-	-	+
13	-	0	+	+	+
15	-	0	+	+	+
16	-	0	+	+	+
17	+	4	-	+	+
18	+++	400	+	+	+
20	++	0	-	-	+
21	+	10	+	-	-
22	-	270	-	-	-
23	++	165	+	+	-
24	+++	0	-	-	-
25	+	0	+	+	+

1. Invasion levels: + = 1 colony g^{-1}; ++ = 2-10 colonies g^{-1}; +++ = more than 10 colonies g^{-1}.
2. Means of duplicate samples.
3. Prediction values: + and - indicate accurate or inaccurate prediction of aflatoxin based on the invasion levels. Aflatoxin contents below 15 $\mu g\ kg^{-1}$ were regarded as negative.

The last three columns in Table 3 show that none of the levels of *A. flavus* selected was an effective predictor of unacceptable aflatoxin levels in dried nuts. The data show a 60% success rate for each level: success rates for all 48 farms were slightly higher, and the '+++' score was slightly more effective, close to 70%. However, the results were not sufficiently good for this technique to have direct practical application. Some sources of inaccuracy are clear. First, 1-kg samples are barely adequate; statistical analysis is needed to determine the appropriate sampling level. Second, farm management may play a role in final aflatoxin levels, although our studies indicate this would be more likely to affect quantitative results rather than presence or absence of aflatoxins. Third, this technique measures extent of invasion, not percentage of kernels invaded. Although from earlier studies percentage of kernels invaded appears to remain quite constant after harvest, the extent of invasion within individual nuts can, and indeed would, be expected to increase. The newer large-scale technique, much more effective from the sampling viewpoint, may well be less effective in estimating final aflatoxin than plating of individual kernels.

Aspergillus flavus versus *A. parasiticus*

Even when the factors discussed above are taken into account, the data from farms 5, 12, 20, and 24 remain intriguing, because high invasion levels have not led to aflatoxin production. A possible explanation lies in the differences now known to exist between the two aflatoxigenic species, *A. flavus* and *A. parasiticus*. Recent studies (Klich and Pitt 1985, Klich and Pitt 1988) have defined the taxonomic distinctions between these two species more accurately than has been previously possible. Critical examination of nearly 200 isolates from sources around the world have confirmed three important points previously alluded to in the literature without adequate basis. First, isolates of *A. parasiticus* invariably produce both B and G aflatoxins, while almost all *A. flavus* isolates produce only the B compounds. Second, the majority of *A. flavus* isolates produce cyclopiazonic acid, while *A. parasiticus* isolates never do. Third, examination of almost 300 *A. parasiticus* isolates showed that they almost always produce aflatoxins when growing under laboratory conditions, while only 40% of the 100 isolates of *A. flavus* examined by Klich and Pitt (1988) did so. It appears probable that many *A. flavus* strains existing in nature do not produce aflatoxins. Hence it is possible for a farm to be heavily invaded by a nontoxigenic *A. flavus* strain, and produce nuts highly contaminated with *A. flavus* but not to have an aflatoxin problem regardless of environmental conditions. Current investigations aim to elucidate this point, as it may provide an explanation for high *A. flavus* invasion without aflatoxin production on farms such as number 24 (Table 3).

Information on the relative abundance of *A. flavus* and *A. parasiticus* on groundnut farms may be obtained in two ways: by isolation of these species from soil or groundnuts, and identification; or by mycotoxin analysis of groundnut samples. *A. flavus* can be reliably distinguished from *A. parasiticus* by conidial wall texture: *A. parasiticus* produces conidia with walls which appear distinctly roughened under 40 × magnification, while those of *A. flavus* are comparatively smooth. Other differences also exist (Klich and Pitt 1988). Detection of cyclopiazonic acid in a groundnut sample indicates the presence of *A. flavus*, but of course does not rule out the simultaneous presence of *A. parasiticus*. The presence of G aflatoxins is indicative of *A. parasiticus*, but does not rule out the simultaneous presence of *A. flavus*. In the Burnett region, G aflatoxins are present in most aflatoxin assays, indicating the widespread occurrence of *A. parasiticus*. Studies are needed on the relative invasivity of these two species, as their ability to compete with each other in groundnuts is an important ecological factor in our understanding of *A. flavus* invasion, and consequent aflatoxin production, in groundnuts.

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Discussion

D. Caley. Can *Aspergillus flavus* and *A. parasiticus* be transmitted systemically from one generation to the next?

J.I. Pitt. I think this may be possible, but there does not appear to be any reliable information.

T. Shanta. If the fungal infection is mainly from the soil, how do you explain that mechanical damage increases the chances of infection by Aspergilli and production of aflatoxin?

J.I. Pitt. Mechanical damage forces soil into close contact with kernels, and so facilitates invasion.

R.V. Bhat. It is important to look into the role of soil as the source of inoculum for preharvest *A. flavus* infection. Recent work has indicated that soil irrigation, the addition of lime to increase soil pH to 7, temperatures above 40° C, and cultivation of legumes or small grains will all reduce *A. flavus* levels in soils.

J.I. Pitt. Irrigation, which greatly reduces drought stress, seems to prevent *A. flavus* invasion, regardless of any other factor. Unfortunately at this stage clear evidence that crop rotation reduces *A. flavus* levels in soils is lacking. More research in this area is needed.

R.C. Nageswara Rao. Your data showed that drying time influences aflatoxin content, fully dried groundnut had higher aflatoxin than partially dried ones. What were the conditions of drying?

J.I. Pitt. These were field studies over 5 years. Drying conditions varied from hot and dry to cold and very wet.

R.C. Nageswara Rao. Were these studies continued to see if aflatoxins levels continued to increase during drying, reached a maximum level, or even decreased after a period of time.

J.I. Pitt. No. These are commercial fields. All the nuts were mechanically handled in large batches.

R.C. Nageswara Rao. Are these results useful in determining time of sampling for quality control purposes.

J.I. Pitt. Quality control sampling should be made before or after shelling, or before sale. This is a commercial and public health matter.

D. Caley . When you said that groundnuts could be stored for several months at moisture contents of 8 to 9% without spoilage, or 11 to 12% without development of aflatoxin, to what temperature range did you refer?

J.I. Pitt. I was referring to ambient temperatures up to approximately 30° C.

D. Caley. What moisture content ranges would be safe when the temperature at the top of bag stacks covered by black tarpaulins is 40° C?

J.I. Pitt. Storage under tarpaulins at high ambient temperatures is not recommended because of moisture migration with diurnal temperature variations. Nuts stored in that way should be very well dried.

Aflatoxin Research at the Indian National Research Centre for Groundnut

M.P. Ghewande, G. Nagaraj, and P.S. Reddy[1]

Abstract

The potential of Aspergillus flavus *isolates to produce aflatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings were studied. Isolates NRRL 3000 and V 3734/10 produced high levels of aflatoxins in culture. Culture filtrates from these isolates and from NRCG AFA were most toxic to seeds and seedlings. Commercial cultivars, advanced breeding lines, and wild* Arachis *species were screened for resistance to in vitro colonization of seeds by* A. flavus *isolates, and to aflatoxin production. Genotypes CGC 2, 1-4, CGC 7, S 230, derivatives of S230 + PI 337394F, Latur 33 × PI 337394F, and the wild species* A. cardenasii *and* A. duranensis *were resistant to seed colonization by* A. flavus. *All genotypes of groundnut and three wild* Arachis *species supported high production of aflatoxins, but only trace levels were produced in* A. cardenasii *and* A. duranensis.

Aflatoxins were found (range of 27-146 μg kg^{-1}) in commercial groundnut cake and in de-oiled cake. Moisture intake capacity, levels of seed coat phenols, and protein content of seeds were considered to influence aflatoxin contamination levels. Soaking seeds in various organic and inorganic substances was found to influence the degree of seed invasion by A. flavus *and of aflatoxin production in in vitro inoculation tests. Several detoxification methods were examined.*

Résumé

Recherches sur les aflatoxines au National Research Centre for Groundnut en Inde : *Le potentiel des isolats d'*Aspergillus flavus *à produire des aflatoxines est étudié ainsi que la toxicité du filtrat pour les semences en germination et les plantules. En culture in vitro les isolats NRRL 3000 et V 3734/10 présentent des niveaux très élevés d'aflatoxine. Les filtrats de ces isolats et ceux de NRCG AFA se sont montrés les plus toxiques pour les semences et les plantules. Les cultivars commerciaux, le matériel en sélection et les espèces sauvages d'*Arachis *ont été évalués pour leur résistance à la colonisation in vitro des graines par les isolats d'*A. flavus *et à la production des aflatoxines. Les génotypes CGC 2, 1-4, CGC 7, S 230 issus des croisements S 230 × PI 337394F et Latur 33 × PI 337394F ainsi que les espèces sauvages* Arachis cardenasii *et* Arachis duranensis *se sont montrés résistants à la colonisation des semences par* A. flavus. *Tous les génotypes et trois espèces sauvages ont supporté une forte production d'aflatoxines;* A. cardenasii *et* A. duranensis *n'ont présenté que des niveaux négligeables.*

Les tourteaux d'arachide commerciaux et deshuilés présentent des taux d'aflatoxines allant de 27 à 146 μg kg^{-1}. La capacité d'absorption de l'eau, les phénols présents sur le tégument séminal et

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la teneur en protéine des semences influenceraient le niveau de contamination. Les tests d'inoculation in vitro ont révélé que le trempage des semences dans plusieurs substances organiques et inorganiques conditionnent l'invasion des graines par A. flavus *et la production des aflatoxines. Plusieurs méthodes de détoxification ont été examinées.*

Resúmene

Investigaciones sobre las aflatoxinas en el Centro Nacional de Investigaciónes sobre el Cacahuate de la India : *Se estudió el potencial de los aislamientos de* Aspergillus flavus *para la producción de aflatoxinas, así como la toxicidad de los filtrados de los medios en los cuales se desarrollaron, sobre semillas en germinación y plántulas. Los aislamientos NRRL 3000 y V 3734/10 produjeron altos niveles de aflatoxinas en sus medios de cultivo. Los filtrados de esos aislamientos y del aislamiento NRCG AFA fueron los más tóxicos a las semillas germinadas y las plántulas. Se seleccionaron algunos cultivares comerciales, varias líneas avanzadas y diversas especies silvestres de* Arachis *para lograr la resistencia a la colonización* in vitro *de sus semillas por aislamientos de* A. flavus, *y baja producción de aflatoxinas. Los genotipos CGC 2, 1-4, CGC 7, S 230, los descendientes de la cruza S 230 × PI 337394F, y de la cruza Latur 33 × PI 337394F, y las especies silvestres* Arachis cardenasii *y* Arachis duranensis *resultaron ser resistentes a la colonización de* Aspergillus flavus *en sus semillas. Todos los genotipos de cacahuate y tres especies silvestres de* Arachis *dieron origen a altas producciones de aflatoxinas, pero* Arachis cardenasii *y* Arachis duranensis *sólo produjeron cantidades insignificantes de estas micotoxinas.*

En torta de cacahuate comercial y en torta de cacahuate desgrasada, se encontraron concentraciones de aflatoxinas que fluctuaron de 27 a 146 μg kg^{-1}. *La capacidad de absorber humedad, los niveles de fenoles presentes en los tegumentos de las semillas y el contenido de proteínas de las semillas aparentemente influyeron en los niveles de contaminación con aflatoxinas. Asimismo, la maceración de las semillas en varios líquidos orgánicos e inorgánicos, influyó en la intensidad con la cual ocurrían las invasiones de la semilla por* A. flavus, *y las cantidades de aflatoxinas producidas en pruebas con inoculaciones realizadas* in vitro. *Se evaluaron varios métodos para la eliminación de las toxinas presentes en materiales contaminados con aflatoxinas.*

Introduction

Aflatoxin contamination is a serious problem affecting the quality of groundnut. Groundnuts and their products are good substrates for the growth of *Aspergillus flavus* and the production of aflatoxins. High levels of aflatoxin contamination in such exportable commodities as HPS-grade kernels and deoiled cake have seriously jeopardized export earnings, thereby depriving India of valuable foreign exchange. This has led to research on different aspects of aflatoxin contamination in groundnut at the National Research Centre for Groundnut (NRCG), Junagadh, since 1982. Investigations have been made over the past 5 years on the aflatoxin production potential of *A. flavus* isolates, identification of sources of resistance to *A. flavus*, seed colonization, and aflatoxin contamination in groundnut cultivars and wild species. Studies have also been carried out on detoxification of aflatoxin-contaminated groundnuts and groundnut products.

Production of Aflatoxins by *Aspergillus flavus* Isolates and Effect of Culture Filtrates on Seed Germination and Seedling Growth

Production of aflatoxins

Five isolates of *Aspergillus flavus* (AF 8-3-2A, AFS-2, V 3734/1-, NRRL 482, and NRRL 3000) were obtained from ICRISAT, and two isolates designated as NRCG-AFJ and NRCG-AFA were obtained from infected seeds of cultivars GUAG 1 received from Junagadh and cultivar TMV 10 from Aliyarnagar. To investigate their aflatoxin-producing potential they were grown in a liquid yeast extract sucrose (YES) culture medium consisting of 2% yeast extract and 20% sucrose (w/w) for 7 days. Cell-free culture filtrates were collected and analyzed for aflatoxin content by thin layer chromatography (Holaday 1984) and photofluorimetry (Nagaraj and Kumar 1986).

Isolate AFS 2 did not produce any aflatoxins. All the other isolates produced significant levels of aflatoxin B_1 plus one or more of aflatoxins B_2, G_1, and G_2 (Table 1).

Table 1. Production of aflatoxins by *Aspergillus flavus* isolates in culture, National Research Centre for Groundnut, Gujarat, India, 1983/84.

A. flavus isolates	Aflatoxins ($\mu g\ kg^{-1}$)				Total aflatoxin content ($\mu g\ kg^{-1}$)
	B_1	B_2	G_1	G_2	
NRCG-AFJ	171	-	43	-	214
NRCG-AFA	400	43	71	-	514
AF 8-3-2A	257	71	-	-	329
AFS 2	-	-	-	-	-
NRRL 482	2543	2900	-	-	5443
NRRL 3000	8614	17542	3543	386	30084
V 3734/10	6828	3971	1471	143	12414

Resistance to *Aspergillus flavus*

The toxigenic isolate of *A. flavus* NRRL 3000 that showed high aflatoxin producing capacity in culture was used in laboratory inoculation studies to screen genotypes derived from a J 11 × Robut 33-1 (Kadiri 3) cross, some released varieties, breeding varieties, breeding lines of S 230 × PI 337394 F, Latur 33 × PI 337394 F, TMV 10 × PI 337394 F, and M 37 × PI 337394 F crosses in F_5 generation, and the wild *Arachis* species *A. cardenasii, A. duranensis, A. monticola, A. pusilla, A. stenosperma*, ICG 8127, and ICG 8128 for resistance to *A. flavus* colonization of rehydrated, mature seeds using the method of Mehan and McDonald (1980). Cultivars J 11 and Ah 7223 were included as resistant cultivars, and TMV 2 as a susceptible cultivar.

Seed colonization by *A. flavus* ranged from 9.1 to 60% (Table 2). Low percentage seed colonization was recorded in CGC 2, 1-4, S 230, Ah 7223, CGC 7, J 11, and 1-7; these are considered to be resistant cultivars. Derivatives of S 230 × PI 337394 F and Latur 33 × PI 337394 F were also found to be resistant.

Table 2. Seed resistance to *Aspergillus flavus* colonization in some groundnut genotypes, National Research Centre for Groundnut, Gujarat, India, 1985/86.

Genotype	Seed colonization (%)
Cultivar	
S 230	8.9 (17.36)[1]
CGC 2	9.1 (17.56)
1-4	9.1 (17.56)
CGC 7	10.6 (19.00)
1-7	14.2 (22.14)
Jyoti	18.2 (25.25)
1-17	18.2 (25.25)
1-19	20.8 (27.13)
TMV 12	21.3 (27.49)
GUAG 1	30.8 (33.71)
PG 1	39.6 (39.00)
Breeding lines	
S 230 × PI 337394 F	2.5 (9.10)
Latur 33 × PI 337394 F	12.0 (20.27)
M 37 × PI 337394 F	22.5 (28.32)
TMV 10 × PI 337394 F	35.0 (36.27)
Control	
Resistant	
Ah 7223	10.0 (18.44)
J 11	14.1 (22.06)
Susceptible	
TMV 2	40.0 (39.23)

1. Figures in parentheses are arc sine transformations.
Source: Ghewande et al. 1986.

Table 3. Seed resistance to *Aspergillus flavus* colonization in wild *Arachis* species, National Research Centre for Groundnut, Gujarat, India, 1985/86.

Arachis species	Seed colonization (%)
Arachis cardenasii	0.0 (0.0)[1]
Arachis duranensis	2.0 (8.13)
Arachis monticola	90.0 (71.56)
Arachis pusilla	51.7 (45.97)
Arachis stenosperma	25.0 (30.00)
Controls	
ICG 8127 (*Arachis appressipila*)	80.0 (63.44)
ICG 8128 [*Arachis* sp (Erectoides ER/PR)]	68.8 (56.04)

1. Figures in parentheses are percentage arc sine transformations.

In the wild *Arachis* species studied, seed colonization ranged from 0 to 90%. Species *A. cardenasii* and *A. duranensis* were found to be highly resistant while others were susceptible (Table 3).

It is evident that some cultivars and wild *Arachis* species have high levels of resistance to *A. flavus* colonization. Dry seed resistance to seed colonization has been reported in such genotypes as PI 337409, PI 337394 F, UF 71513-1 (Mixon and Rogers 1973, Mixon 1976), Ah 7223, Var. 27, Faizpur and Monir 240-30 (Mehan et al. 1981) But this is probably the first report of resistance in the genotypes listed here.

Production of Aflatoxins

Released varieties

Twenty-six varieties of groundnut belonging to different botanical groups i.e., spanish—J 11, GUAG 1, GG 2, TMV 2, TMV 7, TMV 12, Co 1, DH 3-30, KRG 1, JL 24, Jyoti, CGC 3, and Ah 7223 (13); valencia—Gangapuri and MH 2(2); virginia—BG 1, TMV 10, Robut 33-1 (Kadiri 3), C 501, and RSB 87(5); virginia runner—GUAG 10, GG 11, S 230, PG 1, M 13, and RS 1(6) were tested for their capacity to support aflatoxin production. Seed samples (100 g) of each variety were surface sterilized, inoculated with NRRL 3000, and incubated at 26 ± 1° C under laboratory conditions for 8 days. Where the growth of the fungus over the seeds was observed to be sparse, seeds were surface scarified and reinoculated. After incubation samples were analyzed for aflatoxin content.

All the cultivars including those resistant to *A. flavus* colonization of seeds were found to support aflatoxin production (Ghewande et al. 1986). However, genotypic differences were observed for aflatoxin production. Aflatoxin B_1 and G_1 were produced in all the varieties tested. The aflatoxin production ranged from 1 308 to 92 541 $\mu g\ kg^{-1}$. Minimum production of aflatoxin below 2 000 $\mu g\ kg^{-1}$ was supported by RSB 87, TMV 12, TMV 7, S 230, and KRG 1 and the maximum by BG 1 followed by JL 24, and GG 2. Gangapuri, TMV 10, Robut 33-1 (Kadiri 3), GUAG 1, PG 1, RS 1, and Ah 7223 supported the production of all four components (B_1, G_1, B_2, and G_2) of aflatoxin. It has been reported that different varieties of groundnut differ in their ability to support aflatoxin production (Rao and Tulpule 1967, Doupnik 1969, Mehan and McDonald 1980, Mehan et al. 1986).

Wild *Arachis* species

Seed samples of wild *Arachis* species i.e., *A. cardenasii, A. duranensis, A. monticola, A. pusilla,* and *A. stenosperma* were inoculated with *A. flavus* (NRRL 3000) and samples analyzed for aflatoxin content after incubation.

The aflatoxin content in wild *Arachis* species ranged from traces to 24 500 $\mu g\ kg^{-1}$ (Table 4). *A. cardenasii* and *A. duranensis*, which supported aflatoxin production in traces were also found to be resistant to *A. flavus* colonization.

Chemical Composition of Seeds and Aflatoxin Contamination

Some preliminary comparisons were made between varieties for aflatoxin contamination and such characters as polyphenol content and protein content of seeds. There were indications that some varieties with high polyphenol and low protein contents had less aflatoxin contamination

Table 4. Aflatoxin content in wild *Arachis* species, National Research Centre for Groundnut, Gujarat, India, 1985/86.

Arachis species	Aflatoxin content ($\mu g\ kg^{-1}$)		
	B_1	G_1	Total
Arachis cardenasii	Traces	-	Traces
Arachis duranensis	Traces	-	Traces
Arachis monticola	12 000	-	12 400
Arachis pusilla	20 000	4 500	24 500
Arachis stenosperma	18 000	5 250	23 250

of seeds than others that had low polyphenol and high protein contents. However, further investigations are needed before definite conclusions can be made.

Prevention of *A. flavus* Infection and Aflatoxin Contamination of Groundnut Seeds by Application of Organic and Inorganic Substances

Seeds of cultivars J 11 and JL 24 were treated with various organic and inorganic substances (rock salt, sodium chloride, propionic acid, asafoetida, turmeric powder, and extracts from several plants), then inoculated with a spore suspension of the toxigenic *A. flavus* isolate NRRL 3000 and incubated under conditions conducive to fungal invasion and aflatoxin production. Some substances inhibited fungal colonization and/or aflatoxin production. Further investigations would be required to confirm results and examine practical possibilities for use in preventing aflatoxin contamination.

Aflatoxin Contamination at Lifting and during Windrow Drying

The two varieties J 11 and Ah 7223 previously reported to have seed resistance to infection by *A. flavus* had lower levels of seed infection by *A. flavus* than had the two susceptible varieties JL 24 and GAUG 1 both at lifting and after 3 days of windrow drying (Table 5).

Table 5. Seed infection by *Aspergillus flavus* and aflatoxin contamination of groundnuts at harvest and after 3 days of windrow drying, National Research Centre for Groundnut, Gujarat, India, rainy seasons 1985 and 1986.

Cultivar	At harvest		After 3 days of windrow drying	
	Seed infection (%)	Aflatoxin content ($\mu g\ kg^{-1}$)	Seed infection (%)	Aflatoxin content ($\mu g\ kg^{-1}$)
J 11	0.1	29.5	2.4	Trace
Ah 7223	1.6	34.0	0.6	19.5
JL 24	6.1	45.4	8.9	26.9
GUAG 1	3.2	43.8	2.9	28.0

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Discussion

S. Nahdi. What was the objective of making crosses only with J 11. How can you claim that the toxin was reduced in windrow drying?

P.S. Reddy. We used J 11 as a source of resistance to *A. flavus* in our breeding program. We have also established its resistance. It is only the buildup of the toxin in the windrows which was investigated.

J.I. Pitt. The loss of aflatoxin during sun drying is apparent rather than real and is due merely to sample variation.

Aflatoxin Contamination of Groundnuts with Special Reference to Sudan and some Caribbean Countries

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Abstract

Based on analyses using the Velasco and the Tropical Development Research Institute (TDRI) methods, none of the samples collected from farm households in two regions of Sudan; a rainfed area (El Obeid), and an irrigated area (Wad Medani) for the crop year 1983/84, contained more than 15 $\mu g\ kg^{-1}$ of aflatoxin. However, samples collected from the markets in Khartoum and Wad Medani contained aflatoxin up to 945 $\mu g\ kg^{-1}$ in raw groundnuts, up to 517 $\mu g\ kg^{-1}$ in roasted peanuts, and up to 994 $\mu g\ kg^{-1}$ in groundnut paste. Groundnut paste prepared after a careful sorting and cleaning had only 19 μg aflatoxin kg^{-1}.

Analyses of 145 samples in Jamaica and St. Vincent in 1984 indicated only eight samples containing more than 20 $\mu g\ kg^{-1}$ of aflatoxins. Roasted peanuts and peanut butter samples collected from markets in Jamaica and Trinidad did not contain detectable amounts of aflatoxins. However, groundnut products collected from St. Vincent had very high levels of aflatoxins varying from 1 to 469 $\mu g\ kg^{-1}$.

Résumé

Contamination des arachides par les aflatoxines au Soudan et dans les pays des Caraïbes : *Les analyses fondées sur les méthodes de Velasco et du Tropical Development Research Institute (TDRI) ont montré que la teneur en aflatoxine ne dépasse pas 15 $\mu g\ kg^{-1}$ pour les échantillons de la campagne agricole 1983-1984 prélevés au niveau de la ferme dans deux régions au Soudan : une région pluviale (El Obeid) et une région irriguée (Wad Médani). Cependant, dans les échantillons prélevés sur les marchés de Khartoum et de Wad Médani, on trouve de l'aflatoxine dans les arachides crues (945 $\mu g\ kg^{-1}$), les arachides grillées (517 $\mu g\ kg^{-1}$) et la pâte d'arachide (994 $\mu g\ kg^{-1}$). La pâte préparée à partir d'arachides bien triées et nettoyées ne contient que 19 $\mu g\ kg^{-1}$ d'aflatoxines. En 1984, parmi les 145 échantillons provenant de la Jamaïque et de St. Vincent, seulement 8 échantillons ont une teneur en aflatoxines supérieure à 20 $\mu g\ kg^{-1}$. Les échantillons d'arachides grillées et de beurre d'arachide collectés sur les marchés en Jamaïque et au Trinidad n'ont pas une teneur détectable. Cependant, les produits à base d'arachide provenant de St. Vincent présentent parfois des taux très élevés allant jusqu'à 469 $\mu g\ kg^{-1}$.*

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Resúmene

La contaminación con aflatoxinas del cacahuate en Sudán y algunos países del Caribe : *Los análisis realizados con los métodos de Velasco y del Instituto de Investigaciones para el Desarrollo Tropical (Tropical Development Research Institute, TDRI), en muestras recolectadas en predios rurales de dos regiones del Sudán, una de secano (El Obeid), y la otra de riegg (Wad Medani), de materiales de la cosecha del ciclo 1983/84, indicaron que ninguna de las muestras contenía más de 15 $\mu g\ kg^{-1}$ de aflatoxinas. Sin embargo, muestras recolectadas en los mercados de Khartoum y de Wad Medani contenían hasta 945 $\mu g\ kg^{-1}$ en cacahuates crudos; hasta 517 $\mu g\ kg^{-1}$ en cacahuates tostados; y hasta 994 $\mu g\ kg^{-1}$ en torta de cacahuate. La torta de cacahuate preparada después de una cuidadosa selección y depuración de los granos solamente contenía 19 $\mu g\ kg^{-1}$ de aflatoxinas. Análisis realizados en 145 muestras, en Jamaica y San Vicente, durante 1984, indicaron que solamente ocho de las muestras contenían más de 20 $\mu g\ kg^{-1}$ de aflatoxinas. Muestras de cacahuate tostado y de mantequilla de cacahuate, recolectadas en los mercados de Jamaica y Trinidad no contenían cantidades detectables de aflatoxinas. Sin embargo, los productos derivados de cacahuate recolectados en San Vicente tenían niveles muy elevados de aflatoxinas, que variaron desde 1 a 469 $\mu g\ kg^{-1}$.*

Introduction

Since the discovery of aflatoxin in 1961, much progress has been made in research on understanding its toxicological impacts and on developing methods for its detection and prevention (Rodricks 1976, Rodricks and Stoloff 1976, FAO 1979). In developing countries, most of the research has been oriented towards enhancing or maintaining export markets. Aflatoxin contamination within the stored commodity is a more frequent problem than contamination detected at harvest (Nwokolo and Okonkwo 1978, Hendrickse et al. 1982). It is also in these regions that the existing gap between supply and demand forces people and animals to consume what they might otherwise reject, even when it is infected with fungi and is aesthetically and organoleptically unacceptable (FAO 1977). Many people in the developing world are undernourished and may thus be more susceptible to the toxic effects of mycotoxins. The bulk of the population in major groundnut-producing countries is exposed to the toxic effects of aflatoxins. Aflatoxins were detected in 23% of the serum samples of 252 Sudanese children (Hendrickse et al. 1982). Aflatoxins were detected more often, and at higher concentrations in serum from children with kwashiorkor disease than in other malnourished groups (marasmus) and control groups (normal children).

Based on opinion surveys of scientists from groundnut-producing countries, the Peanut Collaborative Research Support Program (CRSP) recognized aflatoxin contamination as one of the 13 major constraints to world groundnut production and utilization (Cummins and Jackson 1982). Aflatoxin research was included as one of the objectives in food technology projects in semi-arid tropical (SAT) regions of Africa and in the Caribbean nations. Results presented in this paper are based on consumption and postharvest surveys in the Sudan and Caribbean countries.

Aflatoxin Contamination of Groundnuts in Sudan

In 1984, a postharvest survey was conducted in groundnut-growing areas of the Sudan to evaluate farmers' stock groundnuts for aflatoxin contamination. The survey plan, described

elsewhere (Singh 1983), included 100 farm families from four villages (Omonainad, Gehbat, El Hamdi, and Mabag) near El Obeid and another 100 families from three villages near Wad Medani (Mobi, El Bastantana, and Kreiba), and two blocks in the Rahad Scheme area. The El Obeid area is rainfed while Wad Medani and the Rahad Scheme areas are irrigated. In both areas, farm size ranged from 0.2 to 4.04 ha with a mean of about 1.22 ha. The average quantity of groundnuts stored for food consumption was about 45 kg and this stock may include groundnuts gleaned after harvest (Singh 1984).

The size of samples collected was inadequate to complete the desired analyses. Therefore, samples from each of the sites were pooled to form 5 samples from each village. Aflatoxin determinations were made by the Velasco method (Anon 1981). Thin layer chromatography was used to test the accuracy of the Velasco method. Results of analyses are presented in Table 1. The data indicate the presence of aflatoxins in all samples, however, 75% of samples contained less than 20 $\mu g\ kg^{-1}$ aflatoxins. Samples from irrigated areas contained relatively higher amounts of aflatoxin G_1.

The samples collected from the markets in Khartoum and Wad Medani contained up to 994 $\mu g\ kg^{-1}$ of aflatoxins. Table 2 presents the data on raw, roasted, and groundnut paste samples collected during 1983 and 1984. Samples of groundnuts and groundnut products contained very high levels of aflatoxins. In general, aflatoxin B_1 was more prevalent than aflatoxins B_2, G_1, and G_2.

The most commonly utilized form of groundnut in the Sudan is roasted groundnuts followed by groundnut paste (Singh 1984), and both as sold in the market place were highly contaminated with aflatoxins (Table 2).

One of the possible sources of contamination is the use of groundnuts gleaned after the main harvest, a common practice in the Sudan. The gleaned groundnuts are consumed by the farm families and are also sold in the markets. If the contaminated groundnuts were visually sorted, contamination levels could be reduced. The groundnut paste produced in the presence of scientists from Alabama A&M University (in which the nuts were carefully sorted and then processed) had much lower amounts of aflatoxins than the commonly sold groundnut pastes

Table 1. Aflatoxin content of groundnut samples[1] collected in the Sudan, crop season 1983/84.

Villages	Aflatoxin content ($\mu g\ kg^{-1}$)				
	B_1	B_2	G_1	G_2	Total
El Obeid area (rainfed)					
Omonainad	4.7±1.79[2]	2.23±1.52	2.5±1.19	2.6±2.09	12.1±6.45
El Hamdi	2.3±0.96	3.9 ±2.69	3.5±0.76	3.1±1.02	12.8±4.12
Gehlbat	2.0±0.80	1.2 ±0.52	2.7±0.80	2.3±0.90	8.2±2.81
Mabag	2.7±1.73	2.0 ±1.11	2.6±2.28	2.7±1.95	10.0±6.96
Wad Medani area (irrigated)					
Mobi	2.7±0.54	1.1 ±0.58	6.4±1.12	0.2±0.04	10.2±1.94
El Bastantana	2.4±1.04	2.5 ±0.97	6.2±3.32	1.9±1.23	13.0±2.12
Kreiba	4.3±2.40	4.3 ±2.94	10.4±3.16	2.7±0.60	21.7±7.72
Rahad Scheme area					
Village 40	4.6±0.69	3.6 ±0.87	12.8±3.04	1.1±0.72	22.1±4.41

1. Average of 5 samples.
2. ± values are standard errors.

Table 2. Aflatoxin contamination of groundnuts collected in Sudanese markets, 1983 and 1984.[1]

Form	City	Year	Aflatoxin content ($\mu g\ kg^{-1}$)				
			B_1	B_2	G_1	G_2	Total
Raw	Khartoum	1983	608	102	58	101	869
Raw	Wad Medani	1983	714	98	69	64	945
Roasted	Khartoum	1983	299	42	20	33	394
Roasted	Wad Medani	1983	406	49	34	28	517
Paste	Khartoum	1983	666	198	64	66	994
Paste	Wad Medani	1983	514	201	39	34	788
Paste[2]	Khartoum	1984	19	10	6	4	39

1. Mean of 3 determinations of a composite sample.
2. Paste prepared after sorting and cleaning of samples.

(Table 2). However, the discarded groundnuts may be quickly eaten by pigeons which if consumed by humans could present an additional source of aflatoxins.

Aflatoxin contamination in Sudanese food items including groundnuts has been recognized (FAO 1979). A study conducted by the Liverpool School of Tropical Medicine, UK indicated that groundnuts contained aflatoxins B_1 up to a level of 59 666 $\mu g\ kg^{-1}$, B_2 to 370 $\mu g\ kg^{-1}$, and G_2 to 23 $\mu g\ kg^{-1}$. Peanut butter contained aflatoxin B_1 at up to 26 300 $\mu g\ kg^{-1}$, G_1 to 84 500 $\mu g\ kg^{-1}$ and B_2 to 9720 $\mu g\ kg^{-1}$ (Hendrickse et al. 1982). Clinical studies indicated that aflatoxins were commonly detected in serum and urine samples of children with kwashiorkor. Aflatoxicol, a derivative of aflatoxin, was detected in the urine of children with kwashiorkor.

This study indicates the urgency of monitoring and prevention of aflatoxin, not only in Sudan but in all SAT-African countries.

Aflatoxin Contamination of Groundnuts in Caribbean Countries

A postharvest survey was conducted in 1984 in St. Elizabeth Parish in Jamaica and in St. George Parish in St. Vincent to identify groundnut production and postharvest practices that might affect seed quality (including incidences of mold and aflatoxin contamination). There were 107 completed questionnaires from Jamaica, and 209 from St. Vincent.

Groundnut samples were also collected for analysis of aflatoxin. In Jamaica, the 107 farmers surveyed produced an average of 932 kg ha^{-1} of groundnuts, while the St. Vincent farmers produced an average of 804 kg ha^{-1}. On an average, 9 kg of groundnuts were harvested for food by individual farmers in both countries (Singh 1985).

Samples of farmers' groundnuts, 100 from Jamaica and 300 from St. Vincent, were analyzed for aflatoxins and for the presence of *A. flavus*-group fungi on seeds at the mycotoxin laboratory of the University of Georgia. *Aspergillus flavus* was present in almost all seed samples. When aflatoxin determinations were made, 8 of 160 samples had aflatoxin contamination ranging from 8 to 7 526 $\mu g\ kg^{-1}$ (Table 3). Four of the toxic samples were from the St. Elizabeth area of Jamaica and four from the growing areas adjacent to Kingstown, St. Vincent.

Because of the very small number of toxic samples detected, tests of significance are handicapped. However, as a preliminary screening, T-tests were run for the two groups: detectable (n=8) and non-detectable (n=133). Several of the practices associated with positive and negative

Table 3. Aflatoxin contamination of farmer's stock groundnut samples collected from St. Vincent and Jamaica, West Indies, postharvest survey, 1984.[1]

Sample no.[2]	Source	Aflatoxin content ($\mu g\ kg^{-1}$)				
		B_1	B_2	G_1	G_2	Total
2	St. Vincent	8	1	<1	<1	8
16	St. Vincent	578	248	<1	<1	826
42	St. Vincent	698	168	<1	<1	862
15	St. Vincent	22	8	<1	<1	30
119	Jamaica	2742	793	<1	<1	3535
146	Jamaica	320	66	<1	<1	386
147	Jamaica	1226	152	4522	626	7526
158	Jamaica	38	32	<1	<1	70

1. The total number of samples analyzed was 160, only the 8 with detectable amounts of aflatoxin are presented.
2. Each sample represents groundnuts from farmers' stock for food or seed.

aflatoxin detections are selected and presented in Table 4. In many instances, the more appropriate contingency tables of nonparametric dichotomous indications are also illustrated with their corrected chi-squared significance. Postharvest data were missing for four non-detectable cases. The practice of washing the nuts after harvest was less frequently performed than was washing by the producers with no detections (37.5% vs 50.8% difference not significant). Alternatively, groundnut producers whose produce was included in the aflatoxin-detected group were more frequently gleaning their fields for food use than producers whose groundnuts were determined to be free of aflatoxin by a comparison of 75% to 52%; but again these comparisons are not significant. Reported losses were higher among the farmers with detectable aflatoxin contamination than those without (4 kg vs 1.8 kg). While these differences appear sizeable, they were again not significant at the 0.05 level. Molds were observed as a food storage problem by two of the eight farmers who supplied samples detected to have aflatoxins. Only eight of the remaining 133 farmers with non-detectable aflatoxin contamination reported mold problems. The two samples suggested significance at the 0.043 level but the chi-square statistics was not significant. The T-test for molds as a seed storage problem was also significant at the 0.037 level even though only one of the eight farmers reported the problem, but the chi-square statistics did not suggest significance and were questionable because of the low numbers of observations of mold on seeds in either class.

There appears to be an interaction between gleaning groundnuts from the field for food and the presence of mold that predicts with near certainty the detection of aflatoxin. There was one such case each in Jamaica and St. Vincent, and both cases showed detectable aflatoxins.

In St. Vincent, groundnuts grown after a sweet potato crop produced three of the four aflatoxin-positive samples, while only 14% of the farmers with aflatoxin-free samples sowed groundnuts in the same field after sweet potato (Table 4). All aflatoxin-positive samples from St. Vincent were harvested in February compared to 41% of the aflatoxin-negative crops. The February-harvested crops in St. Vincent may have been drought stressed at a critical time leading to the formation of aflatoxins. In Jamaica three of the four positive samples were harvested in December. In spite of the small number of aflatoxin-positive samples, the cultural practices associated with the positive samples coupled with the time of harvest suggest that aflatoxin could be largely avoided by paying careful attention to cultural practices.

Table 4. Relationship of production and postharvest practices and problems to aflatoxin incidence in groundnuts, St. Vincent and Jamaica, West Indies.

Selected farm practices and related variables	Aflatoxin positive		Aflatoxin negative	
	Mean (%)	(n)	Mean (%)	(n)
Mold on food groundnut	25.0	(8)	6.0	(133)
Mold on gleaned groundnut	25.0	(8)	1.5	(133)
Mold on seed groundnut	12.5	(8)	1.5	(133)
Sweet potato rotated	37.5	(8)	7.5	(133)
St. Vincent	75.0	(4)	14.1	(64)
Jamaica	0	(4)	1.4	(69)
Groundnut/maize intercrop	75.0	(8)	45.1	(133)
Groundnut/maize intercrop, gleaned	62.5	(8)	24.8	(133)
Aflatoxin-prone, HM[1]	87.5	(8)	31.6	(133)
St. Vincent (Feb)	100.0	(4)	40.6	(64)
Jamaica (Dec)	75.0	(4)	23.2	(69)
Gleaned groundnut, HM, prone	62.5	(8)	15.8	(133)
HM, prone, sweet potato rotated	37.5	(8)	2.3	(133)
St. Vincent	75.0	(4)	4.7	(64)
HM, prone, maize intercrop	75.0	(8)	9.0	(133)
St. Vincent (Feb)	75.0	(4)	10.9	(64)
Jamaica (Dec)	75.0	(4)	7.2	(69)
Gleaned HM, maize intercrop	62.5	(8)	3.8	(133)
Washed groundnut at harvest	37.5	(8)	50.8	(130)
Washed groundnut sweet potato rotated	25.0	(8)	3.8	(130)
Washed, HM, sweet potato rotated	25.0	(8)	0.8	(130)
St. Vincent	50.0	(4)	1.6	(61)
Washed, HM, maize intercrop	25.0	(8)	1.5	(130)
St. Vincent	50.0	(4)	3.3	(61)
Food store loss (lbs)	8.8	(8)	3.9	(133)
Acres 1st crop groundnut	1.88	(8)	1.37	(133)
Acres 2nd crop groundnut	0.59	(8)	1.17	(133)
Acres 3rd crop groundnut	0.09	(8)	0.71	(133)

1. HM indicates the harvest month of December in Jamaica; February in St. Vincent which were identified with positive detections of aflatoxins.

Aflatoxin Contamination of Groundnut and Groundnut Products from the Market

Table 5 includes data on aflatoxin levels in nine groundnut products collected from St. Vincent in 1984. The amount of aflatoxin ranged from 1 to 469 $\mu g\ kg^{-1}$; 2 had less than 20 $\mu g\ kg^{-1}$, 1 had 97 $\mu g\ kg^{-1}$, and 3 had 266 to 469 $\mu g\ kg^{-1}$. The results for samples collected during 1985/86 are presented in Table 6. Apparently, none of the products had more than 20 $\mu g\ kg^{-1}$ of aflatoxin. Only three products from Jamaica had 13 to 19 $\mu g\ kg^{-1}$ of aflatoxin. Most of the raw groundnuts were from the Caribbean Agricultural Research and Development Institute (CARDI) experimental plots; the sources of the groundnuts used in preparation of the various products were not known. It is evident from these data that aflatoxin contamination in groundnut and its products

is common, and there is a need for further research to monitor and manage the aflatoxin problem.

Under the Caribbean Peanut-CRSP project, researchers at the University of Florida have carried out research to decontaminate groundnuts using microwave energy and chlorine gas. Results indicate that microwave roasting reduces aflatoxin B_1 and G_2 contamination to a

Table 5. Aflatoxin contamination of groundnut products, St. Vincent, West Indies, 1984.

	Aflatoxin content ($\mu g\ kg^{-1}$)				
Product types[1]	B_1	B_2	G_1	G_2	Total
Roasted (salted)	63	34	<1	<1	97
Roasted (tops special)	2	<1	<1	<1	10
Roasted (unsalted)	211	55	<1	<1	266
Roasted (salted)	8	<1	<1	<1	9
Peanut butter (crunchy)	1	<1	<1	<1	<1
Peanut butter (no added oil)	392	77	<1	<1	469
Peanut butter (2% added oil)	376	74	<1	<1	450
Peanut (partially processed)	<1	<1	<1	<1	<1
Peanut bar (honey preserved)	<1	<1	<1	<1	<1

1. Brand names of the products omitted for confidentiality.

Table 6. Contamination measured by the Velasco aflatoxin meter method in groundnuts and groundnut products collected in the Caribbean, 1985/86.

Varieties as known	Type material or product	Source code[1]	Aflatoxin content ($\mu g\ kg^{-1}$)
Valencia	Raw	<1	<1
NC 17921	Raw	<1	<1
MH 383	Raw	<1	<1
NC 4	Raw	<1	<1
Florigiant	Raw	<1	<1
NC 2	Raw	<1	<1
NC 5	Raw	<1	<1
NC 17	Raw	<1	<1
Valencia	Raw	<1	19
Valencia (1)	Roasted, in poly bags	<1	13
Valencia (2)	Roasted, in poly bags	<1	<1
Undesignated	Roasted, in recycled bottles	3[2]	<1
Undesignated	Peanut butter, in glass	2	<1
Undesignated	Peanut butter, in glass	2	<1
Undesignated	Peanut butter, in plastic	<1	<1
Undesignated	Peanut butter, in glass	1	<1
Undesignated	Peanut butter, in glass	1	<1
Undesignated	Peanut butter bar, in foil	<1	<1

1. Source codes; 1 = Jamaica, 2 = Trinidad, and 3 = St. Vincent above (2) notes product from St. Vincent.
2. Product from St. Vincent collected in Trinidad.

significant degree. In naturally contaminated groundnuts, both oven and microwave roasting were equally effective in destroying 48 to 61% of aflatoxin B_1 and 32 to 40% of aflatoxin G_1 (Pluyer et al. 1987). A time-course study of chlorine gas treatment (11 mg aflatoxin B_1 with 4 mL chlorine gas) showed that 60 to 75% of aflatoxin B_1 was destroyed within 10 min of exposure (Sen et al. 1987).

Conclusion

Aflatoxin contamination in SAT-African and Caribbean countries is very common. It is important to note that aflatoxin levels in groundnut products are potentially very high and, in most instances, little effort has been directed to avoiding contaminated products. Efforts to decontaminate groundnuts have concentrated on export materials. The population, in general, is left with little choice but to consume contaminated products. Cultural practices and socioeconomic considerations are important in planning interventions and research to avoid or eliminate aflatoxin contamination in food products consumed by the populations of groundnut-producing countries. Considering the urgency of the problem and the potential health hazard to the most vulnerable groups in developing countries, more emphasis should be given to monitoring and managing the aflatoxin contamination of groundnuts during postharvest handling, storage, processing, and preparation.

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Discussion

N.E. Ahmed. Low levels of aflatoxin in groundnuts tested immediately after harvest are probably due to infestation with insects, because most sound groundnuts from sound pods are free of aflatoxin at this stage? Did you trace the sources of groundnuts used in paste production? Groundnuts are always segregated, good material going for export, and small, discolored, blemished seeds for local production of paste and roasted groundnuts.

B. Singh. We did not test the groundnuts used for making paste, and we did not know their source. But the material definitely included rejects, gleaned, and contaminated nuts. In addition, *A. flavus* could grow on paste exposed in pans in the market place.

I.A. Rana. Some breeders insist that the developing countries should first concentrate on increasing production of more food commodities such as groundnut, and bother about aflatoxins, allergic proteins, etc., only at a later stage. Should we ignore the quality aspect for the time being?

B. Singh. No, we should not ignore the quality aspects of groundnut. Breeders should be aware of the needs of consumers. Quality tests such as that for aflatoxin contamination should be a vital part of groundnut evaluation, along with yield and production aspects. I may not recommend inclusion of testing for allergens, but aflatoxin, flavor, size, and acceptability should always be taken into account.

Aspergillus flavus Colonization and Aflatoxin Contamination of Groundnut in Sudan

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Abstract

The effects of irrigation regimes and date of harvesting on preharvest infection by Aspergillus flavus *and aflatoxin contamination of seed of four commercial and two other groundnut cultivars were studied. Groundnuts watered at 1-, 2-, and 3-week intervals and harvested at the normal time, and at 1 week before, and 1 week after this time were free from* A. flavus *colonization and aflatoxin contamination.* Aspergillus flavus *colonized a low percentage (2.7-7%) of groundnuts left in the soil for 6 weeks after harvest. However, no aflatoxin contamination was detected. Wilt diseases and insect damage, mainly by white grubs and termites, predisposed seeds to preharvest* A. flavus *infection (56.4-69.8%) and aflatoxin contamination (18-21 $\mu g\ kg^{-1}$). Groundnuts stored for 3 months in a well-ventilated room with an average temperature of 15°C were colonized at a low level but with no aflatoxin contamination. Infection increased with time in storage. Groundnuts harvested 1 week before maturity were most affected by* A. flavus *infection of seeds in storage, but there were no differences between genotypes. Groundnuts stacked in sacks at shelling sites were sampled, 4% were contaminated, with an average aflatoxin content of 11 $\mu g\ kg^{-1}$. Those sampled at oil mill sites were 15% contaminated with an average aflatoxin content of 20 $\mu g\ kg^{-1}$. Groundnuts left in the soil for 2-3 weeks after harvest in trials on the Gezira and Rahad irrigation schemes had 12% of samples contaminated, with an average aflatoxin content of 10 $\mu g\ kg^{-1}$. This produce is usually allocated for local processing.*

Résumé

Colonisation par *Aspergillus flavus* et contamination par les aflatoxines des arachides au Soudan : *Les effets des régimes d'irrigation et de la date de récolte sur l'infection par* Aspergillus flavus *avant la récolte et la contamination des graines par les aflatoxines ont été étudiés chez quatre cultivars commerciaux et deux autres cultivars d'arachide. Les arachides sont indemnes et non contaminées lorsque les cultures sont irriguées à 1, 2 ou 3 semaines d'intervalle et que la récolte a lieu à la date normale ou bien une semaine avant ou après cette date. Il y a une faible colonisation (2,7-7%) lorsque les arachides sont laissées aux champs six semaines après la récolte, sans, cependant, une contamination par les aflatoxines.*

Les maladies de flétrissement ainsi que les dégâts d'insectes, en particulier les vers blancs et les termites, favorisent l'infection des graines par A. flavus *(56,4-69,8%) avant la récolte et la contamination par les aflatoxines (18-21 $\mu g\ kg^{-1}$). Les arachides stockées pendant trois mois dans un endroit bien aéré où la température moyenne est de 15°C, sont faiblement colonisées et sans*

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contamination. L'infection est d'autant plus intense que la durée de stockage est plus longue. Les arachides récoltées une semaine avant la maturation sont les plus infectées pendant le stockage; il n'y a pas de différence entre les génotypes. Les arachides stockées en sacs aux sites de décorticage ont été analysées; 4% en étaient contaminées avec une teneur moyenne des aflatoxines de 11 μg kg⁻¹. Les échantillons prélevés dans les huileries présentent un niveau de contamination de 15% avec une teneur moyenne en aflatoxines de 20 μg kg⁻¹. 12% des échantillons d'arachides laissées aux champs pendant 2-3 semaines après la récolte des cultures expérimentales sur les périmètres irrigués de Gezira et de Rahad ont été contaminés avec une teneur moyenne en aflatoxine de 10 μg kg⁻¹. Cette production est normalement destinée au traitement local.

Resúmene

Colonización por *Aspergillus flavus* y contaminación con aflatoxinas del cacahuate en el Sudán : *El efecto de diferentes regímenes de riego y fechas de cosecha, sobre la infección por* Aspergillus flavus *en el período precosecha y la contaminación de las semillas con aflatoxinas, se estudió en cuatro cultivares comerciales y dos no comerciales. Los cacahuates regados a intervalos de una, dos y tres semanas y cosechados en la fecha normal, o una semana antes y una semana después de esa fecha estaban sanos y libres de* A. flavus *y de contaminación con aflatoxinas. La colonización con* Aspergillus flavus *fue baja (2.7-7%) en cacahuate que permaneció dentro del suelo seis semanas después de la cosecha; en este caso, tampoco se detectó contaminación con aflatoxinas. Las enfermedades que producen marchitamiento y los daños de insectos, principalmente por larvas de coleópteros y termitas, favorecieron la infección precosecha del grano por* A. flavus *(56.4 a 69.8%) y la contaminación con aflatoxinas (18 a 21 μg kg⁻¹). Los cacahuates almacenados durante tres meses en un local bien ventilado, con una temperatura de 15°C, fueron infectados a un nivel bajo y no hubo contaminación con aflatoxinas. El grado de infección aumenta con el tiempo de almacenamiento. Los cacahuates cosechados una semana antes de su madurez completa fueron los más infectados por* A. flavus, *entre las diversas semillas almacenadas, pero no hubo diferencia en este aspecto entre los diferentes genotipos estudiados. Los cacahuates en costales apilados en los lugares del descascarado se muestrearon y se analizaron, encontrándose que el 4% estaba contaminado y tenía un contenido promedio de 11 μg kg⁻¹ de aflatoxinas. El 15% de las muestras recolectadas en los sitios donde estaban instalados los molinos aceiteros estaba contaminado con un promedio de 20 μg kg⁻¹ de aflatoxinas. Cuando el cacahuate se dejó sobre el suelo durante dos a tres semanas después de la cosecha, en los ensayos en las áreas de riego de Gezira y Rahad, 12% de las muestras recolectadas estaban infectadas y tenían un contenido promedio de 10 μg kg⁻¹. Esta producción normalmente se destina a ser procesada localmente.*

Introduction

Aflatoxin contamination of groundnut is a matter of worldwide concern. Research has shown that toxin-producing strains of *Aspergillus flavus* Link ex. Fries can infect groundnuts and produce aflatoxin before lifting, in the windrow after harvest, and in storage (Diener 1973). Several factors favor aflatoxin contamination of groundnut before lifting. These include damage by insects and mites, drought stress, irrigation practices, maturity at harvest, and climatic conditions (Diener 1965, Diener et al. 1965). Immature and overmature seeds are more susceptible to *A. flavus* infection than just mature seeds and aflatoxin contamination increases with delay in harvesting (Dickens and Pattee 1966, McDonald and Harkness 1967).

In the Sudan, *A. flavus* is a common constituent of the soil microflora (Tarr 1955). The fungus is associated with pre- and postemergence seedling diseases of groundnuts in rainfed areas (Clinton 1960). However, under irrigated conditions in the Gezira, attempts to isolate the fungus and/or the toxin from freshly harvested, intact seeds were not successful (El Nur and Ibrahim 1968, 1970).

Groundnut is an important crop in the Gezira and Rahad irrigation schemes where area allocated to growing the crop is 134.5×10^3 ha. The studies described here were undertaken at the Gezira Agricultural Research Station (GRS) to determine the effects of watering intervals, early, and delayed harvesting on natural contamination by the fungus and on aflatoxin production. Surveys were also conducted in the Gezira and Rahad schemes to determine the level of aflatoxin contamination in irrigated groundnuts under standard field, storage, and processing conditions.

Materials and Methods

Experimental Trials

Effect of watering intervals and harvesting date on seed colonization and aflatoxin contamination

Field experiments were conducted at GRS during the 1984/85 and 1985/86 cropping seasons. Four commercial groundnut varieties (Ashford, Nigerian MH383, and 430B) were chosen for the test on the basis of their known high susceptibility to *A. flavus* invasion. Two genotypes (Early bunch and VA 71-347) were included for comparison. All varieties and genotypes were grown according to standard practices in the Sudan. Two months after sowing they were subjected to different watering regimes, i.e., irrigation at intervals of 1, 2, and 3 weeks. Plants were harvested 1 week before maturity, at maturity, and 1 week after maturity. Treatments were arranged in a randomized complete block design with four replicates.

After harvest, the crop was dried in windrows for 7 days and then in heaps for a further 3 days with pods exposed to the air. The pods were then threshed, handshelled, and the seeds examined for *A. flavus* infection and aflatoxin contamination.

Fungal infection was determined by sowing 50 surface-disinfected seeds (treated for 1 min in a 0.5% aqueous solution of sodium hypochlorite) on potato dextrose agar. The plates were incubated at 30°C for 7 days, then examined for growth of *A. flavus* from the seeds and the percentage of infected seeds was recorded. Aflatoxin contamination was detected by the Velasco minicolumn method (as reported by Mehan and McDonald 1980) while for quantitative measurements, groundnut seeds were extracted using the Best Food (BF) method and aflatoxin contents were estimated by high performance liquid chromatography (HPLC).

Pods which were not lifted during harvest were left in the soil for 6 weeks, then dug and analyzed separately.

Effect of wilts and insect damage on *A. flavus* infection and aflatoxin contamination of seeds

Plants wilt following damage by insects or pathogens; these plants were harvested separately, and the causes of their wilt diagnosed.

Effect of storage time on colonization and seed contamination

Groundnuts from the three watering regimes and harvest dates treatments were handshelled,

placed in cloth bags, and stored for 3 months in well-ventilated stores at an average temperature of 15°C. Samples were examined every month for fungus infection and aflatoxin contamination of seeds.

Pilot Survey

Effect of drying methods on natural contamination

Five hundred samples of groundnut were collected over a period of 7 weeks from the Gezira and Rahad irrigation schemes. Samples were taken from the edges and middles of heaps of unthreshed groundnuts arranged by farmers in the traditional manner.

Effect of storage at shelling sites and in oil mills on aflatoxin contamination

Two hundred samples of unshelled groundnuts were collected over a period of 8 weeks from sacks stacked at shelling sites in central Sudan. Similarly, 120 samples were collected over a period of 12 weeks from oil mills' stores situated near the shelling sites.

Effect of delayed harvest on aflatoxin contamination

Fifty samples of groundnuts left in the soil for 2–3 weeks after the normal harvest time were collected from the Gezira and Rahad irrigation schemes.

Results

Freshly harvested groundnut seeds were not colonized by *A. flavus* and contained no aflatoxin irrespective of the irrigation regime used. Seed of all genotypes left in the soil for 6 weeks after the normal harvest time were colonized by *A. flavus* (Table 1). Colonization of immature seeds was significantly greater than that of mature and overmature seeds ($P<0.05$). Despite the observed *A. flavus* colonization, no aflatoxin was detected in any treatment.

Table 1. *Aspergillus flavus* colonization of gleaned groundnuts left in the soil for 6 weeks after harvest, Hasaheesa, Wad Medani, and Sinar, Sudan, cropping seasons 1984/85 and 1985/86.

	Average seed infection (%)		
Genotype	1 week before maturity	At maturity	1 week after maturity
Ashford	7.3	5.3	6.0
Nigerian	6.7	5.9	5.3
MH 383	6.0	4.7	4.7
430B	6.0	4.0	5.3
Early bunch	6.0	3.3	4.0
VA 71-347	4.0	2.7	3.3
SE	±0.47	±1.2	±0.8

Table 2. Effects of wilts and insect damage on *Aspergillus flavus* infection and aflatoxin contamination of groundnuts, Hasaheesa, Wad Medani, and Sinar, Sudan, cropping seasons 1984/85 and 1985/86.

	Average seed infection (%)				Aflatoxin content ($\mu g\ kg^{-1}$)			
	Wilted		Insect-damaged		Wilted		Insect-damaged	
Genotype	84/85	85/86	84/85	85/86	84/85	85/86	84/85	85/86
Ashford	60	78	75	90	11	13	32	38
Nigerian	45	58	58.5	65	8	19	29	24
MH 383	42	73	65	90	21	12	13	21
430B	48	67	62	80	6	8	16	14
Early bunch	48	65	83.5	87	9	14	11	19
VA 71-347	36.5	57	47.2	35	5	8	7	10
SE	±3.7	±6.0	±3.7	±6.0	±4.8	±4.3	±4.8	±4.3

Table 3. *Aspergillus flavus* infection of gleaned groundnuts stored for 1, 2, and 3 months, Hasaheesa, Wad Medani, and Sinar, Sudan, cropping seasons 1984/85 and 1985/86.

	Average seed infection (%) of plants harvested								
	1 week before maturity			At maturity			1 week after maturity		
Genotype	1	2	3	1	2	3	1	2	3
Ashford	13	16	26	4	6	10	6	8	13
Nigerian	13	13	23	6	6	8	6	9	11
MH 383	16	17	20	4	4	8	4	6	8
430B	12	18	23	3	6	10	4	6	15
Early bunch	10	15	21	4	3	10	4	8	12
VA 71-347	10	13	18	3	5	5	3	6	10
SE	±0.8	±0.9	±1.7	±1.0	±0.4	±0.8	±0.46	±0.6	±0.8

Table 4. Aflatoxin contents of groundnuts stores at shelling sites and in oil mills' stores, Hasaheesa, Wad Medani, and Sinar, Sudan, cropping seasons 1984/85 and 1985/86.

Shelling sites		Oil mills stores			
Sample number	Aflatoxin content ($\mu g\ kg^{-1}$)	Sample number	Aflatoxin content ($\mu g\ kg^{-1}$)	Sample number	Aflatoxin content ($\mu g\ kg^{-1}$)
1	5	1	15	10	11
2	13	2	25	11	23
3	4	3	32	12	14
4	10	4	26	13	10
5	15	5	24	14	10
6	20	6	18	15	28
7	12	7	14	16	8
8	7	8	29	17	18
		9	27	18	12

Groundnut seeds from wilted and insect-damaged plants were colonized by *A. flavus* and contained appreciable amounts of aflatoxin (Table 2).

Seed colonization of groundnuts stored for 3 months was significant, and tended to increase with storage time (Table 3). Differences in colonization between immature and mature seeds were more pronounced than differences between storage times. Immature seeds were more susceptible to colonization than mature and overmature seeds. However, no aflatoxin was detected in seeds from any of the treatments.

No aflatoxin was detected in any of the samples collected from groundnuts heaped in the traditional manner. However, contamination was randomly distributed throughout sacks stored at the shelling sites. Of the 200 samples tested, only 4% were contaminated, with the average aflatoxin contents being 11 $\mu g\ kg^{-1}$ seeds (Table 4).

Of the groundnuts stored in the oil mills' stores 15% were contaminated; their average aflatoxin content was 20 $\mu g\ kg^{-1}$ seeds (Table 4).

Groundnuts left in the soil for 2-3 weeks after harvest showed 12% contamination and an average aflatoxin content of 10 $\mu g\ kg^{-1}$ seeds.

Discussion

Irrespective of crop variety, the level of aflatoxin contamination of groundnuts in the irrigated schemes was below internationally acceptable levels and irrigation practices had no effect on pre- or postharvest colonization and aflatoxin formation. Harvesting at maturity or one week later is a useful way to reduce postharvest contamination.

Contamination at the shelling sites and oil mills' stores could be attributed to farmers washing and improperly drying their produce before delivering it to these sites. Sacking groundnuts with high moisture contents is conducive to *A. flavus* invasion and aflatoxin production in storage.

Storage in dry, clean, and well-ventilated stores at temperatures of 15°C or less will reduce aflatoxin contamination. However, the findings that fungal colonization and toxin production increase with increase in storage time and are influenced by maturity stage at harvest have to be taken into consideration.

The high aflatoxin contents of roasted peanuts and peanut butter reported by Malik (1987), could be attributed to the fact that both are usually processed from groundnuts that remain in the soil after harvest, or from insect-damaged and broken groundnuts. Thus, it becomes evident that aflatoxin contamination in the Sudan is solely due to man-made factors. Although the aflatoxin level is not serious, it could be eliminated if strict control measures, particularly in drying, curing, and storage are implemented.

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Discussion

K.J. Middleton. How did you determine the optimum maturity date?
N.E. Ahmed. The optimum maturity date is determined by our breeders after examining the yields of sample plants. Usually farmers in the Sudan harvest their crops within a week of the optimum maturity date.
T.O.M. Nakayama. You said that the peanut butter sold in local markets was not made by Sudanese people?
N.E. Ahmed. No, it is made mainly by immigrants from Nigeria although some have been in Sudan for several generations.
P. Subrahmanyam. The data you presented on aflatoxin contamination do not agree with the findings reported by Dr B. Singh which indicated that the aflatoxin levels exceeded 50 $\mu g\ kg^{-1}$. You said that in the Sudan aflatoxin is not a problem.
N.E. Ahmed. I did not refer to rainfed areas. All my studies were done on irrigated crops mainly in central Sudan.
B. Singh. May I add that it is not really a contradiction. I also said that most of the groundnuts obtained directly from the farmers contain 15 μg aflatoxin kg^{-1} or less, but you can pick up from the market place samples of roasted peanuts and other products that contain very high levels of aflatoxin. I agree also, that our samples came from rainfed crop areas.
J.A. Wightman. You mentioned insect damage leading to an increase in aflatoxin contamination. I wonder if you could expand on this and tell us what kind of insects were causing the damage.
N.E. Ahmed. The pests were mainly white grubs and termites.
J.A. Wightman. Did you notice any increase in termite damage when you left the pods in the ground a little longer than usual?
N.E. Ahmed Yes.

Traditional Groundnut Storage and Aflatoxin Problems in Côte d'Ivoire: Ecological Approaches

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Abstract

Groundnut storage problems were studied in Côte d'Ivoire during two successive storage periods (1985/86 and 1986/87) and from three different aspects: insect pests; mold damage; and contamination with aflatoxins.

Samples were taken periodically from traditional producers' fields throughout the groundnut-growing areas of the survey and from town and village markets.

Generally, locally stored samples were a little less infested than samples taken from markets. With few exceptions, all the locally sampled material was contaminated with measurable levels of aflatoxin. Over the 2-year survey period, 7.9% of the 434 local stocks examined exceeded the toxicity level threshold of 250 $\mu g\ kg^{-1}$, with 4.4% above 1000 $\mu g\ kg^{-1}$. It was also found that 73% of these samples were above the European Economic Community (EEC) safety level of 10 $\mu g\ kg^{-1}$.

Significant correlations were found between aflatoxin contamination and different storage and meteorological variables. These included physical characteristics and age of the pods, and the influence of the prevailing atmospheric conditions.

Résumé

Stockage traditionnel d'arachides et problèmes d'aflatoxines en Côte d'Ivoire — approches écologiques : *Les problèmes liés au stockage d'arachides ont été étudiés en Côte d'Ivoire pendant deux périodes successives de stockage (1985-1986 et 1986-1987) du point de vue de trois aspects différents : insectes nuisibles, dégâts dus aux moisissures et contamination par les aflatoxines.*

Des échantillons ont été prélevés périodiquement des champs paysans dans les différentes régions arachidières de l'enquête et sur les marchés des villes et villages.

En général, les échantillons stockés localement sont un peu moins infectés que ceux prélevés sur les marchés. A part quelques exceptions, tous les échantillons présentent des taux mesurables d'aflatoxines. Cependant, le taux d'aflatoxines dans 7,9% des 434 stocks locaux examinés au cours de la période de l'étude dépasse le seuil de toxicité de 250 $\mu g\ kg^{-1}$, et s'élève même au-dessus de 1000 $\mu g\ kg^{-1}$ dans 4,4% des échantillons. 73% de ces échantillons présentent des taux supérieurs au niveau autorisé par la CEE de 10 $\mu g\ kg^{-1}$.

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Il y a une corrélation significative entre la contamination par les aflatoxines, d'une part, et les variables météorologiques et de stockage. Parmi ces variables figurent les caractéristiques physiques et l'âge des gousses ainsi que l'influence des conditions atmosphériques.

Resúmene

Almacenamiento tradicional de cacahuates y problemas de aflatoxinas en Côte d'Ivore: Aspectos ecológicos : *Se estudiaron los problemas de almacenamiento de cacahuates en Côte d'Ivore, durante dos períodos sucesivos de almacenamiento (1985/86 y 1986/87), desde tres aspectos diferentes: insectos dañinos, daños causados por hongos y contaminación con aflatoxinas.*

Se recolectaron periódicamente muestras de los campos de productores tradicionales, dentro de las áreas cacahuateras comprendidas en este estudio y de los mercados de pueblos y ciudades.

Generalmente, las muestras procedentes de almacenes locales estaban un poco menos infectadas que las muestras procedentes de los mercados. Con pocas excepciones, casi todos los materiales muestreados localmente estaban contaminados con niveles cuantificables de aflatoxinas. Durante los dos años en que se desarrolló el estudio, 7.9% de los 434 lotes locales de semillas examinadas tuvieron contenidos arriba del valor límite de nivel de toxicidad de 250 $\mu g\ kg^{-1}$ y el 4.4% estuvieron arriba de 1000 $\mu g\ kg^{-1}$. También se encontró que el 73% de estas muestras tenían valores mayores de los 10 $\mu g\ kg^{-1}$, autorizados como nivel sin peligro por la Comunidad Económica Europea (CEE).

Se encontró que existen correlaciones estadísticamente significativas entre la contaminación con aflatoxinas y diferentes variables meteorológicos y de condiciones de almacenamiento. Entre éstas figuran las características físicas y la edad de las vainas, así como el efecto de las condiciones atmosféricas prevalentes.

Introduction

In West Africa, soil pests such as millipedes (Raheja 1975, Pollet 1985), coleoptera larvae (Wyniger 1962, Hill 1983), termites (Feakin 1973, Johnson et al. 1981), and storage pests cause significant losses in yield and quality of groundnuts produced by small farmers. Damage may be direct or indirect (Johnson et al. 1981), direct damage being caused by termites and millipedes penetrating the pods and destroying the developing kernels or exposing them to direct attack by other soil pests and by soil fungi and bacteria (Busnardo and Pollet 1985). Indirect damage occurs when termites or millipedes scarify or wound the outer tissues of the pod but do not penetrate to the kernels. Such damage renders the pods susceptible to invasion by other soil pests and by such fungi as *Fusarium* spp, *Macrophomina phaseolina*, and *Aspergillus spp* (Johnson et al. 1981). Insect-damaged pods are also structurally weakened and so are more liable to crushing during postharvest handling than are undamaged pods. Destruction of pods in the soil reduces yields but has no further significance. However, pods that have been damaged by pests and soil fungi may be further colonized by molds during postharvest drying in the field and during storage. This is of particular importance when the kernels are invaded by toxigenic strains of *Aspergillus flavus* and *A. parasiticus*, and the role of soil fauna in the development of aflatoxin contamination in groundnuts has been indicated by several workers (Wogan 1968, Widstrom 1979, Diener et al. 1963, McDonald and Harkness 1965, Fennell et al. 1975).

Crop production and produce-handling practices, particularly storage methods, are of

obvious importance in relation to aflatoxin contamination, and these interact with pest damage and climatic factors in determining the severity of the aflatoxin problem. The major objective of the research described in this paper was to elucidate such interactions and to see if there were significant correlations between aflatoxin contamination levels in groundnuts and meteorological parameters.

The Côte d'Ivoire Climate

The Côte d'Ivoire is situated on the Gulf of Guinea on the Atlantic coast of Africa (Fig. 1). Rainfall is relatively high throughout the year, and mean temperatures are in the range of 20 to 23° C. In the south there is rain forest, then moving northwards there is a forest-savanna mosaic belt, and further north are Guinea Savanna and Southern Sudan Savanna zones.

In the rain forest zone rain occurs every month (>60mm) but although there is no marked dry season, there are two periods of heavy rainfall with intervening periods of reduced rainfall. Thus, in the Abidjan area there is a long rainy season (April- June), a short "dry" season (July), a short rainy season (August-September), and a long "dry" season (October-March).

In the savanna zones in the north of the country (Ferkessedougou, Odienne, Bouna, etc.) the dry season is long and more severe, stretching from October to June. The rainy season is from July to September. Rainfall is less predictable than in the southern zones and may occasionally be insufficient for the groundnut crop.

Groundnut Cropping Seasons, Production, and Storage

There are no accurate data for total annual groundnut production in Côte d'Ivoire, but this is estimated to be in the region of 100 000 t of dried pods. Groundnuts are grown by smallholders whose farms are of approximately 0.8 ha. They generally follow traditional cropping practices and obtain yields of 800 to 900 kg dried pods ha^{-1} (Pollet 1982a, 1985).

As temperatures are relatively stable throughout the year, rainfall is the critical factor in determining when groundnuts are grown. In the southern and central regions of the country groundnuts can be grown in both rainy seasons, sowing following the first rains; and harvesting and drying being carried out in the subsequent dry seasons. In the north, where the bulk of the groundnut crop is grown, only one crop is possible without irrigation.

In central Côte d'Ivoire at such places as Bouaké, Bondoukou, and Bouaflé, groundnuts are usually sown in March or April in the first rainy season, the second rainy season being reserved for such major crops as cotton and rice. Harvesting takes place in June towards the end of the first rainy season. This leaves time to prepare the ground for sowing the second rainy-season crop, but it is often difficult to dry the groundnut pods thoroughly.

In the north the groundnut crop is sown in July/August at the beginning of the rainy season and it is harvested 3–4 months later (depending upon variety duration) in the dry season. Drying the produce is more rapid and satisfactory than in the central and southern regions.

In the main groundnut-growing areas of the north farmers store the produce in the traditional "senoufo" stores made from boulder clay, while in other areas, and particularly near large towns, groundnuts are stored in sacks. Other methods of storage include keeping pods in earthernware pots, baskets, or barrels, or simply spreading them on the floor.

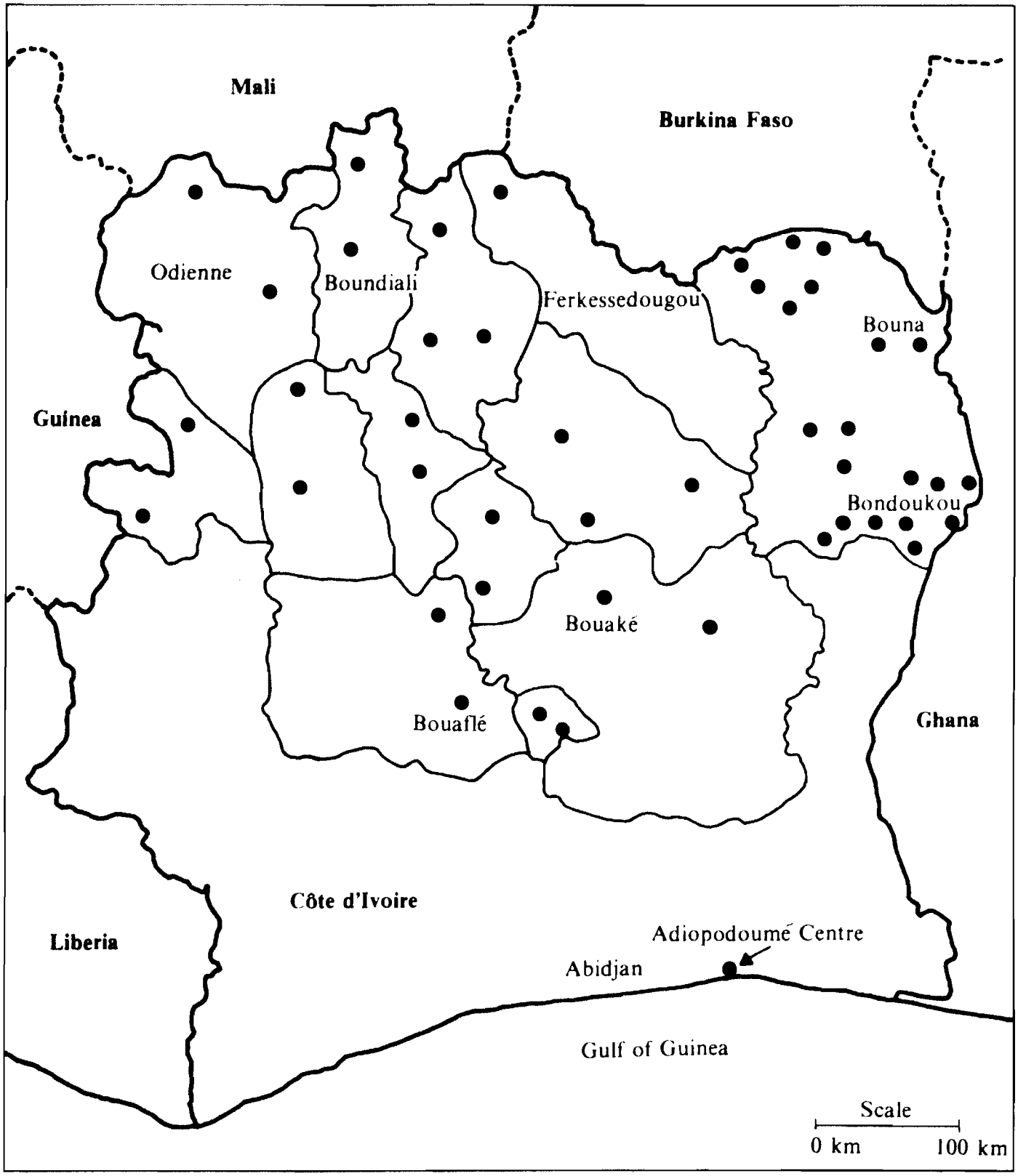

Figure 1. Groundnut-growing regions of Côte d'Ivoire showing administrative divisions, survey officers network locations, and Adiopodoumé Centre of la Compagnie Ivoirienne des Textiles (CIDT).

Materials and Methods

During the 1985/86 and 1986/87 storage periods, pest damage, fungal infection, and aflatoxin levels in farmers' stored groundnuts were investigated.

Meteorological data were recorded for the calendar years 1985, 1986, and 1987.

Pest Damage

Samples from town and village markets collected with assistance from 45 survey officers of the Compagnie Ivoirienne des Textiles (CIDT) were examined for the following:

1. Weight (percentage) and occurence of undamaged pods, termite-scarified pods, broken or penetrated pods.
2. Information on insect pests present in or on each of the above categories of pods at the time of sampling, and 15 days later.

Fungal Infection

Seeds of each sample (16 seeds) were held for 4 days at 28°C on moist, sterile sand, and then assessed for:

1. Viable seeds (germinated) (%)
2. Nongerminated seeds (%)
3. Malformed seedlings (%)
4. Dead seeds (%)
5. Seeds infected by *A. flavus* and/or *Rhizopus* spp (%).

Aflatoxin Contamination

Seeds of each sample (20 g seed) were assessed for aflatoxin content. Aflatoxins were estimated using the high performance liquid chromatography (HPLC) method of Wiegandt et al. (1987).

Results and Discussion

Over the two storage periods, 434 samples of groundnuts from farmers, and 72 samples from markets were examined. Overall, aflatoxin levels were slightly higher in the market samples, 18% of these had levels of toxicity in excess of 50μg kg^{-1} while 14% of farmers' samples had similar levels of toxicity. There were differences between the two storage periods in relation to percentages of samples with aflatoxin contents above 250μg kg^{-1} (5.3% in 1985/86; 10.6% in 1986/87). This could be due to higher levels of insect-damaged pods in the samples taken from the 1986/87 storage period.

The degree of pest damage, particularly termite scarification of shells, was found to be related to rainfall during crop growth, there being a significant ($P<0.05$) negative correlation. Rainfall and humidity during storage also influenced aflatoxin contamination.

Stepwise multiple regression analysis revealed significant correlations between aflatoxin contamination level and such factors as:

- scarified pods
- undamaged pods
- number of broken pods per sample
- seed infection by *Aspergillus* spp.

Factorial analysis of variance revealed interestingly significant correlations between the geographical location of the sampling sites and the risk of aflatoxin contamination. For example, the maximum risk of aflatoxin contamination was recorded from the locations situated in the central region of Côte d'Ivoire.

The sampling method used in these studies is certainly not the best. The unit weight of 500 g of

pods in each sample, is probably too small to ensure a sufficiently accurate measure of the aflatoxin contamination level. Noirot in an unpublished study proved that to obtain a measure of aflatoxin contamination of 10 $\mu g\ kg^{-1}$ (accurate to 5%) it was necessary to take at least 7.0 kg of pods per sample. Thus, with a unit sample of 500 g the sampling error increases to about 35%. In other words, a significant result could only be obtained by chance one time in three.

To reduce or prevent aflatoxin contamination it is necessary to take measures to control pest damage during crop growth, drying, and storage, to adopt curing and drying procedures that ensure rapid drying of pods, and to use effective storage methods. All molded and insect-damaged pods and seeds should be removed before produce enters storage.

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Engineering Aspects of Aflatoxin Research in Groundnuts: Evolution of an Environmental Control Plot Facility

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Abstract

In 1980, an environmental control plot facility was designed and built at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Peanut Research Laboratory to study the preharvest invasion of groundnuts by Aspergillus flavus *and subsequent aflatoxin production. Requirements for the planned research included the ability to induce drought and to manipulate soil temperature. Initially, the facility consisted of six, 12.2-m long × 5.5-m wide × 1.8-m deep, isolated plots with electric-motor-powered roofs for rainfall exclusion as required. Geocarposphere temperature manipulation was accomplished with thermostatically controlled, electrically heated cables; and cooling coils supplied with chilled water. Environmental data were collected using a microprocessor-based, digital data acquisition system that recorded conditions every 2 h during experiments. The facility was recently expanded to investigate the potential of the separate roles of plant stress and pod stress in aflatoxin contamination using two ancillary plots in which pod and root locations in the soil are separated and independently controlled. A microcomputer-based temperature control/alarm system has been designed and installed to replace manual controls for soil temperature manipulation. The functional performance of the facility has to date been adequate to provide a wide variety of required environmental conditions for research.*

Résumé

Aspects techniques de la recherche sur les aflatoxines dans les arachides—évolution d'un dispositif de contrôle des conditions d'environnement : *En 1980, le service de recherche agronomique de l'United States Department of Agriculture (USDA) a étudié et construit un dispositif de contrôle des conditions d'environnement au sein du National Peanut Research Laboratory. L'objectif en est d'examiner l'invasion des arachides par* Aspergillus flavus *avant la récolte, ainsi que la contamination ultérieure par les aflatoxines. Ce dispositif permet de créer des conditions de sécheresse et de contrôler la température du sol. Au départ, il comprenait six parcelles (12,2 m de longueur, 5,5 m de largeur, 1,8 m de profondeur) isolées, pouvant être couvertes par des toits amovibles électriques pour arrêter la pluie. La température de la géocarposphère est réglée grâce à*

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des câbles de chauffage électrique avec contrôle thermostatique, et par des serpentins de refroidissement alimentées par l'eau froide. Les données sur les conditions du milieu sont obtenues par un système informatisé digital d'enregistrement des données à des intervalles de deux heures. Deux autres parcelles ont été récemment ajoutées à ce dispositif afin d'étudier l'importance respective des stress subis par la plante et par les gousses dans la contamination par les aflatoxines. Dans ces parcelles, la zone racinaire a été séparée de celle entourant les gousses dans le sol pour contrôle indépendant des conditions. Un système informatisé de contrôle de température et d'avertissement a été étudié et mis en place pour remplacer l'ancien système manuel. Ce dispositif permet de créer une diversité de conditions d'environnement nécessaires à ces études et les résultats obtenus jusqu'ici sont satisfaisants.

Resúmene

Aspectos técnicos de la investigación sobre las aflatoxinas en el cacahuate: Evolución de dispositivos para controlar las condiciones ambientales en parcelas experimentales : *En 1980, el Departamento de Agricultura de Estados Unidos (USDA) diseñó y construyó un dispositivo para controlar las condiciones ambientales en parcelas experimentales, en el Laboratorio Nacional de Investigación del Cacahuate del Servicio de Investigación Agrícola (ARS), para el estudio de la invasión precosecha de los cacahuates por* Aspergillus flavus, *y la subsecuente producción de aflatoxinas en los granos. Las investigaciones efectuadas requirieron de que se pudiera producir condiciones de sequía en el cultivo, así como variaciones de la temperatura del suelo bajo condiciones estrictamente controladas. Inicialmente, los dispositivos estaban constituidos por seis parcelas aisladas de 12.2 m de longitud por 5.5 m de ancho y 1.8 m de profundidad, equipados con techos accionados por motores eléctricos que permitían la exclusión de la lluvia cuando así se requería. Las variaciones de temperatura deseadas dentro de la geocarpósfera se producían por medio de resistencias eléctricas controladas termostáticamente y serpentines de enfriamiento dentro de los cuales circulaba agua previamente enfriada. Los datos sobre las condiciones del medio ambiente se obtuvieron por medio de un sistema de registro de información digital en una microcomputadora, en la cual se registraban las condiciones citadas cada dos horas, durante el desarrollo de los experimentos.*

Estos dispositivos se han incrementado recientemente para poder investigar separadamente el efecto que tienen los siguientes factores: a) el esfuerzo interno de las plantas; y b) el esfuerzo interno existente en las vainas, sobre la contaminación con aflatoxinas, utilizándose para este fin dos subparcelas adyacentes en las cuales los suelos donde se encuentran situados la vaina y la planta están separados entre sí y existen condiciones independientemente controladas. Se ha diseñado e instalado un sistema de control con alarma de la temperatura, que está conectado a una microcomputadora, y que sustituye los controles manuales para regular la temperatura del suelo. Estos dispositivos han resultado ser muy funcionales hasta la fecha, y han permitido crear la diversidad de condiciones ambientales necesarias para la realización de los estudios deseados.

Introduction

In 1972, 1978, and 1980, severe droughts occurred in southeastern USA where a large portion of the US groundnut crop is grown. Severe economic losses occurred not only from yield reduction but also from aflatoxin contamination in groundnuts. Prior to 1980, considerable research

attention had been given to factors predisposing groundnuts to aflatoxin contamination during various aspects of production and processing (Bampton 1963, Dickens et al. 1973, Diener and Davis 1977, McDonald 1969, McDonald and Harkness 1964, Pettit et al. 1971, Porter and Garren 1968). Preharvest *Aspergillus flavus* invasion and subsequent aflatoxin production in groundnuts had been closely associated with drought; however, the particular contributing factors during drought stress resulting in contamination had not been specifically defined (Bampton 1963, Dickens et al. 1973, Diener and Davis 1977, McDonald 1969, McDonald and Harkness 1964, Pettit et al. 1971, Porter and Garren 1968). To pursue the study of preharvest contamination, an environmental control plot facility was designed and built at the USDA-ARS National Peanut Research Laboratory (NPRL), Dawson, Georgia, and has now been in operation for 7 years (Blankenship et al. 1983). The design criteria for this facility included not only automatic, movable shelters but also an apparatus unique to this facility that manipulates soil temperature. Considerable information relative to the preharvest environmental factors involved in *Aspergillus flavus* invasion and subsequent aflatoxin contamination in groundnuts has been generated from research at this facility (Blankenship et al. 1983, Blankenship et al. 1984, Blankenship et al. 1985, Cole et al. 1982, Cole et al. 1984, Cole et al. 1985, Cole et al. 1986, Hill et al. 1983, Sanders et al. 1981, Sanders et al. 1984, Sanders et al. 1985a, 1985b).

The purpose of this paper is to chronologically review the operation of this facility during the past 7 years, and to describe the evolution of facilities and engineering aspects required to produce the required experimental conditions needed during research to determine the factors involved in preharvest aflatoxin contamination.

Original Facility Design

Initially, the facility consisted of six 12.2-m long by 5.5-m wide plots with a 1.8m-deep topsoil profile of Tifton sandy loam (Blankenship et al. 1983). Plan and cross-section views of the facility are presented in Figures 1, 2, and 3 (Blankenship et al. 1983). Provisions were made in the design to isolate the soil in each plot from surrounding soil and from adjacent plots to prevent surface runoff and groundwater infiltration. Individual, automatic, electric motor driven shelters were provided to exclude rainfall when desired. An irrigation system for each plot was attached underneath the roofs that could be moved over the plots when irrigation was necessary.

Initial research plans required the capability both to elevate the soil temperature above ambient temperature in the groundnut fruiting zone in an irrigated plot, and to lower the soil temperature below ambient temperature in a drought plot (Blankenship et al. 1983). Electrical heating cables placed 10-cm apart, 12.7 cm deep across the entire cross-sectional area of a plot and regulated with an on-off thermostat were used to elevate the soil temperature. Water from a well at approximately 20°C circulated through parallel 0.6-cm diameter epoxy-coated copper pipes spaced 10.2 cm apart and 10.2 cm deep was successfully used to lower the soil temperatures.

A 500-channel, microprocessor-based, digital data acquisition system was installed to collect environmental data. Type T thermocouples were used with the data-collection system for temperature measurements, while gypsum blocks were used for soil-moisture measurements. A lightning protection system for the data-collection equipment was installed. This included two 15.2-m towers placed opposite each other adjacent to the south end of the outermost east and west plots. An aluminum cable was suspended between the towers and attached to the metal framework surrounding the plots. Several 1.8-m long copper ground rods were driven around the perimeter of the plot facility and interconnected to the steel framework with a copper cable.

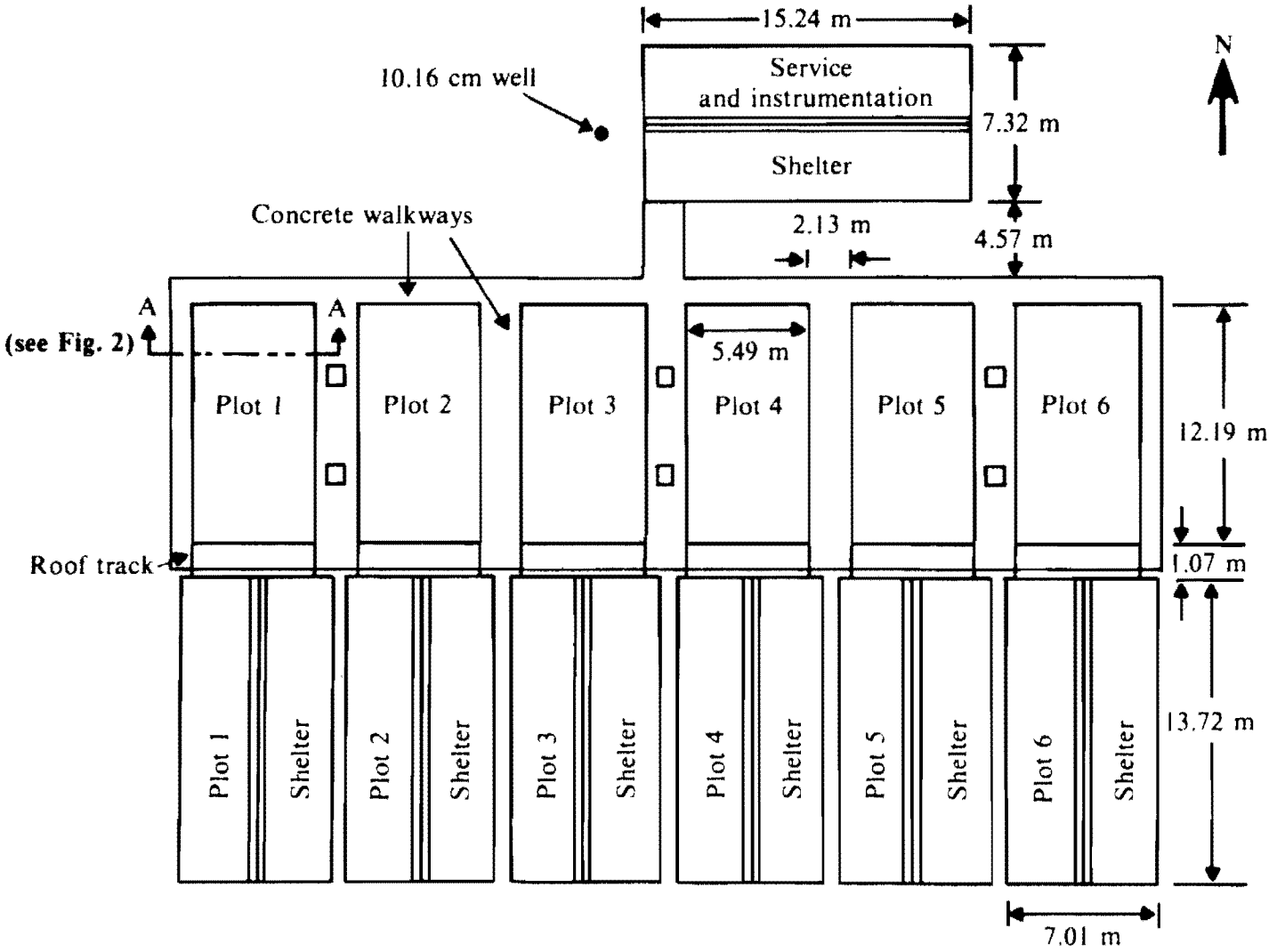

Figure 1. Plan view of the National Peanut Research Laboratory rain control plot facility, Dawson, Georgia, USA.

It was assumed that the cable would intercept a lightning strike or static electricity discharge and dissipate the charge into the surrounding soil instead of into sensor leads in the plots, thus protecting the electronic data-collection equipment.

Performance of the Facility

Inducing drought conditions in the plots at the facility has been relatively easy because of the designed drainage and the isolation of soil profiles of the plots. Generally, after water has been withheld from a plot, soil moisture tensions have reached levels up to -3 to -5 bars in 2 to 3 weeks in the top 30 cm of the soil. Drought periods in experiments longer than 40 days have usually produced tensions greater than -10 bars. Groundnuts typically begin to show drought stress by wilting 7 to 10 days after a final irrigation.

A summary of the accuracy of the soil temperature controlling equipment in obtaining desired treatment period averages is presented in Table 1. After the first year's tests, ensuing research has required that all of the original plots be fitted with soil temperature manipulation equipment.

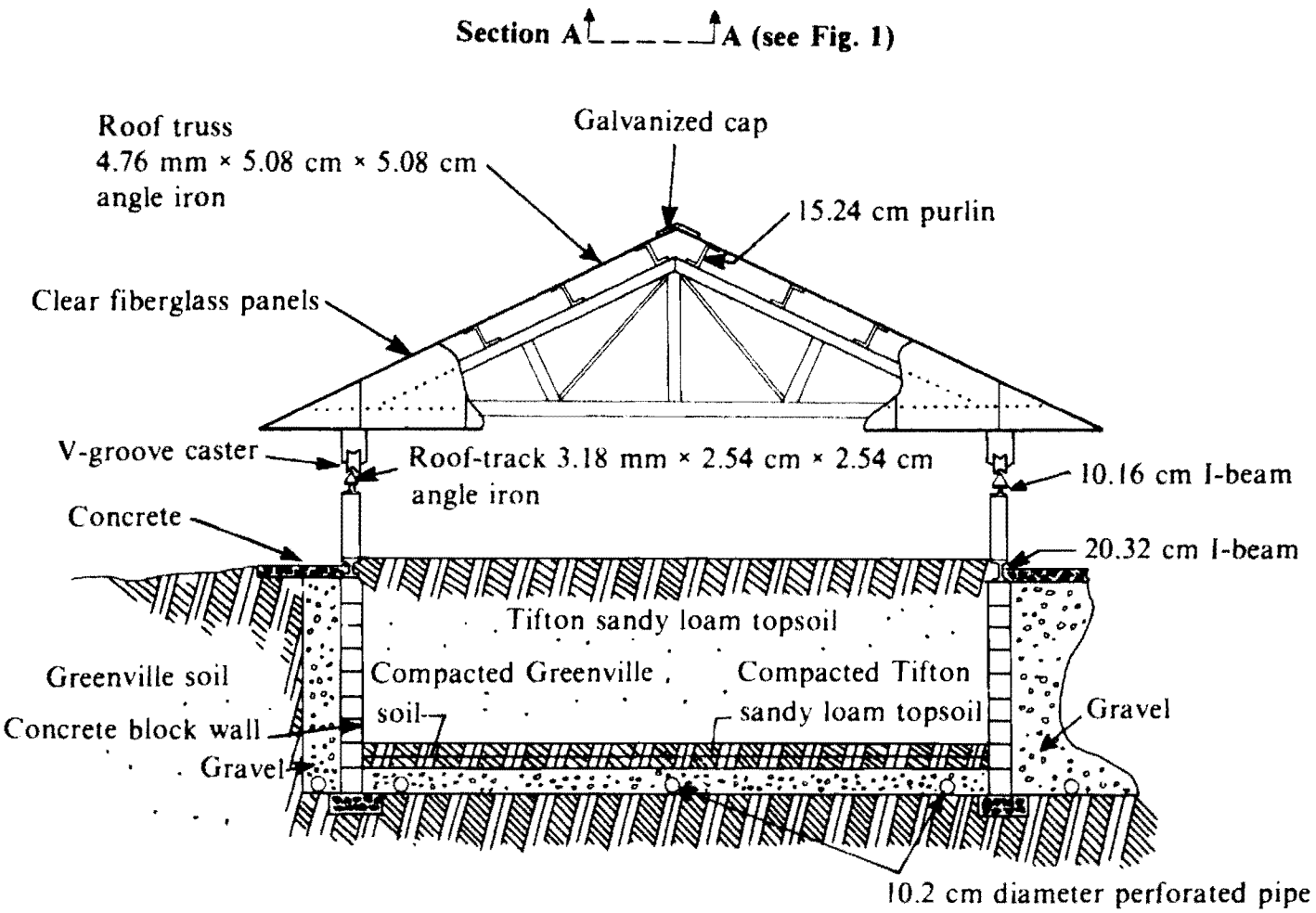

Figure 2. Cross-sectional view (A-A) of Plot 1.

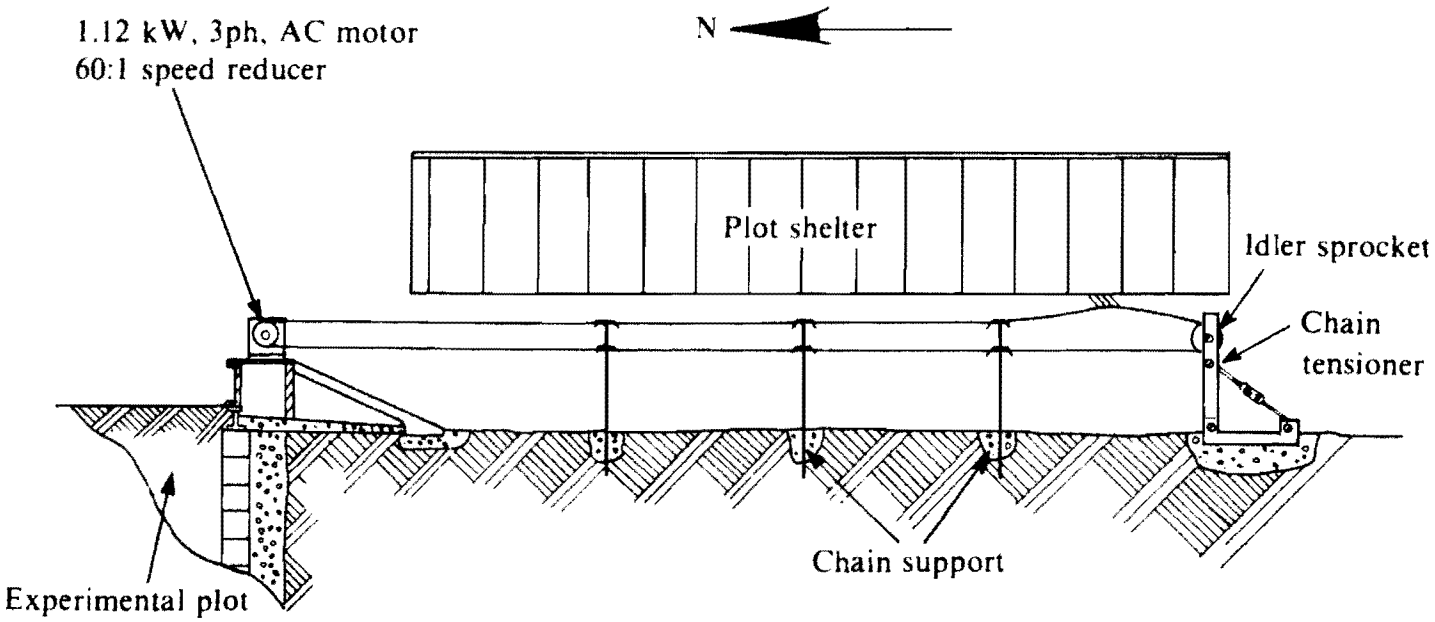

Figure 3. Plot shelter drive system.

Soil heating and cooling was generally accomplished in the plots using apparatus similar to that used in 1980. However, to extend the cooling capacity, an electrical water-chilling unit with two 5.07 hp compressors has been added to supply chilled water for cooling instead of using water

Table 1. Performance of the facility in controlling soil temperature, National Peanut Research Laboratory, Georgia, USA.

Year	Treatment	Desired average temperature (°C)	Treatment period average (°C)
1980	Irrigated	Unmanipulated	24.7
	Drought	Unmanipulated	28.0
	Irrigated/Heated	33.3	34.1
	Drought/Cooled	<25	24.4
	Irrigated	Unmanipulated	25.6
	Drought	Unmanipulated	28.9
1981	Irrigated	Unmanipulated	23.8
	Drought/Heated	30	30.5
	Drought/Heated	25	25.7
	Drought/Cooled	23	22.9
	Drought/Cooled	21	21.3
	Drought/Cooled	19	19.8
1982	Irrigated	Unmanipulated	23.8
	Drought	32	31.3
	Drought	30	29.6
	Drought	28	27.8
	Drought	26	26.3
	Drought	24	24.3
1983	Irrigated	Unmanipulated	25.9
	Irrigated/Heated	30	29.4
	Drought/Heated (20 days)	30	28.9
	Drought/Heated (30 days)	30	29.6
	Drought/Heated (40 days)	30	30.2
	Drought/Heated (50 days)	30	30.5
1984	Drought/Heated	30	28.9
	Drought/Heated	30	29.7
	Drought/Heated	30	29.1
	Drought/Heated	30	28.5
	Drought/Heated	30	28.9
	Irrigated	Unmanipulated	24.7
1985	Irrigated	Unmanipulated	23.1
	Drought	29	30.0
	Drought	29	29.5
	Drought	25	25.1
	Drought	25	24.9
	Drought	25	24.7
1986	Drought	30	29.5
	Drought	30	29.7
	Drought	30	30.2
	Drought	32	32.1
	Drought	28	27.5
	Drought	26	25.5

from the well. Water for cooling is circulated through the pipes in the plots and then returned to the chilling unit instead of being exhausted.

Lightning damage to the data-collection equipment at the facility occurred occasionaly from 1980 through the 1985 season. After the 1985 growing season, the lightning protection system (towers and connecting cables) was dismantled and interconnections between adjacent plot steel frameworks were disconnected where possible. Subsequently, lightning damage has greatly diminished, possibly because the protection system was attracting lightning and dissipating charges of the electricity that exceeded the limits for the analog voltage equipment into the soil around the plots. Other types of difficulties have been previously documented (Blankenship et al. 1983).

Engineering Modifications

Prior to the 1985 growing season, two additional plots 2.4-m wide by 12.2-m long were built; one each adjacent to the outside east and west plots. Shelters for the added plots were provided by increasing the widths of one side of the shelters of the original plots adjacent to the new plots. The same type topsoil profiles were used for the added plots but they were only 0.9-m deep. Provisions were not made to prevent moisture from the surrounding soil from entering the plots, but apparently this has so far not affected experiments. The major design criteria for these plots was to provide facilities for studying the influence of drought and heat stress in the root zone of the soil (plant stress) versus similar stresses in the pod zone (pod stress) on aflatoxin contamination of groundnut pods. The separation between the two depths in the soil has been assumed to be 7.6 cm. A 2.5-cm thick layer of polystyrene was used to separate the zones and to provide insulation from required experimental conditions between zones (Fig. 4). One plot is fitted with heating cables above the polystyrene that heat the pod zone, and the other with cables underneath the polystyrene that heat the root zone. Porous tubing was placed underneath the polystyrene to irrigate the root zones (Blankenship et al. 1985). Pod zones of the soil are irrigated with a sprinkling system. In one of the plots each year during the fruiting period, the controlled conditions have been dry and heated in the root zone, while the pod zone was irrigated and at ambient temperature. The opposite conditions have been provided in the two soil zones in the other plot.

Temperature differences produced between the soil zones for the two types of stress during 1985 and 1986 are shown in Table 2. The two types of stress produced striking differences in aflatoxin contamination of USA edible-grade categories of groundnuts (Table 3). Data indicate that adverse conditions of heat and drought in the pod zone and not in the root zone predispose groundnuts to contamination with aflatoxin.

Apart from adding two plots, another major engineering change was implemented at the facility prior to the 1987 planting season. To reduce the labor requirement in controlling experiments, a microcomputer control and alarm system was designed and installed to control the operation of the facility and to provide telephone alarms for soil temperature discrepancies, improper shelter operation, or electrical power failures (Blankenship et al. in press). The system periodically measures temperatures in plots (every 10 min), and subsequently adjusts the operation of the equipment used to maintain soil temperatures. A measured mathematical approach generates control algorithms to control soil temperatures. The system has provided adequate control of soil temperature in the research plots and has activated telephone alarms for roof drive failures, plot temperature extremes, and a power failure.

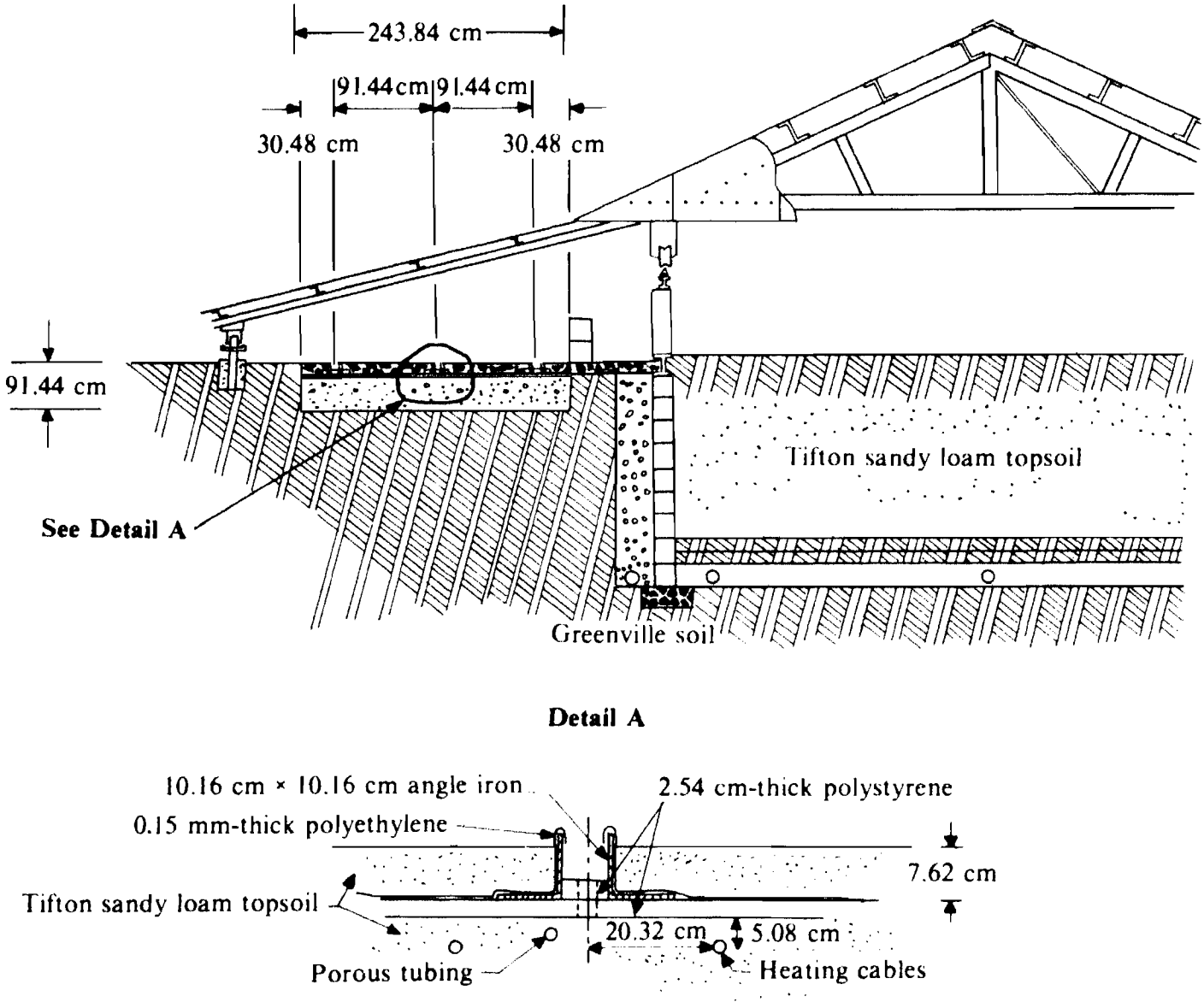

Figure 4. Cross-sectional view of one of the added plots.

Table 2. Comparison of soil environmental conditions for plant and pod stress treatments, National Peanut Research Laboratory, Georgia, USA, 1985 and 1986.

Year	Plot treatment	Soil zone	Soil moisture condition	Temperature (°C)
1985	Plant stress	Pod	Irrigated	24.9
		Root	Drought	28.9
	Pod stress	Pod	Drought	29.1
		Root	Irrigated	27.8
1986	Plant stress	Pod	Irrigated	27.6
		Root	Drought	29.8
	Pod stress	Pod	Drought	29.6
		Root	Irrigated	29.9

Table 3. Effect of plant and pod stress treatments on aflatoxin contamination of USA edible-grade categories of groundnuts, National Peanut Research Laboratory, Georgia, USA, 1985 and 1986.

Year	USA edible-grade category	Aflatoxin content ($\mu g\ kg^{-1}$)	
		Plant stress treatment	Pod stress treatment
1985	Jumbo	0	98
	Medium	0	522
	Number 1	0	1 780
	Other edibles	0	1 833
1986	Jumbo	0	83
	Medium	1	708
	Number 1	1 122	439
	Other edibles	1	691

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Environmental Conditions Required to Induce Preharvest Aflatoxin Contamination of Groundnuts: Summary of Six Years' Research

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Abstract

Environmental conditions necessary for preharvest aflatoxin contamination of visibly sound groundnuts are reviewed on the basis of studies conducted at Dawson, Georgia, USA during 6 consecutive crop years using six environmentally controlled plots. The role of temperature and moisture in preharvest aflatoxin contamination of groundnuts was established. Preventive measures, including the use of so-called 'resistant' varieties, calcium nutrition, and irrigation, were evaluated using environmentally controlled plots. The studies showed that groundnuts do not become contaminated with aflatoxins in the absence of severe and prolonged drought stress in spite of invasion levels of up to 80% by the aflatoxin-producing fungi, Aspergillus flavus *and* A. parasiticus. *Also, larger, more mature groundnut kernels require considerably more drought stress to become contaminated than do smaller, more immature kernels. Phytoalexin-based resistance can readily explain the resistance in immature kernels, but it does not explain the broader-based resistance observed in larger, more mature kernels. Studies during 1983 supported the hypothesis that preharvest contamination with aflatoxin orignates mainly from the soil and not from the air via floral invasion.*

Résumé

Les conditions d'environnement favorables à la contamination des arachides par les aflatoxines avant la récolte—récapitulation de six ans de recherches : *Les conditions requises pour la contamination par les aflatoxines avant la récolte des arachides apparemment indemnes sont rappelées. Ces observations sont fondées sur les études menées à Dawson (Georgia) aux Etats-Unis pendant six campagnes consécutives, sur six parcelles et sous conditions contrôlées. Le rôle de la température et de l'humidité dans la contamination par les aflatoxines avant la récolte a été établi. Les mesures préventives, notamment l'utilisation des variétés dites résistantes, l'apport de calcium et l'irrigation ont été également étudiées sur ces parcelles expérimentales. En l'absence d'une forte sécheresse prolongée, il n'y a pas de contamination malgré des taux d'infection allant jusqu'à 80% par les champignons aflatoxinogènes* Aspergillus flavus *et* A. parasiticus. *Il faut une sécheresse d'autant plus forte que la graine est plus grosse et plus mûre. Bien que la résistance fondée sur les phytoalexines explique clairement la résistance chez les graines immatures, elle*

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n'explique pas celle observée chez les graines plus grosses et plus mures. Les études effectuées en 1983 corroborent l'hypothèse que la contamination par les aflatoxines avant la récolte provient essentiellement du sol et non pas d'une invasion florale suite à la transmission du champignon par l'air.

Resúmena

Condiciones ambientales requeridas para inducir la contaminación del cacahuate con aflatoxinas antes de la cosecha: Resumen de siete años de investigación : *Las condiciones ambientales necesarias para la contaminación con aflatoxinas antes de la cosecha de granos de cacahuate aparentemente no dañados a la simple vista, se reexaminaron con base en los resultados obtenidos en los estudios realizados en Dawson, Georgia, Estados Unidos de América, durante siete ciclos agrícolas consecutivos, empleando seis parcelas con medios ambientes controlados. Los efectos de temperatura y humedad sobre la contaminación de los cacahuates con aflatoxinas durante el período de precosecha fueron establecidos. Se evaluaron diversas medidas preventivas, incluyendo el uso de variedades supuestamente resistentes, aplicaciones de calcio, y diversas prácticas de riego, en parcelas experimentales con condiciones ambientales controladas. Los resultados obtenidos demostraron que los cacahuates no se contaminan con aflatoxinas, si no hay condiciones severas y prolongadas de sequía en el cultivo, a pesar de la presencia de fuertes niveles de infección (hasta de 80%) de los hongos aflatoxígenos,* Aspergillus flavus *y* A. parasiticus. *Asimismo, se determinó que los granos de mayor desarrollo y madurez requieren de condiciones más severas de sequía para llegar a contaminarse, que los granos más pequeños e inmaduros. La resistancia con base en fitoalexina es fácil de entender en los granos inmaduros pero ne se explica la más amplia resistencia observada en granos más grandes, más maduros. Los estudios desarrollados durante 1983 corroboran la hipótesis que la contaminación con aflatoxinas durante el período de precosecha se origina principalmente por infecciones a través del suelo y no a partir de infecciones de los órganos florales, por transmisión de los hongos patógenos a través del aire.*

Introduction

The groundnut (*Arachis hypogaea* L.) is a unique plant in that after flowers are produced and fertilized above ground, the fruit develops underground. The fruit therefore comes in contact with soil microorganisms, and many species of soil fungi can invade groundnut pods and seeds. The nature and degree of invasion are dependent on the soil environment during growth and development of the groundnut fruit. The aflatoxin-producing fungi, *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare, can invade groundnut fruits and produce aflatoxin before harvest, during postharvest handling, drying, and in storage. Preharvest aflatoxin contamination has been associated with severe late season drought stress (Dickens et al. 1973, McDonald and Harkness 1967, Pettit et al. 1971, Wilson and Stansell. 1983). Preharvest aflatoxin contamination can be found in seeds from insect-damaged pods or from pods without obvious damage. The factors involved in aflatoxin contamination associated with insect damage are more obvious than those associated with the absence of visible damage.

This paper reviews results of 6 years of experimentation concerned with elucidating the environmental factors (soil moisture and temperature) responsible for preharvest aflatoxin contamination of visibly intact groundnuts, and with evaluating potential preventive measures.

Materials and Methods

Studies were conducted over a 6-year period (1980–1985) using six environmentally controlled plots, each provided with independent control and monitoring of soil temperature and soil moisture (Blankenship et al. 1983). Soil moisture and temperature data were collected every 2 h throughout the crop season, and subsequently analyzed using the Statistical Analysis System (SAS-79).

The groundnut variety primarily used in these studies was 'Florunner', grown extensively in the USA, and particularly in the southeastern peanut belt. The fruit of this variety develops from 3.5 to 5.0 cm beneath the soil surface and matures between 145 and 165 days after sowing (DAS). On the basis of soil analyses, soil fertility was adjusted annually before sowing. Fungicides, insecticides, and herbicides were applied as needed, according to Federal-State Extension recommendations.

Soil moisture tension was measured with Delmhorst gypsum blocks, and soil temperature was measured with copper constantan thermocouples.

The soil temperature in the pod zone was modified using two methods. Soil temperature was raised by thermostatically-controlled, lead-shielded heating cables arranged 10 cm apart at a depth of approximately 12.5 cm, and reduction was achieved by circulating cooled water through 6.35-mm copper tubing coated with chemical resistant paint, installed in a similar fashion.

Groundnuts were dug by hand, dried, combined, and shelled. They were categorized into commercial grades prior to analyses. The grade categories were loose-shelled kernels (LSK) (shelled during combining), jumbo, medium, number 1, other edible, and oil stock. Edible categories included jumbo, medium, number 1, and other edible. Damaged kernels were handpicked from all categories, combined and analyzed separately. Samples were analyzed for aflatoxin by the high performance liquid chromatography (HPLC) method of Dorner and Cole (1988).

Crop Season Treatment Regimes

1980

All plots were provided with adequate moisture until 94 DAS when four different treatment regimes were applied. Treatments were: irrigation provided throughout the growing season (2 plots), irrigation provided throughout but with elevated soil temperature in the pod zone (1 plot), no water applied after 94 DAS (2 plots), and no water applied after 94 DAS but with reduced soil temperature in the pod zone (1 plot). Groundnuts were dug 145 DAS.

1981

One plot was provided with adequate moisture throughout the growing season (146 DAS); the five remaining plots were provided with water until 85 DAS after which no additional irrigation was provided. The five drought treatments were distinguished by varied soil temperature in the pod zone. One plot was drought stressed without soil temperature modification, another was heated to provide a mean soil temperature of 30°C throughout the treatment period, the three remaining plots were cooled to provide 20°C, 22°C, and 24°C mean pod-zone temperatures during the treatment period.

1982

One plot was again provided with moisture throughout the growing season (138 DAS). The treatment strategy in this year was to induce drought stress in all five remaining plots 91 DAS, but to vary the overall mean pod-zone temperature to provide mean temperatures of 24°C, 26°C, 28°C, 30°C, and 32°C.

1983

One plot was provided with adequate moisture throughout the growing season (143 DAS). Drought stress was induced in four plots for 20, 30, 40, and 50 days before harvest. Optimum mean pod-zone temperature for aflatoxin development (approximately 29-30°C) was provided for each plot during the various treatment periods. Studies were also designed to investigate the relative significance of pathways of infection by *A. flavus* , either aerial flower and peg invasion or direct invasion from the soil. These studies used two color mutants, one each of *A. flavus* and *A. parasiticus* .

1984

Four experimental groundnut genotypes, which were deemed resistant to *A. flavus* invasion were evaluated and compared with the Florunner cultivar for resistance to *A. flavus* invasion and aflatoxin contamination. A study was also conducted to evaluate the role of soluble calcium in protecting groundnuts from preharvest aflatoxin contamination. Both tests evaluated effectiveness of resistance when subjected to environmental conditions ideal for aflatoxin contamination (29°C for approximately 45 days of drought stress).

1985

This year's study was designed to test the role of groundnut phytoalexins in resistance or susceptibility to preharvest aflatoxin contamination. The hypothesis was tested by monitoring kernel moisture, capacity for phytoalexin production, and aflatoxin contamination in groundnuts at different stages of maturity during a drought period with two different mean soil temperatures (25°C and 29°C). Three plots were drought treatments maintained over the treatment period at a mean pod-zone temperature of 25°C; two were drought treatments at a mean temperature of 29°C; while one control plot was provided adequate moisture throughout at an ambient pod-zone temperature. Plants from the 29°C treatment were initially sampled after 17 treatment days then sampled weekly for 6 weeks (59 treatment days). The 25°C treatment was initially sampled after 18 treatment days and then weekly for 9 weeks (80 treatment days). The irrigated control plot was sampled weekly throughout the test period.

Results and Discussion

Table 1 summarizes the experimental results of the 1980 crop season (Hill et al. 1983). Aflatoxin contamination of visibly intact kernels in the edible categories occurred only in the drought-stressed groundnuts without pod zone soil temperature reduction. Therefore, it was concluded that both dry soil and elevated soil temperatures in the pod zone were required to induce preharvest aflatoxin contamination of edible category groundnuts. Other observations were that damaged kernels were considerably higher in aflatoxin content than were visibly undamaged kernels and that the larger kernels, presumably more mature, were less contaminated than were the smaller, less mature kernels.

Table 1. Total aflatoxin contents of groundnuts from various commercial seed grades and mean pod zone soil temperatures of treatments, National Peanut Research Laboratory, Georgia, USA, crop year 1980.

	Treatment[1]/ temperature[2]			
	I 25.1°C	D 28.4°C	IH 34.1°C	DC 24.4°C
Seed grade	Total aflatoxin content ($\mu g\ kg^{-1}$)			
Edible				
Jumbo	0	29	0	0
Medium	0	127	0	0
Number 1	0	188	0	
Other edible	0	629	Trace	0
Other				
Oil stock	0	2 109	0	11
LSK[3]	0	2692	0	0
Damaged	365	22900	12	594

1. I = irrigated; D = drought; IH = irrigated heated; DC = drought cooled.
2. Mean soil temperature 2.5 cm under rows (94-145 DAS).
3. LSK = Loose-shelled kernels.
Source: Hill et al. 1983.

Crop Years

1981

The results from 1981 are summarized in Table 2 (Blankenship et al. 1984). In drought treatments significant aflatoxin contamination occurred in edible and nonedible categories in

Table 2. Total aflatoxin content of groundnuts from various commercial seed grades and mean pod zone soil temperatures of treatments, National Peanut Research Laboratory, Georgia, USA, crop year 1981.

	Treatment[1] temperature[2]					
	I 23.8°C	D 30.5°C	D 25.7°C	D 19.8°C	D 21.3°C	D 22.9°C
Seed grade	Total aflatoxin content ($\mu g\ kg^{-1}$)					
Edible						
Jumbo	0	315	0	0	0	0
Medium	0	816	72	0	0	0
Number 1	0	135	Trace	0	0	0
Other edible	0	620	4	0	0	0
Other						
Oil stock	0	3400	980	0	0	0
LSK	0	1350	1680	0	270	119
Damaged	0	26800	5000	200	815	4500

1. I = Irrigated D = Drought.
2. Mean pod zone soil temperature 2.5 cm under rows (85-146 DAS).
Source : Blankenship et al. 1984.

the treatment with elevated pod zone soil temperature (30.5°C), while from the cooler treatments, only the nonedible categories were contaminated (Table 2). No aflatoxin developed in visibly intact groundnuts grown with adequate moisture throughout the growing season.

1982

The treatment strategy for the 1982 crop year was to cover the critical range from 24°C to 32°C with 2.0°C increments. The resultant temperatures for the treatments were in excellent agreement with the treatment objectives (Table 3, Cole et al. 1985b). The mean pod-zone temperature range for aflatoxin development during the stress period was between 26.3 and 29.6°C with the latter being optimum. A mean pod-zone temperature of less than 26.3°C was insufficient to cause significant aflatoxin contamination during the stress period, while a mean temperature of 31.3°C was apparently too high for aflatoxin development. The optimum temperature for aflatoxin development was very close to the upper temperature limit for contamination. This skewed curve is similar to that obtained when *A. flavus* growth or aflatoxin production vs water activity and temperature are plotted (R.A. Hill, personal communication).

Table 3. Total aflatoxin content of groundnuts from various commercial seed grades and mean pod zone soil temperatures of treatments, National Peanut Research Laboratory, Georgia, USA, crop year 1982.

	Treatment[1]/temperature[2]					
	I 25.6°C	D 31.3°C	D 29.6°C	D 27.8°C	D 26.3°C	D 24.6°C
Seed grade	Total aflatoxin content ($\mu g\ kg^{-1}$)					
Edible						
Jumbo	0	0	275	0	0	0
Medium	0	0	10	2	0	0
Number 1	0	0	3100	250	83	0
Other edible	0	0	6660	0	330	20
Other						
Oil stock	0	0	440	220	360	0
LSK[3]	0	0	40	0	0	0
Damaged	0	0	650	(-)[4]	(-)	(-)

1. Treatments : I = Irrigated; D = Drought.
2. Mean pod zone soil temperature at a depth of 5 cm (90-138 DAS).
3. LSK = Loose-shelled kernels.
4. (-) = No damaged groundnuts available for analysis.

Source : Cole et al. 1984.

1983

Results from 1983 (Table 4) demonstrated that 20 days of drought and heat stress were insufficient for aflatoxin to develop in edible categories (Sanders et al. 1985, Cole et al. 1984). However, 30 days of stress was sufficient for contamination while 40 and 50 days of stress were increasingly more conducive to aflatoxin development. A comparison of the invasion of flowers, aerial pegs, and kernels by wild-type and mutant strains of *A. flavus* or *A. parasiticus*, along with aflatoxin analyses of kernels from different drought treatments, supported the hypothesis that

Table 4. Total aflatoxin concentration ($\mu g\ kg^{-1}$) of grade-size categories of groundnuts from five plot treatments, National Peanut Research Laboratory, Georgia, USA, crop year 1983.

	Treatment[1]				
Seed grade	I	D20	D30	D40	D50
Edible					
Jumbo	0	0	0	0	570
Medium	0	0	4	275	120
Number 1	0	0	155	260	430
Other edible	0	0	80	780	190
Other					
Oil stock	0	0	2600	1000	350
LSK[2]	0	0	590	146	1600
Damaged	0	600	2100	24000	14000

1. I = Irrigated. D20, D30, etc., = drought and elevated soil temperatures (~=29-30°C) for 20, 30, etc., days before harvest.
2. LSK = Loose-shelled kernels.
Source : Sanders et al. 1985.

preharvest contamination with aflatoxin originates mainly from the soil (Cole et al. 1986). The following evidence supports soil invasion as opposed to aerial invasion : (1) a greater percentage invasion of kernels rather than flowers or aerial pegs by either wild-type *A. flavus* or mutants; (2) significant invasion by an *A. parasiticus* color mutant occurred only in groundnut from soil supplemented with the mutant, whereas adjacent plants in close proximity but in nontreated soil were only invaded by wild-type *A. flavus* or *A. parasiticus* ; (3) aflatoxin data from drought-stressed, visibly intact groundnut kernels showed that samples from soil not supplemented with a mutant strain contained a preponderance of aflatoxin Bs (from wild-type *A. flavus*) whereas adjacent samples from mutant-supplemented soil contained a preponderance of B's plus G's (from wild-type and mutant *A. parasiticus*); and (4) preliminary data from two air samplings showed no propagules of *A. flavus* or *A. parasiticus* in the air around the experimental facility.

1984

Results from 1984 demonstrated conclusively that certain lines or cultivars deemed resistant to seed invasion and colonization by *A. flavus* were highly susceptible to preharvest contamination when challenged with environmental conditions conducive to aflatoxin contamination (Blankenship et al. 1985). These studies indicated the need for further research to develop an accurate screening method to identify the possible existence of genetic resistance to preharvest aflatoxin contamination in groundnut germplasm. The application of soluble calcium is not a feasible preventive measure against preharvest aflatoxin contamination of groundnuts (Table 5; Cole et al. 1985a).

1985

Results (Table 6) from these studies showed that as kernel water activity (Aw) decreased because of drought stress, kernels lost the capacity to produce phytoalexins at an Aw of 0.95 and lower and then, after a delay of approximately 1 week, became contaminated with aflatoxin (Dorner et al. in press). Immature groundnuts were more likely to become contaminated than mature groundnuts, and phytoalexin production was well correlated with the natural resistance of

Table 5. Effect of calcium (Ca) on total aflatoxin content of various seed grades, National Peanut Research Laboratory, Georgia, USA.

Seed grade	Aflatoxin content ($\mu g\ kg^{-1}$)		
	0 kg Ca ha^{-1}	250 kg Ca ha^{-1}	1000 kg Ca ha^{-1}
Jumbo	45[1]	336	319
Medium	457	262	579
Number 1	111	737	446
Splits	162	319	370
Other edible	1427	505	847
Oil stock	4804	3218	3093

1. Each value ($\mu g\ kg^{-1}$) is the average of at least 6 or as many as 12 analyses.
Source : Cole et al. 1987.

immature groundnuts to contamination. Mature groundnuts possessed resistance to contamination that could not be attributed solely to phytoalexin production. Kernel moisture loss was accelerated in the 29°C treatment compared to the 25°C treatment, and data indicated that the higher soil tempeature also favored growth and aflatoxin production by *A. flavus* in groundnuts susceptible to contamination, because of their reduced Aw and inability to produce phytoalexins.

Table 6. Phytoalexin production and aflatoxin contamination in five maturities of groundnuts from two drought treatments, National Peanut Research Laboratory, Georgia, USA.

Trt	Total phytoalexins (area counts)					Total aflatoxins ($\mu g\ kg^{-1}$)				
days	Yellow 1	Yellow 2	Orange	Brown	Black	Yellow 1	Yellow 2	Orange	Brown	Black
29° Treatment										
17	17 000	10 700	8 600	4800	9200	0	0	0	0	0
24	0	13 700	11 800	2900	3200	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	861	15	0	0	6
45	0	0	0	0	0	1190	624	0	12	0
51	0	0	0	0	0	2419	118	329	0	0
59	0	0	0	0	0	785	1915	33	291	0
25° Treatment										
18	11 000	4 600	5 000	7000	1000	0	0	0	0	0
25	11 500	11 300	4400	5300	6100	0	0	0	0	0
32	35 000	500	0	4700	6100	0	0	0	0	0
39	3200	200	0	0	0	12	0	0	0	0
46	0	0	0	0	0	784	62	0	0	0
52	0	0	0	0	0	0	447	0	0	0
60	0	0	0	0	0	0	484	0	0	0
66	0	0	0	0	0	5	8	13	0	0
73	0	0	0	0	0	1189	234	173	0	0
80	0	0	0	0	0	0	291	0	0	82

Source: Dorner et al. 1988.

Conclusion

Environmental control plots have been used to provide valuable information on preharvest aflatoxin contamination of groundnuts. The optimum soil stress conditions of moisture and temperature for aflatoxin contamination have been elucidated. The plots provide valuable and conclusive test facilities to evaluate potential resistance strategies for preharvest aflatoxin contamination, including those of so-called resistant varieties.

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Session VIII

Research on Aflatoxin Contamination of Groundnut: Genetic Resistance

Screening Groundnut Cultivars for Resistance to *Aspergillus flavus, Aspergillus parasiticus,* and Aflatoxin Contamination

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Abstract

Screening groundnut cultivars for aflatoxin resistance involves a consideration of the environmental conditions that favor activity by the Aspergillus flavus *group of fungi. The plant tissues penetrated, time of penetration, and the physical and biochemical factors that restrict invasion and aflatoxin formation also require consideration.* Aspergillus*-invaded cotyledonary leaves may be a primary source of inoculum.*

Developing shells of all cultivars examined were easily invaded; but penetration through the shell into the pod cavity varied with cultivar. Pods that formed lignified sclerenchyma bands early in their development were less susceptible to hyphal penetration than those without such bands. Kernel invasion is influenced by features of the hilum and seed coat. Small, covered hila, and compact seed coats with a thick wax deposition are important in relation to resistance. The content of the seed coats and pods varied among cultivars. Inhibitory compounds in the cotyledons slow fungal growth or inhibit aflatoxin formation. Tannin-like compounds (umbelliferone and methyl catechol) found in some groundnut seed coats were found to inhibit A. flavus *growth and aflatoxin formation. Electrophoretic separation under sodium dodecyl sulfate denaturation has revealed the presence of 20 polypeptides that vary among cultivars differing in susceptibility to* A. flavus. *Isolation of various plant constituents to detect the presence of specific proteins, tannin-like compounds, lignins, phytoalexins, and other compounds may correlate with levels of resistance and should be helpful in screening cultivars.*

Résumé

Evaluation des cultivars d'arachide pour leur résistance à *Aspergillus flavus, Aspergillus parasiticus* et à la contamination par les aflatoxines : *L'évaluation des cultivars d'arachide pour leur résistance aux aflatoxines doit considérer les conditions du milieu favorables au développement du groupe de champignons* Aspergillus flavus. *Les tissus végétaux infectés, l'époque de la pénétration, ainsi que les éléments physiques et biochimiques qui limitent l'invasion et la formation des aflatoxines doivent également être pris en compte. Les feuilles cotylédonaires infectées peuvent servir de source primaire d'inoculum. Les coques de tous les cultivars étudiés sont très*

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sensibles à l'infection pendant leur développement; cependant, la pénétration du champignon à travers la coque dans la gousse varie selon le cultivar. La formation précoce des bandes sclérenchymatiques lignifiées rend les gousses plus résistantes à la pénétration des hyphes. L'infection des graines est influencée par les couverts du hile et du tégument séminal. De petits hiles et un tégument compact avec une couche cireuse épaisse sont essentiels à la résistance. La composition du tégument et de la gousse varie selon les cultivars. Les composés inhibiteurs dans les cotylédons ralentissent la croissance du champignon ou limitent la formation des aflatoxines. Des substances de la famille des tanins (umbelliferone et methyl catechol) présentes parfois dans le tégument séminal permettent d'inhiber la croissance de A. flavus. *et la formation des aflatoxines. La séparation électrophorétique par la dénaturation du sulfate dodecyl de sodium a révélé la présence de 20 polypeptides qui varient en fonction de la sensibilité des cultivars à* A. flavus. *L'isolation des différents constituants de la plante afin de détecter la présence de protéines spécifiques, de composés de la famille des tanins, de lignines, de phytoalexines et d'autres composés permettra d'établir une corrélation avec le niveau de résistance, facilitant ainsi la sélection des cultivars.*

Resúmene

La selección de cultivares de cacahuate por su resistencia a *Aspergillus flavus*, *Aspergillus parasiticus* y a la contaminación con aflatoxinas : *La selección de cultivares de cacahuate por su resistencia a las aflatoxinas requiere de la debida consideración de las condiciones ambientales que favorecen el desarrollo del grupo de hongos* Aspergillus flavus. *También deben tomarse en cuenta los tejidos vegetales invadidos, la época en la cual ocurre la infección, y los factores físicos y bioquímicos que limitan la invasión del patógeno y la formación de aflatoxinas. Las hojas cotiledonarias infectadas por* Aspergillus *pueden constituir una fuente primaria de inóculo.*

Las cáscaras en desarrollo de todos los cultivares estudiados fueron fácilmente invadidas; pero la penetración a través de las cáscaras hacia el interior de las vainas variaba de cultivar a cultivar. Las vainas que formaban franjas de esclerénquima lignificadas en las etapas iniciales de su desarrollo fueron menos susceptibles a la penetración de las hifas que aquellas que carecían de dichas franjas. La intensidad de invasión de la semilla varía con las características del hilo y el tegumento seminal. La presencia de hilos pequeños y cubiertos y de tegumentos compactos, con gruesas deposiciones cerosas, son importantes en la resistencia de un cultivar dado. La composición de los tegumentos y de las vainas varía entre cultivares. Compuestos inhibitorios presentes en los cotiledones deprimen el desarrollo fungoso o inhiben la formación de aflatoxinas. Los compuestos de la familia de los taninos (umbeliferona y catecol metílico), que se identificaron en algunos de los tegumentos de semillas de cacahuate, se observó que inhiben el desarrollo de A. flavus *y la formación de aflatoxinas. La separación electroforética por desnaturalización con sulfato-dodecilsódico ha revelado la presencia de 20 polipéptidos en cantidades que varían entre los cultivares que difieren en sus susceptibilidad a* A. flavus. *La separación de varios constituyentes vegetales para detectar la presencia de proteínas específicas, compuestos de la familia de los taninos, las ligninas, las fitoalexinas y otros compuestos, quizá pueda correlacionarse con los niveles de resistencia y podría facilitar los trabajos de selección de cultivares.*

Introduction

Some groundnut genotypes have shells and seed testae which resist penetration by *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare, thus reducing the chance of subsequent aflatoxin production in seeds. In the late 1960s to early 1970s it was reported that the cultivars Asiriya Mwitunde, Kobaka (US 26), and TMV 2 exhibited some resistance to aflatoxin production (Kulkarni et al. 1967, Rao and Tulpule 1967, Nagarajan and Bhat 1973). Subsequent studies indicated that these cultivars lacked resistance. The results may have been because the isolates of aflatoxin-producing fungi varied in their ability to produce aflatoxins, or due to unfavorable conditions for a compatible host-pathogen interaction (Doupnik and Bell 1969).

Mixon and Rogers (1975) discovered that seed colonization by *A. flavus* in PI 337409 and PI 337394 F was lower than in the other germplasm accessions they tested. Bartz et al. (1978) noted considerable variability in *A. flavus* colonization of different groundnut genotypes and that susceptibility varied with harvest dates and curing methods. Other reports have described structural and biochemical features of seed testae which could account for the observed differences in susceptibility (Bartz et al. 1976, 1978, LaPrade et al. 1973, Sanders 1977, Taber et al. 1973, Pettit et al. 1977). Genotypes with resistant seed testae had compact palisade cellular arrangements, small hila, extensive surface wax, and a deposition of tannin-like compounds.

Mehan and McDonald (1984) reported five new sources of resistance, including cultivar J 11. This cultivar exhibited resistance to kernel colonization. However, the kernels supported aflatoxin production after invasion. Zambettakis et al. (1981) reported that the degree of natural *A. flavus* infection of groundnuts in Senegalese field plots was correlated with the rate of contamination of seeds artificially inoculated the laboratory. More recently, Davidson et al. (1983) reported that kernels of cultivar Sunbelt Runner that was reported to exhibit resistance in a laboratory test were highly susceptible to *A. flavus* under field conditions.

Florunner kernels were shown to have a moderate level of resistance to aflatoxin contamination (Amaya et al. 1980). Evidence indicates that kernels of Florunner produce phytoalexins, compounds inhibitory to aflatoxin formation, so long as they are not subjected to drought stress (Dorner et al. 1987). The development of phytoalexins in groundnut kernels has been reported by several researchers as one possible mechanism of resistance to fungal invasion (Turner et al. 1975, Ingham 1976, Keen and Ingham 1976, Wooton and Strange 1985, 1987).

Our studies were designed to determine; the extent to which pegs, shells, and kernels of different groundnut cultivars are invaded by *A. flavus* and *A. parasiticus*, and the structural and biochemical features of groundnut plant parts that could be used for screening purposes.

Materials and Methods

Field trials

Field trials were conducted in a South Texas field soil known to contain viable propagules of *A. flavus*. During the 1985/86 growing season, 20 groundnut cultivars (with some resistance to soilborne pathogens) were sown in a randomized complete block with 3–4 replications. Plant parts were sampled 3–4 times, either during the growing season, or at harvest, and after windrow or aritificial curing. In one trial the following plant parts were sampled: (1) aerial immature pegs, (2) pegs which had entered the soil, (3) immature pods (3 mm diameter), and (4) mature shells and kernels at harvest time. In another trial, the haulms of 20 cultivars were placed in windrows and inoculated with *A. flavus* and *A. parasiticus* conidia, and allowed to cure for 5 weeks. Plant

parts were surface sterilized by successive 1-min submersions in 70% ethanol, 10% NaOCl, and sterile distilled water. They were then plated onto Griffins medium (peptone, 5 g; NaCl, 60 g; agar, 20 g; Rose Bengal, 33 mg; H_2O 1 L; streptomycin, 50 mg; and chlorotetracycline, 50 mg). Following 6–10 days incubation the number of *Aspergillus* spp colonies was recorded.

Microplot Trials

Microplot trials were conducted in 30 boxes (90 cm × 150 cm × 60 cm deep) subdivided to provide 60 microplots (90 cm × 75 cm). The boxes were filled with sandy loam soil and covered with a greenhouse-type polyethylene cover. Conidia of *A. flavus* and *A. parasiticus* (1500 conidia g^{-1} soil) were mixed in the topsoil. Six groundnut cultivars were grown in a randomized complete block with four replications. Two treatments were imposed on these plots: (1) a 15-day drought stress imposed 100 days after sowing followed by irrigations as needed, and (2) no stress throughout the growth period. Pegs, shells, and kernels were sampled for fungal invasion, and seeds were analyzed for aflatoxin content by a high pressure liquid chromatographic (HPLC) technique (Pons and Franz 1978, Shepherd et al. 1982) using HPLC Model 510, (Waters Millipore Corporation) and absorbance detector Model 44.

Seed Colonization Tests

Initially seeds of 50 groundnut cultivars were remoistened in a humidity chamber at 90±2% relative humidity (RH) and 28±2°C to determine the ability of *A. parasiticus* to invade these seeds and produce aflatoxin. Following a determination of the degree of seed colonization, the seeds were frozen and replicate samples analyzed for aflatoxin using HPLC.

Tannin Extracts of Seed Testae

Testae were removed from kernels of 23 cultivars and the tannins extracted with methanol. Known weights of testae were ground in a Waring blender, added to 150 mL flasks with 50 mL of methanol, and allowed to stand at room temperature for 48 h. A portion of the extract was used to determine the tannin content using the Folin-Denis reagent (AOAC 1975). A standard curve for tannic acid was prepared, and a spectrophometer (760 nm) used to obtain comparative absorbance levels. The remaining methanol extract was dried under nitrogen at 40°C. The fungistatic effect of tannin-related dried extracts (100 mg L^{-1}) on fungal growth was tested after resuspension in distilled water, filter-sterilization, and addition to a liquid yeast extract sucrose medium (YES). Flasks containing 15mL of YES were seeded with a spore suspension of *A. parasiticus* and incubated for 7 days. Mycelial mat dry weights were determined, and the YES solution assayed for aflatoxin by HPLC.

Composition of Seed Testae

Testae of 12 groundnut cultivars were examined for the presence of phosphorus, sulfur, potassium, and calcium. Testae were mounted on aluminum stubs, evaporatively coated with carbon, and mounted in a JEOL 35 CF scanning electron microscope. X-ray analysis was

determined with a Tracor Northern TN 2000 energy dispersive X-ray spectometer system, operating at 15 KV. X-rays from the testae were collected and computer-analyzed according to their respective energies to provide an elemental signature of each test sample. Computer-generated curves showed the relative levels of the four elements.

Protein Composition of Kernels and Shells

Kernels and mature shells from the following groundnut cultivars were tested: Starr, PI 341885, PI 337409, Tamnut 74, Florunner, TX AG3, Toalson, TX 798736, 73-33, and 73-30, Pronto, PI 343419, PI 337394 F and 55-437. The testae and embryonic axes were removed and the cotyledons were ground in a pestle and mortar. The shells were finely ground in an electric grinder. Samples were extracted with a buffer containing 10mM Tris-HCl, 1 mM EDTA (pH 8.0), then filtered and centrifuged at 20 000 g for 30 min. After adding anionic detergent-SDS (2.5% w/v), and 2-mercaptoethanol (5.0% v/v, to reduce disulfide linkages) samples were heated for 5 min at 100° C, bromophenbol blue was added, and they were subjected to polyacrylamide discontinuous gel electrophoresis. The gel buffer in both zones contained 0.112 M acetate and 0.112 M Tris, pH 6.4. The electrophoresis buffer system consisted of 0.20 M Tricine, 0.20 M Tris, and 0.55 % SDS. The gel stacking zone consisted of an acrylamide monomer and the crosslinker, was 4.5% T, crosslinker 3% C, and the gradient zone was 8-25%. Isoelectric focusing of groundnut cotyledonary proteins included: (1) sample preparation of ground cotyledons in presence of buffer containing 0.1 M Tris (hydroxymethyl) aminomethane, 0.1 M KCl, 0.005 M EDTA, 0.04 M 2-mercaptoethanol, and 0.1 M sucrose; (2) filtration of the sample; and (3) centrifugation. Proteins were separated in a pH gradient gel matrix (43 × 50 × 0.35 mm with a 37 mm separation length) containing 5% and 3% C, along with isoelectric point protein markers. The gels covered a pH gradient from 3 to 9.

Pod Maturation Rate Trials

Pods of five groundnut cultivars were periodically harvested from field plots during the growing season to obtain pods from nine maturity classes. Pods were introduced into a humidity chamber (90±5%RH, 24-28° C) and inoculated with spores of *A. flavus*. Following incubation, pods of each maturity class (with and without *A. flavus* inoculation) were dehydrated, embedded in paraffin, sectioned, stained for fungal hyphae and structural features, and photographed in cross section.

Results and Discussion

Field Plot Trials

The isolation frequency of *A. flavus* from aerial pegs of field-grown groundnut cultivars was relatively high (13-32%) and for soilborne pegs lower (5-22%) (Table 1). The decreased recovery of *A. flavus* following soil penetration is attributed to an increase in the activity of other fungi which grew from these pegs. Examination of immature pods revealed a similar recovery rate (10-27%) of *A. flavus*. As the pods matured the incidence of *A. flavus* increased. All pods appeared to be equally susceptible, with a recovery rate of 20-72%. Some differences were noted

Table 1. Isolation frequency of *Aspergillus flavus* in groundnut plant parts collected from field-grown plants, 1986.

Cultivar	Isolation frequency of *Aspergillus flavus*[1]				
	Aerial pegs (%)	Soil pegs (%)	Immature shells (%)	Mature shells (%)	Mature kernels (%)
UF 73-4022 (610)	16	22	15	20	10
AH 7223	22	12	12	33	10
PI 337409	28	12	12	23	12
J 11	20	10	18	32	7
55-437	18	10	27	38	2
Toalson	32	10	10	72	15
Toalson × UF 73-4022 (736)	13	7	10	27	5
PI 365553 × Tamnut 74 (157)	22	5	10	70	0

1. Isolation frequency reported as a mean of three replication where 20 plant parts were tested for each replication.

in the degree of mature kernel infection. Kernels of 55–437 had 2% infection and those of selection 157 (from a cross of PI 365553 × Tamnut 74) were free of *A. flavus*.

The recovery rate of *A. flavus* from mature pods decreased following windrow curing and varied from 0–13% at 2 weeks to 3–8% at 5 weeks (Table 2). The reduced recovery rate appeared to be related to an increased recovery rate of other fungi. During windrow curing, environmental conditions did not favor *A. flavus* growth.

At digging, half of the groundnut haulms from each plot were bagged and forced-air dried. After drying the infection levels remained at a relatively high level (23–72%) (Table 2); these levels being similar to the recovery rates noted at lifting time (Table 1). The extent of kernel infection following forced-air drying remained fairly constant (0–15%). Kernels of 55–437 showed 2% infection whereas kernels of selection 157 (from a cross of PI 365553 × Tamnut 74) were free of *A. flavus*.

Kernels from windrow-cured samples had a low incidence (0–13%) of *A. flavus* infection (Table 3). Kernels from some cultivars, bagged at digging and then forced-air dried, had a higher

Table 2. Isolation frequency (%) of *Aspergillus flavus* from pods and groundnut kernels harvested from fields plots, 1986.

Cultivar	Windrow dried				Forced-air slow dried	
	2 weeks		5 weeks			
	Pods	Kernels	Pods	Kernels	Pods	Kernels
Toalson × UF 73-4022 (731)	13	2	3	22	30	5
AH 7223	0	10	7	15	33	10
UF 73-4022 (610)	8	0	7	12	20	10
PI 365553	0	0	5	10	70	0
55-437	3	0	3	7	38	2
PI 337409	5	2	10	3	23	12
J 11	8	3	3	0	32	7
Toalson	8	2	8	0	72	15

Table 3. Isolation frequency of *Aspergillus flavus* detected in groundnut kernels harvested from field-grown plants, 1986.

Cultivar	Isolation frequency (%)		
	Forced-air dried	Windrow dried	
		2 weeks	4 weeks
Starr	28	0	1
Toalson × UF 73-4022 (956) F_8	21	0	3
G-A 7045 F_{13}	19	5	0
Tamnut 74 × PI 337409 (922) F_8	18	0	0
Tamnut 74 × PI 337409 (921) F_8	5	3	1
Tamnut 74	5	3	1
Toalson	4	3	3
Toalson × UF 73-4022 (736)	3	1	1
G-A 7404 F_{13}	1	0	1
G-A 72115 F_{14}	1	0	13
PI 337409	1	2	0
Toalson × PI 365553 × Tamnut 74	0	1	0
Toalson × UF 73-4022 (472)	0	0	1
T × 771108	0	1	0
Tamnut	0	0	1

1. Isolation frequency reported as a mean of four replications where 20 groundnut kernels were tested in each replication.

incidence (0–28%) of *A. flavus* infection. *Aspergillus flavus* recovery from kernels of several Texas breeding lines was equal to, or lower than, those from the cultivar PI 337409.

Microplot Trials

The recovery of *A. flavus* from shells harvested directly from the microplot soil at lifting ranged from 22 to 91%, a relatively high recovery rate that was higher in 1986 (Table 4). These data suggest that under high inoculum levels, invasion of shells is quite extensive and the short drought-stress treatment imposed failed to significantly increase *A. flavus* incidence.

The degree to which kernel infection was influenced by the imposed drought stress was more significant. Kernels of cultivars 55-437, PI 337409, and J 11 from the stress treatment were more frequently infected. Levels of aflatoxin detected were relatively low (0–22 $\mu g\ kg^{-1}$, indicating that physiological conditions did not favor aflatoxin formation. The interrupted 15-day stress period encouraged *A. flavus* invasion of the kernels. However, it was not favorable for continued *A. flavus* growth and aflatoxin formation.

Seed Colonization

Distinct differences were observed in the degree of *A. parasiticus* infection of re-moistened seed of different groundnut cultivars. Seed of PI 337409 were the most resistant (8% of the seed were colonized by 7 days). Kernels from a cross of Tamnut-74/PI 337409 (84B3820) were the most

Table 4. Influence of soil moisture regimes on the incidence of *Aspergillus flavus*, *Aspergillus parasiticus*, and aflatoxin contamination of groundnut kernels harvested from covered microplots, 1985 and 1986.

Cultivar	Moisture regime	Isolation frequency (%) 1985 Shells	1985 Kernels	1986 Shells	1986 Kernels	Aflatoxin content ($\mu g\ kg^{-1}$) 1985	1986
Starr	Stress[1]	37	17	77	11	11	0
	Optimum	36	23	91	6	0	8
Toalson	Stress	30	13	73	7	7	4
	Optimum	38	13	91	11	0	13
55-437	Stress	42	34	87	15	0	16
	Optimum	28	6	70	10	0	16
PI 337409	Stress	34	18	79	14	22	4
	Optimum	22	6	78	4	0	6
J 11	Stress	-	-	69	13	-	8
	Optimum	-	-	81	3	-	4

1. Fifteen days of drought stress initiated 100 days after sowing followed by optimum irrigations.

Table 5. Seed colonization by *Aspergillus parasiticus* and aflatoxin production in different groundnut cultivars.

Cultivar	Seed colonized (%)	Aflatoxin production ($\mu g\ kg^{-1}$)
Tamnut 74×337409 (84B 3820)	87[1]	153.2[2]
US 330	81	336.1
Tamnut 74×337409 (84B 4366)	77	252.8
Toalson	62	433.6
TMV 2	56	75.1
US 822-1	51	57.5
Florunner × Tamnut 74	48	147.2
US 216	41	124.3
Manfredi 107	39	123.8
Florunner × PI 337394	38	151.2
TAG 3	35	53.7
J 11	35	54.4
Starr	34	83.1
55-437	25	60.3
Florunner	11	32.7
PI 337409 (86B 3209)	8	9.1

1. Kernels incubated for 7 days at 28±2°C and 90±2% RH.
2. Mean of three replications.

severely colonized (87%) (Table 5). 55-437 and Florunner had 25% or less *A. flavus* seed colonization.

Aflatoxin levels in kernels ranged from 9.1 $\mu g\ kg^{-1}$ for kernels of PI 337409 to 433.6 $\mu g\ kg^{-1}$ for kernels of Toalson. Kernels of J 11 had 35% infestation and an aflatoxin content of 54.4 $\mu g\ kg^{-1}$. Kernels of Florunner also contained a relatively low level of aflatoxin (32.7 $\mu g\ kg^{-1}$).

Tannin Extracts of Testae

The influence of tannin extracts from kernel testae on the growth of and aflatoxin production by *A. parasiticus* in a liquid culture is summarized in Table 6. Mean dry weight of mycelial mats from the control flasks was 0.33 g. The lowest mat weights were from flasks containing tannins from testae of 55-437, US 216, and selections from three crosses (Florunner × Tamnut 74, Florunner × PI 337409, and Tamnut 74 × PI 337409 (84B 4366).

The influence of tannin extracts on aflatoxin production was more pronounced. Extracts from testae of 10 groundnut cultivars significantly reduced aflatoxin levels. Extracts from testae

Table 6. Influence of tannin extracts from testae of different groundnut genotypes on growth of *Aspergillus parasiticus* and aflatoxin production.

Genotype	Mycelial dry mass (g)[1]	Aflatoxin production[2] ($\mu g\ kg^{-1}$)
PI 337409 (86B 3209)	0.29	83.1
US 330	0.28	107.9
US 822-1	0.28	115.2
TX AG3 (PI 365553 Seln.)	0.26	78.5
Florunner × Tamnut 74	0.22	80.9
Florunner × 337394F	0.25	110.8
TMV 2	0.30	121.0
US 216	0.22	91.4
Manfredi 701	0.29	85.7
Tamnut 74×337409 (84B 3870)	0.29	118.9
Starr	0.31	90.3
Florunner	0.27	74.9
55-437	0.24	95.4
Tamnut 74×337409 (84B 4366)	0.23	116.9
J 11	0.30	75.0
A72118	0.28	107.3
Toalson	0.30	81.2
Control		
Water	0.33	116.3
LSD (a=0.005)	0.05	15.2

1. Growth for 7 days at 28°C.
2. Mean of 3 replications.

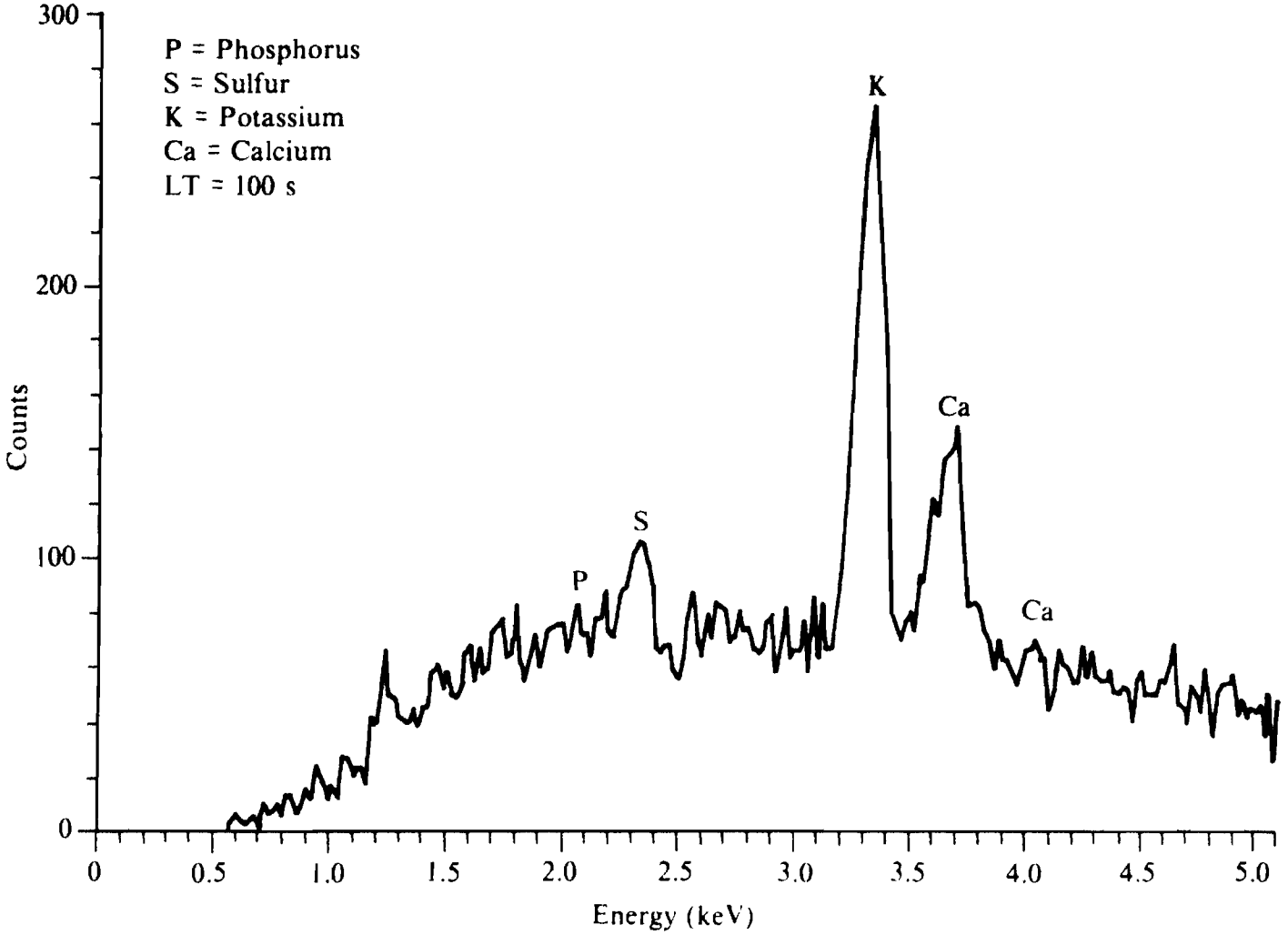

Figure 1. Elemental signature of testa of the groundnut cultivar PI 365553 detected by energy dispersive X-ray analysis.

of Florunner caused a reduction in aflatoxin production. In addition, extracts from testae of the following cultivars caused significant reductions in aflatoxin production: PI 337409, PI 365553 (TX-AG3), Starr, 55-437, J 11, Toalson, Manfredi 701 (cv 129), and a selection from the cross Florunner × Tamnut 74.

Elemental Composition of Seed Testae

The elemental composition of testae, as detected by energy dispersive X-ray analysis in terms of four major elements present (those above sodium in the periodic table) included phosphorus, calcium, sulfur, and potassium. Differences among cultivars were found in the elemental compositions of the testae. For example, testae of PI 365553 contained relatively high levels of phosphorus, sulfur, and potassium, and a low level of calcium (Fig. 1). In contrast, testae of PI 337409 (Fig. 2) contained intermediate levels of phosphorus, sulfur, and potassium, and a higher level of calcium. The increased resistance of testae of PI 337409 to *A. flavus* invasion may be partly attributed to its increased calcium content.

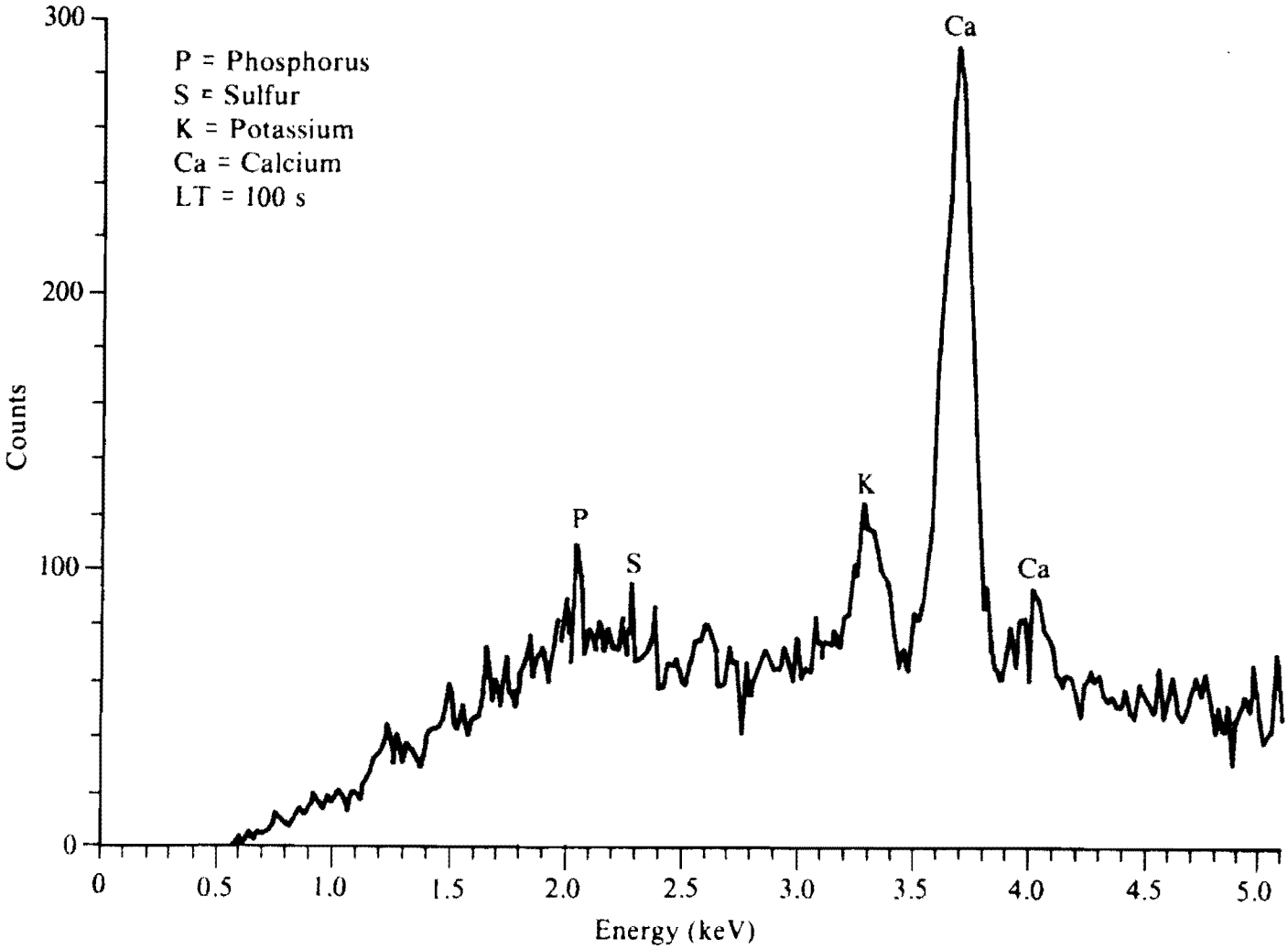

Figure 2. Elemental signature of testa of the groundnut cultivar PI 337409 detected by energy dispersive X-ray analysis.

Protein Composition of Kernels

Electrophoresis in presence of SDS. Thirty-four proteins were separated from groundnut cotyledons. The majority of the proteins had molecular weights (MW) between 12 100 and 94 000. Using silver staining, it was possible to identify very low quantities of proteins which had MWs between 102 300 and 218 700. Electrophoretic patterns of proteins and variations among cultivars are shown in Table 7. Florunner cotyledons lacked a significant protein.

Mature shells contained fewer proteins compared to cotyledonary proteins. Sixteen major proteins were identified with MWs between 82 000 to 190 000. Shells of PI 337409 and TX 798736 showed different protein patterns from those of other shells.

Isoelectric focusing (IEF). Isoelectric focusing revealed 40 distinct protein bands which had isoelectric points (pI) between pH 3.5 and 7.9. Cotyledonary proteins of Florunner and Tamnut showed different pI patterns compared to other groundnut cultivars. The quantity of proteins showing the same pI varied among cultivars.

Table 7. Electrophoretic analysis of groundnut cotyledon proteins in 13 cultivars.

Protein molecular weight	Cultivar[1] minus protein band	Quantity[2] of protein	Protein molecular weight	Cultivar minus certain protein band	Quantity of protein	Protein molecular weight	Cultivar minus certain protein band	Quantity of protein
12 100	-	high	33 100	-	high	76 700	-	moderate
15 200	-	high	34 700	-	high	81 200	-	moderate
17 000	-	high	37 100	Florunner	very high	82 200	-	moderate
18 000	-	high	40 700	-	very high	90 100	-	high
19 300	-	high	43 600	-	very high	94 000	-	high
21 900	-	high	51 500	-	moderate	102 300	-	very low
24 000	-	very high	56 200	-	moderate	109 600	-	very low
26 900	-	moderate	60 200	-	moderate	116 100	-	very low
28 800	-	moderate	65 300	-	very high	120 200	-	very low
30 000	-	moderate	69 200	Starr, PI 341885, TX-AG 3, Toalson	low	142 900	73-30	very low
30 900	-	high	71 600	Starr, PI 341885 TX-AG 2, Toalson	low	215 200	PI 337409	very low
						218 700	PI 337409	very low

1. Cultivars investigated: Starr, PI 341885, PI 337409, Tamnut, Florunner, TX-AG 1, Toalson, 55-437, TX 798736, 73-33, 73-30, Pronto, and PI 343419.
2. Quantity estimated according to intensity scale: very high, high, moderate, low, very low.

Pod Maturation Rate Trials

The rate and degree of lignification in the sclerenchyma tissues differed among cultivars and maturity classes. Sclerenchyma bands developed earlier and thickened more extensively in Toalson and PI 365553 compared to Starr and Florunner.

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Resistance of Groundnut Varieties to *Aspergillus flavus* in Senegal

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Abstract

In four rainy seasons (1977-1980) some 40 groundnut genotypes were screened for field resistance to seed invasion by the aflatoxigenic fungus, Aspergillus flavus *in trials at Bambey and Darou research stations in Senegal. Significant varietal differences were observed at harvest in respect of levels of naturally occurring seed infestation by* A. flavus. *Field resistances were positively correlated with previously measured resistance to in vitro seed colonization by* A. flavus *in laboratory inoculation tests.*

The commercially grown variety 55-437 had high levels of resistance to A. flavus *in both field and laboratory screening, while two other varieties (73-30 and 73-33) also grown in Senegal had moderate levels of resistance.*

In associated investigations it was found that genotypes with seed resistance to A. flavus *had a lower proportion of* A. flavus *in their rhizosphere mycoflorae than had genotypes susceptible to seed invasion by this fungus. Varieties, through their effects on rhizosphere mycoflorae may influence the composition of the soil mycoflora of groundnut fields.*

Résumé

Recherche sur la résistance des variétés d'arachide à *Aspergillus flavus* : *Une quarantaine de génotypes ont été évalués au cours de quatre campagnes pluviales (1977-1980) pour la sélection de matériel résistant à l'invasion des graines par le champignon aflatoxinogène* Aspergillus flavus *dans les champs. Ces essais ont été entrepris sur les stations de recherche de Bambey et de Darou au Sénégal. Des différences variétales significatives ont été observées dans le taux de contamination naturelle des variétés à la récolte. Il y a une corrélation positive entre la résistance dans les champs et la résistance mesurée antérieurement à la colonisation in vitro par* A. flavus *au cours des tests d'inoculation, réalisés au laboratoire.*

La variété commerciale 55-437 s'est montrée très résistante à la fois dans les champs et au laboratoire. Deux autres variétés (73-30 et 73-33) également cultivées au Sénégal présentent une résistance moyenne.

*D'autre part, le pourcentage d'*A. flavus *dans la mycoflore de la rhizosphère est inférieur pour les génotypes résistant à l'invasion des graines par* A. flavus *par rapport aux génotypes sensibles.*

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Compte tenu de l'interaction entre les variétés et la mycoflore de leur rhizosphère, celles-ci peuvent également influencer la composition de la mycoflore du sol des champs d'arachide.

Resúmene

La resistencia de variedades de cacahuate a *Aspergillus flavus* en Senegal : *Durante cuatro temporadas de lluvias (1977-1980), aproximadamente 40 genotipos de cacahuate fueron evaluados y seleccionados por su resistencia, bajo condiciones de campo, a la invasión de sus semillas por el hongo aflatoxigénico* Aspergillus flavus, *en pruebas realizadas en las estaciones experimentales de Bambey y Darou, en Senegal. Se observaron diferencias significativas entre las variedades estudiadas en el momento de la cosecha, en los niveles de infección con* A. flavus que ocurren en la semilla bajo condiciones de campo. Las resistencias de campo observadas resultaron estar positivamente correlacionadas con las resistencias a la colonización de las semillas por A. flavus, *observadas previamente bajo condiciones* in vitro *en pruebas de inoculación realizadas en el laboratorio.*

La variedad comercial 55-437 tuvo altos niveles de resistencia a A. flavus, *tanto en las pruebas de campo como de laboratorio, mientras que otras dos variedades (73-30 y 73-33), que también se siembran en Senegal, solamente tuvieron niveles medianos de resistencia.*

En investigaciones complementarias, se ha encontrado que los genotipos que poseen resistencia en sus semillas a A. flavus *tenían una menor cantidad de* A. flavus *en la microflora de sus rhizosferas que los genotipos susceptibles a la invasión de sus semillas por el hongo citado. Las diferentes variedades, a través de los efectos sobre la micoflora de sus rhizosferas, pueden influir en la composición de la micoflora de los campos cacahuateros.*

Introduction

Groundnuts are very important to the economy of Senegal, both for local consumption and for export to obtain foreign exchange. The significance of the aflatoxin problem in groundnuts in relation to public health and to the future of the export trade has been recognized in Senegal. High priority was given to research on the problem and this was undertaken in a collaborative program involving the Institut senegalais de recherches agricoles (ISRA), the Institut de recherches pour les huiles et oléagineux (IRHO), and the Cryptogamy Laboratory of the Museé national d'histoire naturelle (MNHN) in Paris. This paper describes some of the research carried out during the years 1977–79 on varietal resistance in groundnut to seed infection by the aflatoxin-producing *Aspergillus flavus* Link ex Fries together with preliminary studies in 1981/82 of the rhizosphere mycofloras of two genotypes, one resistant and one susceptible to seed infection by *A. flavus*.

Materials and Methods

Varietal Trials

During the rainy seasons of 1977, 1978, and 1979, two field trials with 36 groundnut genotypes were carried out at two locations (Bambey and Darou) in Senegal. At each location, one trial

was sown at the normal time and the other was sown one month later. Genotypes ranged in duration from 90 to 130 days, and were harvested when judged to have attained optimum maturity.

Bambey is in the north central region of Senegal where the rainy season is short (104±34 days) whereas Darou is in southern Senegal and has a rather longer rainy season (115±18 days). Soils at Bambey are light and sandy, and at Darou are typical Alfisols.

Trials were laid out as randomized blocks with two replications of treatments (genotypes). Five seeds were sown in each hole at 40-cm spacing along 50-cm wide ridges. Plots were composed of six ridges, each 6m-long.

After lifting, plants were dried in windrows in the field until seed moisture contents were below 10%. Samples of undamaged, mature pods were collected from each plot for testing for natural seed infection by *Aspergillus flavus*, and for screening for resistance in rehydrated seed to colonization by *A. flavus*.

To determine natural infection of seeds by *A. flavus*, 300 undamaged mature pods were handshelled and the seeds surface sterilized by immersion for 3 min in a 0.2% aqueous solution of sodium hypochlorite. Following three rinses in sterile distilled water the seeds were transferred aseptically onto moistened filter paper in sterilized petri dishes. The dishes were incubated at room temperature (25 ± 1°C) for 7 days and fungi growing from the seeds were identified and recorded.

The method of Mixon and Rogers (1973) was followed to determine resistance to seed colonization by *A. flavus* in laboratory inoculation tests. Seeds sterilized as described above were hydrated to 20% water content and surface inoculated with a suspension of spores of *A. flavus*. The inoculated seeds were then incubated in petri dishes at 25 ± 1°C for 7 days and the numbers of seed colonized by *A. flavus* were then recorded.

Rhizosphere Studies

Two groundnut genotypes, 55-437 which had been found resistant to seed infection by *A. flavus*, and 75-16 (PI 343419) which had been found susceptible to *A. flavus*, were grown in the 1981 and 1982 rainy seasons at Darou. On the day of sowing, the soil mycoflora of the field was examined, and the rhizosphere mycoflora was examined 15 days later and then at 15-day intervals until harvest. Three samples were taken at each examination from each of three replicate plots. The soil dilution plate technique was employed using acidified potato dextrose agar medium. Colonies of various genera of fungi were subcultured onto specific media for identification to species (Rouxel 1978, Alabouvette 1983). Mycofloras were described both quantitatively and qualitatively.

Results

Field and Laboratory Screening

Full information on all varieties screened for natural seed infection by *A. flavus* and for reaction to seed colonization has been presented in a series of publications (Waliyar 1978, Zambettakis et al. 1977, 1981). In this paper we review data from work in 1977, 1978 and 1979 on 36 genotypes that were common to the trials.

Natural seed infection with *A. flavus* was greater in the late sowings than in the earlier sowings in all but the 1977 trials in Bambey where infection was much higher in the seeds from the earlier sowing (Table 1). This result from Bambey was probably due to the drought that occurred in the late stages of pod maturation in the early-sown crop. Mean levels of *A. flavus* infection were highest in 1978 which was a drought year in Senegal. This again is a strong indication as to the influence of drought stress on seed infection. Of the 36 genotypes scored, 5 showed significantly lower than average levels of *A. flavus* seed infection, and the mean infection levels for these genotypes over years are compared with those of the susceptible control cultivar PI 343419. Three of the resistant genotypes, 55-437, 73-30, and 73-33, are released commercial cultivars in Senegal, 55-437 being the most widely grown.

The mean levels of *A. flavus* infection for combined genotypes were generally higher in Bambey than in Darou, and this was also the case for the resistant genotypes (Tables 1 and 2).

The genotypes with low levels of natural seed infection by *A. flavus* had lower than average levels of seed colonization by this fungus in laboratory inoculation tests (Waliyar 1978). Dates of sowing influenced seed colonization by *A. flavus* in inoculation trials for all genotypes, levels being higher from seed produced by the late-sown crops. This is thought to be occasioned by higher seed testa damage occurring in the late-sown material, which results from greater drought stress in that material and possibly quicker drying of plants later in the year.

In addition to varietal differences, other factors also play a role in the contamination of seed by *A. flavus*. Among these factors soil type (Mehan et al. 1986) influences the groundnut

Table 1. Field contamination by *Aspergillus flavus* of groundnut seeds from two sowings, Bambey and Darou, Senegal, 1977-79.

	Contaminated seed (%)			
	Bambey		Darou	
Year	1st sowing	2nd sowing	1st sowing	2nd sowing
1977	23.5	9.1	9.9	12.2
1978	39.4	42.5	19.1	30.3
1979	9.2	20.3	3.2	18.1

Table 2. Field contamination of various groundnut genotypes by *Aspergillus flavus*, Bambey and Darou, Senegal, 1977-79.

	Contaminated seed (%)			
	Bambey		Darou	
Genotypes	1st sowing	2nd sowing	1st sowing	2nd sowing
PI 337409	1.23	1.70	0.14	1.40
PI 337394 F	1.43	2.57	0.37	2.30
55-437	0.84	0.74	0.30	0.90
73-30	0.54	0.87	0.43	2.30
73-33	0.57	3.10	0.22	0.56
PI 343419[1]	4.13	5.09	3.81	9.49

1. Susceptible control genotype.

infection. Other factors such as damage caused by different insects, termites, and millipedes facilitate seed infection by *A. flavus*.

The underground development of groundnut pods makes them particularly vulnerable to attacks by different fungi likely to facilitate *A. flavus* contamination. Soil mycoflora increases in the presence of groundnut and this may influence the next crop's reaction to diseases.

Rhizosphere Studies

Comprehensive reports on studies on groundnut rhizospheres have been published by Waliyar (1986a, 1986b). From 45 days after sowing (DAS) until harvest, numbers of fungal propagules per gram of dry soil were considerably higher in the rhizosphere of the *A. flavus*-susceptible cultivar 75-16 than in the rhizosphere of the *A. flavus*-resistant cultivar 55-437. Numbers of species of fungi present in the rhizosphere were also greater in the cultivar 75-16 from 45 DAS. These changes in numbers and composition of the components of the mycofloras may be related to the onset of pod development.

Numbers of propagules of *A. flavus* g^{-1} of dry rhizosphere soil were fairly similar for the two cultivars at the first three times of sampling but in the samples taken from 45 DAS until harvest the numbers were higher for the susceptible cultivar 75-16 (Table 3).

Table 3. Numbers of propagules (× 10^3) of *Aspergillus flavus* g^{-1} dry rhizosphere soil, Senegal, 1981.

	Time of sampling (DAS)[1]						
Variety	0	15	30	45	60	75	90
55-437	8	3	7	4	12	24	58
75-16	4	2	6	14	82	42	96

1. DAS = Days after sowing.

Conclusions

The variety 55-437, which is a commercial variety of excellent agronomic character, well suited to conditions in Senegal, is as resistant to natural seed infection by the aflatoxigenic *A. flavus* as the two resistant genotypes PI 337409 and PI 337394F. The varieties 73-30 and 73-33, also commercial varieties in Senegal, have tolerance to seed invasion by *A. flavus* and are also drought tolerant. The three varieties now represent around 80% of the groundnut crop area in Senegal, and their use on this scale should have a significant effect in reducing the overall aflatoxin contamination levels in Senegal's groundnut crop.

The positive correlation found between resistance to natural infection in the field and resistance in laboratory inoculation tests indicates that either method could be used in evaluating groundnut cultivars and breeding lines for seed resistance to infection by *A. flavus*.

There are indications that varieties may have differential influences upon the buildup and constitution of soil mycoflora including *A. flavus*. Effects may be manifest on the infection of pods and seeds of the growing crop or on those of subsequent crops.

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Occurrence of Aflatoxins and Aflatoxin-producing Strains of *Aspergillus flavus* in Groundnut Cultivars in Egypt

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Abstract

The use of groundnut cultivars resistant to seed invasion and colonization by Aspergillus flavus *is a possible means of preventing or reducing contamination by aflatoxin. Twenty-one groundnut cultivars obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, and one cultivar (Giza 4) from Egypt included as a local susceptible control, were tested for their ability to support aflatoxin production. All the cultivars supported production of aflatoxins B_1 and B_2, although the amounts produced differed between cultivars. The lowest level of total aflatoxin production being 19 180 $\mu g\ kg^{-1}$ seed in cultivar Ah 7223, and the highest 44 290 $\mu g\ kg^{-1}$ seed in cultivar Giza 4.*

Résumé

Présence d'aflatoxines et de souches aflatoxinogènes d'*Aspergillus flavus* dans des cultivars d'arachide en Egypte : *L'utilisation de cultivars d'arachide résistants à l'invasion et à la colonisation des semences par* Aspergillus flavus *est un moyen pour empêcher ou réduire la contamination par les aflatoxines. Vingt-et-un cultivars obtenus de l'ICRISAT en Inde et un cultivar (Giza 4) provenant d'Egypte, qui servait de témoin, ont été testés pour leur capacité à être contaminés par les aflatoxines. Tous les cultivars peuvent être contaminés par les aflatoxines B_1 et B_2. Les quantités produites varient cependant selon les cultivars. Le taux le plus bas de la production totale des aflatoxines a été de 19 180 $\mu g\ kg^{-1}$ de graines (cultivar Ah 7223) et le taux le plus élevé de 44 290 $\mu g\ kg^{-1}$ (cultivar Giza 4).*

Resúmene

La presencia de aflatoxinas y cepas aflatoxinógenas de *Aspergillus flavus* en cultivares de cacahuate en Egipto : *El uso de cultivares de cacahuate que son resistentes a la invasión y colonización de la semilla por* Aspergillus flavus, *es un posible medio de evitar o reducir la contaminación con aflatoxinas. Veintiún cultivares de cacahuate obtenidos del Instituto Interna-*

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cional de Investigación sobre Cultivos en los Trópicos Semiáridos (ICRISAT), Patancheru, Andhra Pradesh, India, y un cultivar (Giza 4) de Egipto, que se incluyó como control susceptible de origen local, se probaron para evaluar su capacidad de generar aflatoxinas. Todos los cultivares generaron las aflatoxinas B_1 y B_2, aunque las cantidades generadas variaron entre los diversos cultivares. El nivel más bajo de aflatoxinas totales producidas fue de 19180 μg kg^{-1} en semilla del cultivar Ah 7223; y el más alto, 44290 μg kg^{-1} de semilla, en el cultivo Giza 4.

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important leguminous crops in Egypt and in many other parts of the world. Groundnuts are used for human consumption as confectionery nuts, edible oil, and processed as peanut butter, and for animal feed as oilseed cake.

In Egypt, the crop is cultivated in the governorates of Assuit, El-Behera, El-Minia, El-Sharkia, Giza, Sohage, and Ismailia.

The Ministry of Agriculture estimated that the total cultivated area producing groundnut in Egypt was 12 147 ha in 1986 with an average yield of 2.15 t ha^{-1}. Approximately 40% of the national production is exported, and the rest is used for local consumption.

Much attention has been focused on contamination of groundnuts with aflatoxins produced either by *Aspergillus flavus* Link ex Fries or *A. parasiticus* Speare. This is also a serious problem in many other parts of the world.

Garren and Christensen (1969) reported that many aflatoxin-producing isolates of *Aspergillus* spp can contaminate the groundnut fruit with aflatoxin, and most strains of *A. flavus* and *A. parasiticus* can produce aflatoxin on groundnuts.

Mixon and Rogers (1973) reported that two groundnut genotypes (PI 337394 F and PI 337409) were resistant to seed colonization by *A. flavus* in laboratory inoculation tests. Mehan and McDonald (1983) suggested that the use of groundnut cultivars resistant to seed invasion and colonization by *A. flavus* could be a possible means of preventing or reducing contamination by aflatoxins. They found no direct relationship between resistance to seed colonization by *A. flavus* and the quantity of aflatoxin produced when seeds were colonized by toxigenic strains of the fungus.

This paper reports seed inoculation tests carried out on a range of groundnut genotypes to determine seed resistance to invasion and colonization by a toxigenic strain of *A. flavus*, and the abilities of these genotypes to support aflatoxin production.

Materials and Methods

Source of Seeds

Seeds were obtained from a combined nursery of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the United States Department of Agriculture (USDA). Seeds of 11 cultivars came from ICRISAT, Patancheru, Andhra Pradesh, India and Giza 4 was used as a local susceptible control. Seeds of 10 other genotypes (A 7404, A 72118, A 7405,

A 7309, A 7223, A 7717, PI 337458, A 7715, A 7716, and Florunner) were provided by Dr A.C. Mixon of the USDA, Georgia Coastal Plain Station, Georgia, USA.

The experiment was conducted in Ismailia Province, one of the two main groundnut-producing regions of Egypt.

A randomized complete block design with two replications was used. Each plot comprised two 6-m rows and seeds were sown singly at 10-cm spacing giving a total of 120 seeds plot $^{-1}$ as suggested in the ICRISAT International Groundnut *A. flavus* Nursery (IGAFN) guide. Seeds of all cultivars were treated with a Vitavax/Captan® fungicide (2 g kg^{-1} seed) as seed protectant 2 days before sowing. Recommended agronomic practices were followed, i.e., irrigation, fertilizer application, and application of insecticides and/or fungicides to the foliage as required to control pests and diseases.

Cultivars were harvested at optimum maturity, and the plants were arranged in windrows in the field with pods exposed to the sun to dry. After windrow drying for 3 days, the dried pods were handpicked and stored in cloth bags in a well-ventilated room, with protection from insects and rodents until required for samples.

Determination of Seed Resistance to Invasion and Colonization by *A. flavus*

From the stored intact seeds of each plot 20-g samples of seeds were taken, placed in a clean beaker, and covered with a 0.5% aqueous solution of sodium hypochlorite. Seeds were soaked for 2 min, the excess solution drained off, and the seeds rinsed in two changes of sterile distilled water. The water was then drained off and the seeds hydrated to about 20% moisture content by soaking them for 10-15 min in sterile distilled water. Seeds were aseptically placed in a sterile 9-cm diameter petri dish, and 1 mL of a spore suspension of the toxigenic *A. flavus* strain IMI 187005 (approximately 4×10^6 conidia mL^{-1}) was applied. The spore suspension was prepared from 8-10 day-old cultures; the seeds were gently rolled around the dish to spread the inoculum evenly over their surfaces. The petri dishes were arranged over water in semi-rigid plastic boxes provided with closely fitting lids, and the lids were sealed with adhesive tape. The boxes were then placed in an incubator at 25°C. After 8 days, the seeds were visually examined for invasion and colonization by *A. flavus* by recording:

a. the number of seeds per plate;
b. the number of seeds with sporulating growth of *A. flavus* on their surfaces; and
c. the degree of sporulating growth of *A. flavus* classified as sparse, moderate, or dense.

The percentage of seeds invaded and colonized as shown by the presence of sporulating surface growth was calculated.

Testing Efficiency of Seeds as Substrates for Aflatoxin Production

The method used to determine seed resistance to invasion and colonization by *A. flavus* (IMI 187005) was also used to determine the efficiency of seeds of the different genotypes to support aflatoxin production, but the boxes were incubated at 25°C for 10 instead of 8 days.

Aflatoxin contents of the groundnuts were determined using the Best Food (BF) method described by the Association of Official Analytical Chemists (AOAC 1980).

Results and Discussion

Resistance to Seed Colonization by *A. flavus*

The mean percentages of seed colonized by *A. flavus* ranged from 31% in cultivar A 7405 to 96% in Giza 4 (Table 1). All cultivars differed in their resistance to seed colonization. The cultivars A 7405, NC Ac 841, PI 337458, A 7223, UF 71513, and Ah 7223 were most resistant to invasion and colonization of seeds by *A. flavus*, while Giza 4, Faizpur, A7404, Monir 240-30, and J 11, in descending order, were most susceptible.

Sporulation and growth of *A. flavus* was dense on the seeds of Giza 4, Faizpur, A 7404, Var 27, and J 11, but was sparse on Monir 240-30, PI 337409, A 7309, Ah 7223, and UF 71513.

Aflatoxin Contents of Seeds Inoculated with a Toxigenic Strain of *A. flavus*

Seeds of all cultivars supported production of aflatoxins B_1 and B_2, although the amounts produced differed (Table 2). The lowest level of total aflatoxin production was 19 180 $\mu g\ kg^{-1}$ in Ah 7223 and the highest level was 44 290 $\mu g\ kg^{-1}$ in Giza 4.

Table 1. Groundnut seed colonization by *Aspergillus flavus* in laboratory inoculation tests, Ismailia Governate, Egypt, 1983.[1]

Cultivars	Seeds colonized (%)	Growth/sporulation of *A. flavus* on colonized seed		
		Sparse	Moderate	Dense
UF 71513	40.0	11.0	9.0	-
PI 337394 F	55.0	-	11.5	16.0
PI 337409	78.0	18.0	7.5	13.5
J II	83.0	4.0	14.0	23.5
Ah 7223	46.0	12.5	7.5	3.0
Var 27	77.0	7.5	7.0	24.0
Faizpur	88.0	6.0	6.0	32.0
TMV 2	72.0	5.0	7.0	24.0
Monir 240-30	83.0	21.5	10.0	10.0
Giza 4	96.0	7.0	3.5	37.5
55-437	44.0	2.5	8.0	11.5
NC Ac 841	35.0	3.0	3.0	11.5
A 7404	85.0	9.0	6.5	27.0
A 72118	68.0	10.5	4.0	20.5
A 7405	31.0	13.5	-	7.0
A 7309	67.0	16.5	5.0	12.0
A 7223	40.0	2.5	7.5	10.0
A 7717	70.0	8.5	7.5	19.0
PI 337458	35.0	5.0	6.0	6.5
A 7715	67.0	5.5	20.5	7.5
A 7716	53.0	9.0	8.0	9.5
Florunner	65.0	7.5	12.5	12.5

1. Seeds (20-g samples) were taken from the crop grown in the Ismailia Governate, and were stored for a month prior to testing.

Table 2. Occurrence of aflatoxins in seeds of different groundnut cultivars inoculated with the toxigenic *Aspergillus flavus* strain IMI 187005, Ismailia Governate, Egypt, 1983.[1]

Cultivars	Original/source	Aflatoxin content ($\mu g\ kg^{-1}$)		
		B_1	B_2	Total
UF 71513	USA	20 680	11 190	31 870
PI 337394 F	Argentina	19 340	10 460	29 800
PI 337409	Argentina	23 730	12 840	36 570
J 11	India	34 100	9 720	43 820
Ah 7223	Nigeria	13 570	5 610	19 180
Var 27	Australia	17 960	9 700	27 660
Faizpur	India	22 850	8 220	31 070
TMV 2	India	21 960	7 920	29 880
Monir 240-30	India	20 040	7 230	27 270
Giza 4	Egypt	35 440	8 850	44 290
55-437	Uganda	16 040	8 680	24 720
NC Ac 841	Uganda	20 410	5 670	26 080
A 7404	USA	24 250	6 750	31 000
A 72118	USA	19 820	7 320	27 140
A 7405	USA	19 330	7 140	26 470
A 7309	USA	32 020	8 910	40 930
A 7223	USA	21 170	5 880	27 050
A 7717	USA	21 550	7 980	29 530
PI 337458	Argentina	17 960	9 360	27 350
A 7715	USA	18 360	6 810	25 170
A 7716	USA	25 380	7 050	32 430
Florunner	India	19 120	7 080	26 200

1. Seeds (20-g samples) were taken from the crop grown in the Ismailia Governate.

These results agreed with those obtained by Mehan and McDonald (1983) who reported that there was no direct relationship between resistance to seed colonization by *A. flavus* and the quantity of aflatoxins produced when seeds were colonized by toxigenic strains of *A. flavus*.

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The ICRISAT Approach to Research on the Groundnut Aflatoxin Problem

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Abstract

Research in a number of countries in the 1960s and early 1970s provided an excellent understanding of the effects of cultural practices, produce handling, and storage conditions on aflatoxin contamination in groundnuts and groundnut products. But the recommendations for management of the problem evolved from the early research, while readily adopted by progressive farmers in countries with advanced agriculture, were not being taken up by the majority of small-scale groundnut farmers in developing countries. This influenced the decision of groundnut scientists at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to concentrate on identification and utilization of genetic resistance to seed invasion by Aspergillus flavus *and to production of aflatoxins.*

Résumé

Approche de l'ICRISAT pour les recherches sur le problème des aflatoxines chez l'arachide : *Les recherches menées dans un grand nombre de pays dans les années 60 et au début des années 70 a permis une excellente compréhension des effets des pratiques culturales, du conditionnement de la production et des conditions de stockage sur la contamination des arachides et de leurs produits par les aflatoxines. Cependant, les recommandations proposées pour maîtriser ce problème ont été appliquées rapidement par les cultivateurs à forte technicité dans les pays à agriculture avancée, sans être adoptées par la majorité des petits paysans dans les pays en voie de développement. Les chercheurs travaillant sur l'arachide à l'ICRISAT ont donc décidé de concentrer leurs efforts sur l'identification et l'utilisation de la résistance à l'invasion des graines par* Aspergillus flavus *et à la production des aflatoxines.*

Resúmene

El método del ICRISAT en sus investigaciones sobre el problema de las aflatoxinas en el cacahuate : *Las investigaciones desarrolladas en un gran número de países durante los años 60 y principios de los años 70, han proporcionado un excelente conocimiento de los efectos de las prácticas de manejo del cultivo, el manejo de las cosechas y las condiciones de almacenamiento sobre la contaminación con aflatoxinas del cacahuate y sus derivados. Sin embargo, las recomen-*

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daciones propuestas para manejar el problema derivadas de las investigaciones iniciales, aun cuando fueron rápidamente adoptadas por los agricultores progresistas en países con una agricultura avanzada, no lo fueron por la mayoría de los pequeños productores de cacahuate en los países en desarrollo. Lo anterior influyó en la decisión de los investigadores de cacahuate del Instituto Internacional de Investigación sobre Cultivos en los Trópicos Semiáridos (ICRISAT), de concentrar sus esfuerzos sobre la identificación y utilización de materiales con resistencia genética a la invasión de las semillas de cacahuate por Aspergillus flavus *y a la producción de aflatoxinas.*

Introduction

Following recognition of the groundnut aflatoxin problem in 1960, research institutions in a number of groundnut-producing countries gave high priority to determining the stage or stages in crop production at which groundnuts were invaded by the aflatoxin-producing fungus *Aspergillus flavus* Link ex Fries and became contaminated with aflatoxins.

By the mid-1970s this objective had been largely achieved. It was evident that, depending upon environmental and cultural factors, groundnut seed could be invaded by *A. flavus*, and subsequently become contaminated with aflatoxin, before harvest, during postharvest curing and drying, or during storage. In some regions/countries the problem was predominantly postharvest while in others it was largely preharvest. Considerable data were assembled on the influence of environmental factors, crop-production, and produce-handling procedures on seed invasion by *A. flavus*. This was used to formulate recommended practices to be used by growers and those concerned with purchase, storage, and processing of groundnuts and groundnut products. There was evidence that adherence to the recommended practices could minimize risk of aflatoxin contamination of groundnuts. Some of the more commonly recommended practices were:

- to adjust sowing dates so that crops mature towards the end of the rainy season;
- to avoid damage to plants and pods from cultivations late in the crop's development;
- to control soil pests such as termites and pod borers that scarify and/or penetrate shells;
- to control diseases that cause wilting and death of plants;
- to provide uniform irrigation in the event of drought in the late stages of pod maturation;
- to lift the crop as soon as the majority of the pods have matured, and to glean any detached pods as soon as possible;
- to discard moldy and damaged pods, and pods from plants that had died before harvest;
- to dry pods to below 8% water content within 5–10 days of lifting;
- to avoid damage to pods when threshing and handling in transit to store;
- to store pods in cool, clean, dry, pest-free conditions;
- to shell as efficiently as possible to minimize damage to testae (seed coats);
- to sort out and destroy moldy seeds;
- to divert aflatoxin-contaminated seed lots to nonfood uses; and
- to reduce aflatoxin levels in seed lots by removal of moldy and discolored seeds.

It is obvious that recommendations have to be tailored to fit particular situations. Some may be useful in countries with highly developed agriculture and agricultural industries, but may not be suited to small-scale farming situations in developing countries where the industry may not be equipped for effective monitoring, segregation, or detoxification. Also, some procedures such as hand-sorting of produce can readily be done by small farmers with hand labor, but are not possible in a highly mechanized system.

Research Objectives

When groundnut research started at ICRISAT in 1976, the groundnut aflatoxin situation was reviewed and it was concluded that while adoption of recommended cultural and produce handling procedures had been reasonably effective in reducing levels of aflatoxin in groundnut products reaching the consumer in developed countries, there was little evidence of small-holder farmers in tropical developing countries adopting the improved practices. This situation was taken into consideration when planning the approach to the aflatoxin problem to be followed in the ICRISAT research program. It was suggested that effective solutions to the problem would be to grow groundnut cultivars that were immune to infection by the aflatoxigenic *A. flavus*, or cultivars that, if colonized by the fungus, did not support production of aflatoxins. No such cultivars were available. Researchers in the USA had shown in laboratory inoculation tests that rehydrated, mature, sound seed of certain genotypes had marked resistance to infection and colonization by *A. flavus*. This resistance was dependent upon the seed coat (testa) being entire and undamaged. The practical limitations of this type of resistance were realized but it did present a potential for breeding cultivars with higher than average resistance to seed invasion by *A. flavus*. There was very little evidence, and that conflicting, for existence of resistance to aflatoxin contamination based on cultivars being inefficient substrates for aflatoxin production. Nonetheless, it was felt that this also could be a useful avenue to explore. It was decided that resistance breeding should form the main line of ICRISAT's research to control aflatoxin contamination of groundnut, and this was considered to be particularly appropriate in view of ICRISAT's mandate to maintain the world collection of groundnut and wild *Arachis* spp germplasm.

Accordingly, plans were formulated in 1977 for research on aflatoxin contamination of groundnut with the following major objectives:

- to select qualitative (rapid) and quantitative (highly accurate) methods for estimation of aflatoxins in groundnuts;
- to develop laboratory techniques to screen groundnuts for resistance to seed invasion by *A. flavus* and to aflatoxin production;
- to screen germplasm and breeding lines in laboratory inoculation tests for resistance to *A. flavus* invasion of rehydrated, stored seeds;
- to test lines found resistant to seed invasion by *A. flavus* in laboratory tests for resistance to natural seed infection by the fungus in the field;
- to breed high-yielding cultivars with seed resistance to infection by *A. flavus*; and
- to study the inheritance of this resistance.

Following recruitment of staff in 1978, laboratory facilities were developed and research was started.

In 1980 the project was reviewed and the following objectives were added:

- to develop methods for testing pods at various stages in development for resistance to invasion by toxigenic fungi, and to study the mechanisms of resistance;
- to record all mycotoxin-producing fungi occurring in groundnuts and to test their abilities to produce toxins in groundnuts; and
- to study the effects of foliar diseases, pod rots, and damage to pods by soil fauna on invasion of shells and seeds by toxigenic fungi and on production of mycotoxins.

Following a further review in 1985, the objectives of the research project on aflatoxin contamination of groundnut were summarized in their current form as follows:

- to elucidate factors influencing pod and seed invasion by *A. flavus*, especially preharvest invasion, and aflatoxin production;

- to identify further sources of resistance to seed invasion and aflatoxin production; and
- to breed for aflatoxin resistance.

The objectives of the project have shown minor changes over the past 10 years, but the approach to the problem has remained firmly focussed upon identification of genetic resistance and its utilization in breeding cultivars with resistance in seed to invasion by *A. flavus* and/or resistance to production of aflatoxin.

Progress

Progress at ICRISAT in the identification of genotypes with resistance in seed to invasion by *A. flavus* and to aflatoxin production, and in the use of these genotypes in a resistance breeding program is provided in detail in other papers (see Mehan, pages 323–334, Vasudeva Rao, et. al. pages 345–355 as is progress on investigation of possible mechanisms of resistance (Jambunathan et al., 357–364, Nahdi, pages 365–378). In general, there have been significant advances along most of the lines of research pursued. This has been considerably aided by the facility at ICRISAT Center of being able to grow two crops in the year, a rainy-season crop (Jun–Oct) and a postrainy- season irrigated crop (Nov–Apr). Access to two very different soils (Alfisols and Vertisols) on the ICRISAT Center farm gave additional environments for research. Cooperation between pathologists and physiologists in developing field resistance screening methods using imposed drought stress has been particularly rewarding, providing a greater insight into the factors involved in interactions between drought stress and seed infection by *A. flavus* , as well as evolving effective germplasm-screening facilities.

Limited investigations were made on the possible occurrence in groundnut seed of mycotoxins other than aflatoxin. Particular attention was paid to known mycotoxins produced by fungi commonly found in groundnut pod and seed mycoflorae. Only citrinin and zearalenone could be identified, and these only at very low incidence. It was therefore decided to concentrate upon aflatoxin contamination.

Many different methods for aflatoxin analysis have been tested, and standard methods have been adopted for rapid detection and for accurate quantification as required for specific screening purposes. The paper given earlier in this workshop by Goto and Manabe (pages 177–186) indicates the continued interest in new methods of analysis. The immunochemical method (Chu, pages 163–175) shows excellent promise for development of a simple, rapid, and relatively inexpensive aflatoxin analysis system to facilitate extensive screening of germplasm for resistance in seed to aflatoxin production.

Although emphasis has been on genetic resistance, the broader aspects of the problem have not been forgotten. Information obtained on interactions between environmental factors, biotic and abiotic, and seed infection by *A. flavus* and aflatoxin contamination will be used in developing aflatoxin management programs which, hopefully, will soon include use of resistant cultivars.

Prospects, and Future Research

There appear to be excellent prospects of breeding groundnut cultivars with useful levels of resistance to seed invasion by *A. flavus* and/or poor capacity to support aflatoxin production. When such cultivars are available, they should be tested in as many different environments as possible and under different levels of farm management. It will still be necessary to use already

developed cultural and produce-handling procedures to reduce risk of aflatoxin contamination. Ideally the use of resistant cultivars should form part of an integrated management system. Obviously, resistant cultivars will have to be bred to meet particular producer and user requirements (vegetable oil, confectionery, multi-purpose), and seed will have to be multiplied and made available to farmers, a considerable problem in some countries that lack well-developed seed multiplication systems. Aflatoxin-free produce should attract premium prices from processors, and should provide high quality seed for sowing. It should be remembered that any cultivar bred for *A. flavus* resistance is likely to also have pod/seed resistance to a large number of other soil fungi. This could confer considerable benefits in terms of reduced free fatty acid content, better viability and germinability, and reduced incidence of seed and seedling diseases. It is noteworthy that several of the cultivars that have testa resistance and preharvest resistance to seed invasion by *A. flavus* have good levels of resistance to a *Fusarium*-dominated pod rot that occurs in Alfisols on ICRISAT Center farm.

Research at ICRISAT should continue to focus upon breeding cultivars with resistance to seed invasion by *A. flavus* and to aflatoxin production. Research will be intensified into elucidation of resistance mechanisms and determining their inheritance. Studies will continue on the effects of environmental factors upon *A. flavus* invasion of seeds and aflatoxin production and, if possible, the effects of cropping systems will be examined. As resistant cultivars become available they will be examined for stability of resistance in different environments and under a range of inputs, and compared with existing cultivars in on-farm situations. This should lead to development of basic principles for setting up integrated aflatoxin-management programs suited to particular situations.

Rapid progress in research on aflatoxin contamination of groundnut will depend upon effective cooperation between scientists in all the institutions involved with the problem. Exchange of information can be improved and this has influenced the setting up at ICRISAT of a data base on aflatoxin in groundnut that can be made available to all interested parties. The present workshop is another vehicle for communication and it is hoped that it will result in increased cooperative research in such fields as analytical methods and their validation, assessment of stability of resistance, and exchange of germplasm.

Discussion

K.K. Shresta. Regarding awareness, in our country, aflatoxin is still not given importance. So, it would help if some international organizations like FAO could take steps to create awareness.
D. McDonald. We did invite FAO to send representatives to the meeting but they were too busy. We could certainly pass on the request to them.
R.V. Bhat. FAO has in the recently concluded meeting at Bangkok already made a strong recommendation on the need to create political awareness regarding aflatoxins. The idea of an intersectoral mycotoxin research group is excellent, but past experience in India has proved that there is a need for financial backup for such an organization.
D. Mc.Donald. Agreed that FAO and other organizations are doing much to awaken interest at the govenment level, but more needs to be done to alert all concerned from growers to consumers. National programs can be very useful. One was set up in Nigeria in 1961 and served a very useful purpose in bringing together all concerned. Basic funding is essential.

Screening Groundnuts for Resistance to Seed Invasion by *Aspergillus flavus* and to Aflatoxin Production

V.K. Mehan[1]

Abstract

Research in several countries into evaluation of responses of groundnuts to seed colonization and infection by Aspergillus flavus *and/or aflatoxin production is reviewed, and progress made in this field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is summarized. Several laboratory and field screening procedures have been developed to screen groundnuts for resistance to* A. flavus *infection and/or aflatoxin production. Research on the effects of environmental factors on pod and seed invasion by* A. flavus *has produced information useful in the development of field screening methods. For instance, imposed drought stress has been used to improve large-scale field screening of groundnut genotypes for resistance to preharvest infection of seeds by* A. flavus. *Several genotypes were found resistant to infection, and some of them were also resistant to in vitro seed colonization by* A. flavus *in laboratory inoculation tests. Two genotypes supported only very low levels of aflatoxin B_1 production when seeds were colonized by an aflatoxin-producing strain of* A. flavus.

Résumé

Sélection des arachides résistant à l'invasion par *Aspergillus flavus* et à la production des aflatoxines : *Les recherches menées dans plusieurs pays portant sur l'évaluation des réponses des arachides à la colonisation et à l'invasion par* Aspergillus flavus *et/ou à la production des aflatoxines, sont récapitulées ainsi que les acquis de l'ICRISAT dans ce domaine. Plusieurs méthodes de sélection au laboratoire et au champ ont été mises au point pour étudier cette résistance. Les études sur les effets des facteurs d'environnement sur l'invasion des gousses et des graines ont fourni des informations utiles au développement des méthodes de sélection au champ. Par exemple, la création d'une sécheresse artificielle a permis d'améliorer la sélection au champ à grande échelle de la résistance à l'infection avant la récolte. Plusieurs génotypes se sont montrés résistants, dont certains sont également résistants à la colonisation in vitro par* A. flavus, *dans les tests d'inoculation au laboratoire. Deux génotypes ont présenté de très bas niveaux d'aflatoxine B_1, lorsque les graines ont été colonisées par une souche d'* A. flavus *productrice d'aflatoxine.*

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Resúmene

La selección de cacahuate resistente a la invasión de sus semillas por *Aspergillus flavus* y a la producción de aflatoxinas: *En este trabajo se examinan las investigaciones realizadas en varios países sobre la evaluación de las respuestas del cacahuate a la colonización e infección de sus semillas por* Aspergillus flavus *y/o la producción de aflatoxinas y se resumen los avances logrados en este rubro por el Instituto Internacional de Investigación sobre Cultivos en los Trópicos Semiáridos (ICRISAT). Varios procedimientos de selección para uso en el laboratorio y en el campo se han desarrollado para la selección de cacahuate resistente a la infección de* Aspergillus flavus *y/o la producción de aflatoxinas. Los estudios realizados sobre los efectos de los factores ambientales en la invasión de las vainas y las semillas por* A. flavus *han producido información útil para el desarrollo de métodos de selección en el campo. Por ejemplo, condiciones de sequía creadas artificialmente han sido utilizadas para mejorar la selección en el campo, a gran escala, de genotipos de cacahuate con resistencia en sus semillas a la infección por* A. flavus, *en el período de precosecha.*

Se identificaron varios genotipos resistentes a la infección mencionada, y algunos de estos resultaron ser también resistentes a la colonización in vitro *por* A. flavus *en sus semillas, en pruebas de inoculación efectuadas dentro del laboratorio. Dos de los genotipos presentaron muy bajos niveles de producción de la aflatoxina B_1 cuando sus semillas fueron colonizados por un cepa aflatoxinógena de* Aspergillus flavus.

Introduction

Aflatoxin contamination of groundnut (*Arachis hypogaea* L.) is a serious problem in most groundnut-producing countries. Invasion of groundnut seed by the aflatoxin-producing fungi *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare, and subsequent contamination with aflatoxins, may occur pre- or postharvest. Preharvest aflatoxin contamination is important in the semi-arid tropics (SAT), particularly under drought conditions, while postharvest contamination is significant under wet and humid conditions (Dickens 1977, Mehan 1987). Aflatoxin contamination can be minimized by adopting some cultural, produce-handling and storage practices (Dickens 1977). These practices have been readily adopted by progressive farmers and those concerned with storage and processing of the produce in developed countries, but unfortunately have not been widely adopted by small farmers in developing countries. The use of cultivars resistant to seed invasion by aflatoxin-producing fungi, or resistant to aflatoxin production (Mixon and Rogers 1973, Mehan and McDonald 1984) would be of value to farmers in both developed and developing countries. This has focused research on identification and utilization of genetic resistance to seed invasion by *A. flavus* and/or aflatoxin production. This paper summarizes progress worldwide in selecting groundnuts resistant to "seed colonization" and "seed infection" by *A. flavus*/*A. parasiticus* and to aflatoxin production, and describes research in this field at ICRISAT. *Aspergillus flavus* is used in this paper in a collective sense for both *A. flavus* and *A. parasiticus*.

Resistance to Seed Invasion and Colonization by *Aspergillus flavus*

Mixon and Rogers (1973) first suggested that use of groundnut cultivars resistant to seed invasion and colonization by *A. flavus* could be an effective means of preventing aflatoxin

contamination. The existence of seed resistance was a logical assumption, considering that seeds with damaged testae are more easily and rapidly invaded by the fungus than are seeds with intact testae, and colored testae conferred greater resistance to invasion by *A. flavus* than white or variegated testae (Carter 1970,1973).

Mixon and Rogers (1973) developed a laboratory inoculation method for screening groundnut genotypes for resistance to *A. flavus* invasion and colonization of rehydrated, mature, sound, stored seeds. They selected two valencia-type genotypes, PI 337394F and PI 337409, that showed a high level of resistance to in vitro seed colonization by *A. flavus*. Six more breeding lines (GFA 1, GFA 2, AR 1, AR 2, AR 3, and AR 4) were later reported resistant (Mixon 1986). The mean seed colonization levels in the resistant genotypes tested over several years ranged from 8–13% (Mixon 1986).

Several other researchers have used Mixon and Rogers' method, or modifications of it, to screen groundnuts for resistance to seed colonization by aflatoxin-producing strains of *A. flavus* (LaPrade et al. 1973, Bartz et al. 1978, Zambettakis et al. 1981, Mehan and McDonald 1980, Tsai and Yeh 1985, Pua and Medalla 1986).

At ICRISAT we have used a modification of the method to screen 850 germplasm accessions for their reaction to seed invasion and colonization by *A. flavus*. The tests were carried out on sound, mature seeds from intact pods that were dried and stored for about 1 month. Seeds (20-g samples) were surface sterilized with a 0.1% aqueous solution of mercuric chloride, rinsed in sterile deionized water, hydrated to 20% moisture content, and surface inoculated with a conidial suspension of an aflatoxigenic strain of *A. flavus* (AF 8-3-2A), and then incubated at 25°C for 8 days under 98 ± 2% relative humidity. The percentages of seeds of different genotypes with sporulating colonies of *A. flavus* ranged from 6 to 100%. Genotypes with 15% or fewer seeds colonized were regarded as resistant (Mehan and McDonald 1980). Resistance of the three genotypes, PI 337394F, PI 337409, and UF 71513, was confirmed, and six new sources of resistance (Ah 7223, J 11, U 4-47-7, Var. 27, Faizpur, and Monir 240-30) were identified. In various tests on seed from rainy-season groundnut crops produced on the ICRISAT Center farm from 1980 to 1986 these genotypes consistently had low percentages of seed colonized (8–14%). Resistance in three of them (PI 337394F, PI 337409, and J 11) has also been confirmed by other workers (Wynne 1983, Zambettakis et al. 1981, Kisyombe et al. 1985). A comprehensive list of genotypes reported from different countries to have resistance to seed colonization by *A. flavus* is given in Table 1.

It was observed that absolute percentage incidence of seeds colonized by *A. flavus* varied considerably for specific genotypes within trials in the same season, and between seasons. Effects of environment (climate, location, soil type) and postharvest drying procedures on in vitro seed colonization were examined. In various genotypes tested, seeds from the postrainy-season irrigated crops had significantly higher colonization than seeds from rainy-season crops (Mehan et al. 1983). This may be due to fluctuation in soil moisture during pod development and very rapid drying under the hot, dry conditions during harvest of the postrainy-season crop. Several workers (Dickens and Pattee 1973, Glueck et al. 1977, Woodward 1973) have reported that rapid drying weakens the seed testa, and testa damage decreases resistance to fungal penetration.

In all reported cases of rehydrated, cured, sound, mature seed resisting invasion and colonization by *A. flavus* the protective role of the seed testa has been emphasized (Dieckert and Dieckert 1977, Mixon and Rogers 1975, Mehan et al. 1983), the resistance depending upon the seed testa being intact. The resistance to seed colonization may be of value if groundnuts dried in the field or in storage are wetted, or absorb moisture from the atmosphere. The resistance may be of less value for decorticated seed that may have suffered damage to the testa in processing. It is significant that in spite of considerable differences in seed colonization levels caused by variation

Table 1. Groundnut genotypes reported resistant to seed invasion and colonization by *Aspergillus flavus* in laboratory inoculation tests.

Genotypes	Origin	Reference(s)
PI 337394F, PI 337409	Argentina Argentina	Mixon and Rogers (1973), Mehan et al. (1981), Zambettakis et al. (1981)
UF 71513	USA	Bartz et al. (1978), Mehan et al. (1981)
J 11	India	Mehan et al. (1981), Wynne (1983), Kisyombe et al. (1985)
Ah 7223, Var. 27, Faizpur, Monir 240-30	Nigeria Cuba India ?	Mehan and McDonald (1984!
55-437, 73-30	Senegal Senegal	Zambettakis et al. (1981)
U4-47-7	Uganda	Mehan et al. (1986 b)
GFA 1, GFA 2, AR 1, AR 2, AR 3, AR 4	USA USA USA USA USA USA	Mixon (1986)
Basse, C116(R), M395 C184, F-7, GE 652, Ah 6487 Maria-B, Roxo (Sal.), NC 449, NC 482 PI 196621, PI 196626 RMP 12, Sp. 218, Sp. 424	Gambia India ? ? ? USA ? Burkina Faso ?	Tsai and Yeh (1985)
ACC 63 CES 48-30, Celebes UPL PN 4	? ? Indonesia Philippines	Pua and Medalla (1986)

in environmental and crop handling methods the resistance in certain genotypes holds good (Mixon 1981, 1986).

In the last 15 years there has been much research into genetic resistance to *A. flavus* colonization of rehydrated, mature, sound, stored seed. This has possibly been stimulated by the aflatoxin problem being regarded largely as a postharvest phenomenon. This is no longer valid, as significant invasion by *A. flavus* of intact groundnut pods, and subsequent aflatoxin contamination, is known to occur before harvest (Davidson et al. 1983, Blaney 1985, Mehan et al. 1986b), and identification and possible use of seed testa resistance is definitely regarded as worthwhile.

Resistance to Pod Infection by *Aspergillus flavus*

The groundnut shell has logically been considered as a barrier to penetration by *A. flavus*, as seeds from pods with damaged shells are more frequently contaminated with aflatoxin than those from intact pods (McDonald and Harkness 1967).

Zambettakis (1975) reported that two cultivars, Darou IV and Shulamit, had lower levels of pod infection by *A. flavus* than other cultivars field tested in Senegal. Varietal differences in pod infection were confirmed in subsequent studies, and the differences in resistance appeared to be linked to varietal differences in pod shell structure (Zambettakis et al. 1981). They also reported a significant correlation between natural pod infection and seed infection by *A. flavus* in various genotypes tested in Senegal from 1976 to 1979 (Zambettakis et al. 1981). Pod and seed infection was estimated as sporulating colonies of *A. flavus* on surfaces of dried pods and seeds (examined under a binocular stereoscope in the laboratory). The percentages of seeds with colonies of *A. flavus* observed on their surfaces were consistently lower than those of pods with colonies showing on their surfaces, indicating that the shell acts as a barrier to fungal invasion of seeds. However, internal infections of seeds with *A. flavus* may be present without visible external growth of the fungus.

Considering the concept of the existence of pod shell resistance to *A. flavus*, two groups of workers in the USA, Kushalappa et al. (1979) and Mixon (1980) examined the effects of pod inoculation with *A. flavus* on shell infection and subsequent seed infection in various genotypes in the laboratory. They concluded that resistance to pod infection was highly variable, and appeared to be caused by the presence of antagonistic microflora. At ICRISAT we found that in some genotypes seeds were colonized or infected by the test fungus in pods which did not show colonies of *A. flavus* on their surfaces, while in others seeds were not colonized or infected in pods which showed one or more colonies of *A. flavus* . Colonies of several commonly occurring fungi in groundnut shells such as *Macrophomina phaseolina*, *Fusarium* spp, and *Aspergillus niger* were found on surfaces of both *A. flavus*-inoculated and noninoculated, intact, rehydrated, mature, stored pods of genotypes used in these studies (Mehan, McDonald, and Lalitha, unpublished data). Although the laboratory pod-inoculation method was not pursued, we have used pod inoculation to field test genotypes for resistance to seed infection and subsequent aflatoxin contamination. This aspect is further discussed in the section on resistance to natural seed infection to *A. flavus* in the field.

Resistance to *A. flavus* Seed Infection/Aflatoxin Contamination in the Field

In recent years, realization of the importance of preharvest *A. flavus* infection and aflatoxin contamination stimulated considerable research into possible genetic resistance in groundnuts to *A. flavus* seed infection in the field (Blankenship et al. 1985, Davidson et al. 1983, Kisyombe et al. 1985, Mehan and McDonald 1984, Mehan et al. 1986b). A few studies (Mixon 1980, 1983, 1986) indicated that the genotypes PI 337394F, PI 337409, GFA 1 and GFA 2, resistant to in vitro seed colonization by *A. flavus* (IVSCAF-resistant), showed considerably lower levels of natural seed infection with *A. flavus* and of aflatoxin contamination than the susceptible (IVSCAF-susceptible) genotypes, Florunner and PI 331326. In these studies, observations on natural seed infection were made primarily to determine the "initial" levels of *A. flavus* infection that could interfere with the seed inoculation tests for resistance in the laboratory. The natural seed infection was estimated from sporulating colonies of *A. flavus* on rehydrated seeds that had not been inoculated.

Davidson et al. (1983) could not show significant differences in *A. flavus* infection or in aflatoxin contamination of seed of two cultivars, Sunbelt Runner (reported to be resistant to *A. flavus* colonization of seeds) and Florunner (susceptible to seed colonization) at harvest. Blankenship et al. (1985) reported that four genotypes (A 72118 (GFA 1), A 7404 (AR 3), UF 77316 and UF 791041) resistant to seed colonization, and the cultivar Florunner grown under late-season drought stress were all highly susceptible to aflatoxin contamination. Other workers (Zambettakis et al. 1981) have reported several IVSCAF-resistant genotypes as having field resistance to *A. flavus* infection in Senegal, significant correlations being found between seed colonization in the laboratory and field infection. Kisyombe et al. (1985) demonstrated a correlation between field resistance to *A. flavus* seed infection and in vitro seed colonization in only one of 14 genotypes tested.

At ICRISAT,we give high priority to screening of groundnuts for resistance to seed infection by *A. flavus* in the field. We estimate levels of natural infection, i.e., infection resulting from invasion of pod and seed in the ground prior to harvest, or during postharvest drying. Genotypes that have received similar treatment in the field are compared for natural seed infection by *A. flavus* at either of these two stages. Seeds from intact, mature pods, are surface sterilized in 0.1% aqueous mercuric chloride solution for 3 min, rinsed in sterile distilled water, then incubated on Czapek Dox Rose Bengal Streptomycin Agar at 25°C for 5–7 days. Fungi growing from the seeds are recorded.

We tested various genotypes (IVSCAF-resistant, -susceptible, and -highly susceptible) for natural seed infection by *A. flavus* in replicated field trials at ICRISAT Center, from 1979 to 1982. In all four rainy seasons, the IVSCAF-resistant genotypes PI 337394F, PI 337409, and J-11, had significantly lower percentages of seed infected with *A. flavus* than the IVSCAF-susceptible or highly susceptible genotypes both at normal harvest (at optimum maturity) and late harvest (10 days after maturity) (Mehan et al. 1986b).

We also evaluated six IVSCAF-resistant (PI 337394F, UF 71513, J 11, Ah 7223, Var. 27, and U 4-47-7) and five IVSCAF-susceptible (TMV 2, Gangapuri, EC 76446(292), NC Ac 17090, and F1-5 × NC Ac 17090) genotypes for resistance to field infection of seed by *A. flavus*, and for aflatoxin contamination, in four drought-prone sites in Andhra Pradesh, India. All IVSCAF-resistant genotypes except Var. 27 had significantly lower percentages of seed infected (0.8–1.5%) than IVSCAF-susceptible genotypes (4.2–19.1%) over environments (sites and seasons). Resistance to field infection of seed by *A. flavus* in five of the six IVSCAF-resistant genotypes was stable across environments (Mehan et al. 1987). The IVSCAF-resistant genotypes, Ah 7223, J 11, U 4-47-7, and UF 71513, had significantly lower levels of aflatoxin B_1 (5-9 $\mu g\ kg^{-1}$ seed) than the IVSCAF-susceptible genotypes (39–151 $\mu g\ kg^{-1}$ seed).

We confirmed resistance to preharvest *A. flavus* seed infection in five of the six IVSCAF-resistant genotypes grown under imposed drought stress during pod maturation (30 days before harvest) in the 1984/85 and 1985/86 postrainy seasons (ICRISAT 1987).

Of 37 IVSCAF-resistant genotypes (Table 1), only 10 (PI 337394F, PI 337409, UF 71513, Ah 7223, J 11, Var. 27, 55-437, 73-30, Monir 240-30, and RMP 12) have been tested for resistance to seed infection by *A. flavus* in field trials (Kisyombe et al. 1985, Mehan et al. 1986, Zambettakis et al. 1981). Only three genotypes, PI 337409, PI 337394F, and J 11, have been evaluated in more than one country. J 11 was found resistant to *A. flavus* seed infection in North Carolina, USA (Kisyombe et al. 1985) and in India (Mehan et al. 1987). PI 337409 showed resistance in Senegal (Zambettakis et al. 1981) and in India, but was susceptible in the USA (Kisyombe et al. 1985).

Zambettakis et al. (1981) reported highly significant correlations between seed colonization in the laboratory and field infection of seed by *A. flavus* in 101 genotypes tested in several field trials in Senegal. It should not be assumed that all IVSCAF-resistant genotypes will have

resistance to seed infection by *A. flavus* in the field, or that all IVSCAF-susceptible genotypes will show susceptibility to field infection by the fungus. For example, the IVSCAF-resistant genotypes Var. 27, Monir 24-30, and RMP 12 showed similar susceptibility to *A. flavus* infection in the field to that of the IVSCAF-susceptible genotypes TMV 2 and F1-5 × NC Ac 17090. Similar findings have been reported by Kisyombe et al. (1985). On the other hand, some IVSCAF-susceptible genotypes such as Lampang (Kisyombe et al. 1985) and Exotic 6 (Mehan, V.K., unpublished data) have been found to have low levels of seed infection by *A. flavus* in the field.

Methods for Screening Groundnuts for Resistance to *A. flavus* Infection and Aflatoxin Contamination

Some distinctive problems are encountered when screening groundnuts for resistance to *A. flavus* and/or aflatoxin production under natural field conditions. Only intact pods can be used as damage of any kind is likely to override resistances. *A. flavus* is a weak pathogen and its ability to invade intact pods and seeds is strongly influenced by environmental conditions. Little is known of the comparative pathogenicity of different strains of the fungus, and their capacity to produce aflatoxin. Some environments are conducive to *A. flavus* infection of groundnuts, and extra attention is required to ensure uniform levels of infection for effective resistance screening. For environments where levels of *A. flavus* and aflatoxin contamination of susceptible cultivars are usually low, it is necessary to modify the environment to ensure high levels of infection/ contamination.

Screening trials should be on a light sandy soil, preferably with high populations of *A. flavus* in the soil mycoflora. A test site in a drought-prone area where late-season drought stress is common would be most effective as it provides a congenial environment for the fungus. Otherwise, the screening might have to be carried out on early or late-sown crops, or on irrigated dry-season crops where control of soil moisture during late stages of pod development can be assured.

At ICRISAT, we grow an irrigated dry-season crop and it is relatively simple to impose drought stress when required, it is thus possible to screen large numbers of genotypes for field resistance.

We screened over 500 genotypes for resistance to field infection of seed by *A. flavus* in the 1984, 1985, and 1986 rainy seasons, when severe to moderate drought stress occurred during pod maturation. Levels of *A. flavus* infection ranged from less than 2 to 38% (ICRISAT 1987). In the 1985/86 postrainy season, we used imposed drought stress (95–125 DAS) to field screen 432 additional genotypes for resistance to field infection of seed by *A. flavus*. Levels of seed infection ranged from 1.7 to 47% (ICRISAT 1987).

As drought stress during pod maturation predisposes groundnuts to *A. flavus* invasion it was thought that drought-tolerant cultivars might be resistant to preharvest infection by the fungus. However, several drought-tolerant genotypes tested to date (e.g., NC Ac 17090, Gangapuri, Manfredi × M13) are quite susceptible. Most genotypes found tolerant to end-of-season drought are of the valencia type, many of which appear to have weak pod shell structures. It is of interest that the drought-tolerant spanish cultivar C55-437 shows relatively low levels of seed infection at harvest. More research is needed to answer the important question: can the drought-tolerance of a cultivar reduce stress on pod and seeds and so reduce the chances of invasion by *A. flavus* in the soil? The resistance of the groundnut pod to *A. flavus* invasion appears to be associated with

certain structural and biochemical characters of the pod and seed, and there is a possibility that genotypes may have differential effects upon the populations of *A. flavus* in the geocarposphere. It would be interesting to determine if cultivars of different botanical types and pod characters show substantial differences in their reactions to *A. flavus*.

We also used a line-source sprinkler irrigation system that imposes a water deficit gradient to further evaluate some 40 genotypes for their reaction to *A. flavus* (ICRISAT 1987). A significant, positive, linear relationship was found between water deficits and *A. flavus* seed infection in all genotypes. Genotypic differences for infection were clearly evident over a considerable range of water deficits (62–99%), indicating the value of this method for screening genotypes for their reaction to *A. flavus* over a wide range of water deficits (drought intensities). Simultaneous screening for *A. flavus* seed infection and for drought tolerance is particularly useful as drought stress strongly influences seed infection by *A. flavus*. A pod zone soil temperature gradient is associated with the water deficit gradient, and this is important when considering aflatoxin production. Temperatures between 25° C and 31° C are reported to favor aflatoxin production in groundnuts subjected to drought stress during pod development and maturation (Blankenship et al. 1984, Cole et al. 1985). The position on the stress gradient (water deficit and temperature gradients) can be chosen in the field from which material is collected for resistance screening for *A. flavus* infection or aflatoxin production. High levels of *A. flavus* seed infection can be obtained under severe water-deficit conditions accompanied by high maximum temperatures (38–41° C) in the pod-zone soil, conditions that favor *A. flavus* growth and fungal invasion by suppressing other microbial competitors.

A useful evaluation of genotypes for resistance to aflatoxin contamination can be obtained by comparing the aflatoxin contents of seeds across genotypes. Resistance to *A. flavus* seed infection may be used as an index of possible resistance to aflatoxin contamination, but not all strains have a similar aflatoxin-producing ability. The combination of *A. flavus* strain and host genotype can influence aflatoxin production. However, for all practical screening purposes field resistance to the fungus is important in conferring resistance to aflatoxin contamination.

Genotypes can also be evaluated for resistance to *A. flavus* infection and aflatoxin contamination under artificial inoculation conditions by applying inoculum of an aflatoxin-producing strain of *A. flavus* to the soil around developing pods (20 to 30 days before harvest) to produce uniform, high levels of infection and aflatoxin contamination. Care should be taken to avoid injury to the pegs and pods while adding inoculum. We have used this technique to evaluate selected genotypes (IVSCAF-resistant and IVSCAF-susceptible genotypes) for their reaction to *A. flavus* infection and aflatoxin contamination in the field. Four IVSCAF-resistant genotypes (Ah 7223, J 11, PI 337394F, and UF 71513) had significantly lower levels of infection and aflatoxin contamination than the IVSCAF-susceptible genotypes we tested (Mehan and McDonald 1984).

Resistance to Aflatoxin Production

Rao and Tulpule (1967) reported varietal resistance in groundnut to aflatoxin production. The genotype US 26 (PI 246388) did not support aflatoxin production when seeds were colonized by aflatoxigenic strains of *A. flavus* in laboratory inoculation tests. Kulkarni et al. (1967) reported that the cultivar Asiriya Mwitunde supported very low levels of aflatoxin production under field conditions. These findings were not confirmed by other workers, but did stimulate research on possible varietal resistance (Doupnik 1969, Aujla et al. 1978, Doupnik and Bell 1969, Nagarajan and Bhat 1973, Tulpule et al. 1977). Most of these researchers used autoclaved seeds of

groundnut inoculated with aflatoxigenic strains of *A. flavus* to test genotypes for their ability to support aflatoxin production.

At ICRISAT, we developed a laboratory inoculation method to screen groundnuts for resistance to aflatoxin production (Mehan and McDonald 1980). The method is similar to that used for the seed colonization test. Intact mature seeds are surface sterilized in a 0.1% aqueous solution of mercuric chloride, rinsed in sterile distilled water, and hydrated to 20% moisture. The seeds (20-g samples) are then placed in 9-cm diameter petri dishes, their testae scarified with a sterile needle, and inoculated with 1 mL of a conidial suspension (4×10^6) conidia mL^{-1} of an 8-day-old culture of an aflatoxin-producing strain (AF 8-3-2A) of *A. flavus*. After incubation at 25°C for 10 days the seeds are tested for aflatoxin content. We have tested 502 genotypes for their ability to support aflatoxin B_1 production (Mehan et al. 1986a), and found significant differences in rate and total accumulation of aflatoxin. Levels of aflatoxin B_1 produced in different genotypes ranged from below 10 to 195 $\mu g\ g^{-1}$ seed. We identified two genotypes, U 4-7-5 and VRR 245, that supported production of very low levels of aflatoxin B_1 (7–10 $\mu g\ g^{-1}$ seed). There were indications that aflatoxin production levels were slightly lower in seed (of some genotypes tested) from rainy-season crops than in seed from postrainy-season crops, indicating possible environmental effects. Comparisons of the chemical constituents (such as phytate, zinc, boron) of seed of different genotypes grown in different environments may indicate possible mechanisms of resistance to aflatoxin production.

We tested 30 more genotypes with oil contents that ranged from 33.7 to 48.4% for their ability to support aflatoxin production. No correlation was found between oil content and capacity to support aflatoxin production.

We also tested 16 wild *Arachis* species (9 in section *Arachis*, 3 in section *Erectoides*, 2 in section *Rhizomatosae*, and one each in sections *Extranervosae* and *Triseminale*). All supported production of aflatoxin B_1 (34–110 $\mu g\ g^{-1}$ seed).

Some genotypes resistant to seed colonization by aflatoxigenic fungi are good substrates for aflatoxin production, while others that are susceptible to fungal colonization do not support high levels of aflatoxin production. For example, the IVSCAF-resistant genotypes, PI 337394F, PI 337409, J 11, and UF 71513 support high levels of aflatoxin B_1 production, while some IVSCAF-susceptible genotypes (U 4-7-5 and VRR 245) support only low levels of aflatoxin B_1 production. No correlation was observed between fungal growth (estimated visually or based on ergosterol contents of colonized seeds of J 11, U 4-7-5, and VRR 245) and aflatoxin production. Similar findings have been reported by Priyadarshini and Tulpule (1978) with regard to fungal growth (based on chitin content) and aflatoxin production in several varieties of groundnut and maize.

How Can Genetic Resistance be Applied to Aflatoxin Management?

The ideal solution would be to identify or breed a groundnut cultivar immune to invasion by *A. flavus*, or one that would not support aflatoxin production. But this is not likely to be achieved, at least in the near future, and it is more logical to aim for cultivars with a high degree of resistance to *A. flavus* invasion before and after harvest, that support only low levels of aflatoxin production. *Aspergillus flavus* resistance should be incorporated into both oil and confectionery groundnut cultivars adapted to particular agroecological regions. Such cultivars could be grown using cultural and crop-handling procedures that were found useful in reducing *A. flavus* invasion. Cultivars resistant to fungal invasion in the soil would be particularly desirable for the semi-arid regions where preharvest aflatoxin contamination is a serious

problem. The good level of resistance in the commercial cultivars J 11 and C 55-437 could be useful in minimizing aflatoxin contamination in some environments.

Resistance to *A. flavus* infection is also important in order to maintain seed quality as the fungus also causes seed rots and aflaroot seedling disease. Cultivars with resistance to *A. flavus* invasion are also likely to have resistance to seed invasion by other soilborne pathogens that reduce produce quality and cause seed and seedling diseases.

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Discussion

J.I. Pitt. Do you have a carefully standardized procedure for raising the moisture to 20%? What is the extent of variation?

V.K. Mehan. The seeds are immersed in water for a duration that varies with cultivar, e.g., 8-9 min for the spanish types; this being determined by weighing. Variation from seed to seed is less than 1%.

T. Shantha. Is there any variety which is susceptible to fungal colonization but does not support aflatoxin production?

V.K. Mehan. This is a good question. We have found genotypes which are poor substrates for aflatoxin production, but none of them has marked resistance to fungal colonization. When testing seeds for ability to support aflatoxin production, we scarify the testa to remove resistance to fungal colonization and we inoculate with a highly toxigenic strain of *A. flavus*. Production of aflatoxin in the substrate is obviously dependent upon the fungal growth and this can be estimated using the chitin or ergosterol determination techniques.

K.K. Shresta. Although *A. flavus* is said to be a weak pathogen, it causes aflaroot disease and reduces crop yield. How can we control this disease?

V.K. Mehan. Incidence of aflaroot disease can be reduced by sowing clean seed. This can be ensured by careful attention to harvesting, drying, and storing of sowing materials from the previous season. Use of suitable seed-protectant fungicides can also help.

R.E. Pettit. In 1986, a severe outbreak of aflaroot disease totally destroyed a farmer's crop in South Texas. This outbreak was due to sowing of seed heavily infected with *A. flavus*.

Screening Groundnuts for Seed Resistance to *Aspergillus flavus*: Statistical Approaches to Data Evaluation

Murari Singh , V.K. Mehan , and D. McDonald[1]

Abstract

Environmental factors influence the degree of groundnut seed infection by Aspergillus flavus *and other fungi. This complicates resistance screening over seasons and locations as levels of infection can vary considerably within a genotype. Statistical methods were used to separate genotypes into different resistance/susceptibility categories and to ensure a stable basis for comparisons of control cultivar and test genotypes across environments. An approach was also adopted for comparing the degree and distribution of resistance in spanish and valencia type groundnuts. The establishment of such procedures would facilitate interpretation of screening data from different environments.*

Résumé

Sélection des arachides pour la résistance des graines à *Aspergillus flavus*—analyse statistique des données : *Les facteurs d'environnement influencent l'intensité de l'infection des graines d'arachides par* Aspergillus flavus *et d'autres champignons. Ce phénomène complique la sélection du matériel résistant lorsque les essais sont effectués pendant plusieurs saisons et à divers sites, puisque les niveaux d'infection varient considérablement pour le même génotype. Des méthodes statistiques ont permis de classer les génotypes dans différentes catégories de résistance/sensibilité, et de donner une base sûre de comparaison entre les nouveaux génotypes à tester et les temoins pour différents sites. Cette approche a été également adoptée pour comparer le degré et la distribution de la résistance des types Spanish et Valencia. La mise au point de ces procédés statistiques facilitera l'interprétation des données obtenues dans différents sites expérimentaux.*

Resúmene

La selección de cacahuates basada en la resistencia de sus semillas a *Aspergillus flavus*: Métodos estadísticos para evaluar datos : *Los factores del medio ambiente influyen en la severidad con que ocurre la infección de la semilla de cacahuate por* Aspergillus flavus *y otros hongos. Esto complica la selección para lograr la resistencia a través de varios ciclos de cultivo y en diferentes localidades, debido a que los niveles de infección pueden variar considerablemente dentro de los genotipos. Se utilizaron métodos estadísticos para separar los genotipos en diferentes categorías*

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de la relación resistencia/susceptibilidad, y en esta forma asegurar una base de comparación estable entre el cultivar testigo y los genotipos bajo prueba, en todos los ambientes. Se adoptó, asimismo, un enfoque específico para la comparación del grado y distribución de la resistencia, en cacahuates de los tipos Spanish y Valencia. La adopción de estos procedimientos facilitarían la interpretación de los resultados de las pruebas de selección efectuados en diferentes condiciones ambientales.

Introduction

By screening groundnut genotypes for resistance to seed colonization by *Aspergillus flavus* Link ex Fries, in vitro, they can easily be classified as resistant, susceptible, or highly susceptible on the basis of arbitrarily set percentages of seeds colonized (LaPrade et al. 1973, Mixon and Rogers 1973, Mehan and McDonald 1980). However, when screening groundnuts for resistance to natural seed infection by the fungus in the field, it is not easy to identify resistant genotypes on the basis of arbitrarily set levels of seed infection, because environmental factors such as soil moisture, soil temperature in the pod zone, and soil type can influence *A. flavus* infection of a genotype. Levels of seed infection within specific genotypes show very little variation between replicate samples, but levels of infection can vary considerably between trials, locations, or seasons. In such situations, genotypic resistance can best be measured in relation to the reactions of standard resistant and susceptible control genotypes. Thus a genotype can be considered resistant to the fungus if its reaction to seed infection is similar to that of a resistant control genotype in the same environment. In view of this concept, the reaction of a genotype to *A. flavus* can be represented by the probability distribution of *A. flavus* seed infection levels in a given environment, and one of the following approaches used for resistance screening.

Three situations frequently met in practice are discussed, and ways to screen genotypes resistant to seed infection by *A. flavus* considered.

The levels of resistance distributed in spanish and valencia types of groundnut are also compared.

Statistical Approaches for Screening Genotypes for Seed Resistance to Infection by *Aspergillus flavus*

Statistical methods are discussed in relation to three types of situation prevalent in experimentation.

Comparison of Distribution Functions

Let T stand for a test genotype and R for the resistant control genotype. The frequency distribution of the level of seed infection by *A. flavus* follows that shown in Figure 1.

A test genotype (T) can be considered resistant if the probability distribution of its seed infection level is identical to that of a resistant control genotype (R) in a given environment. To illustrate this point, let the probability distribution functions of R and T be denoted by $F_R(x)$ and $F_T(x)$,

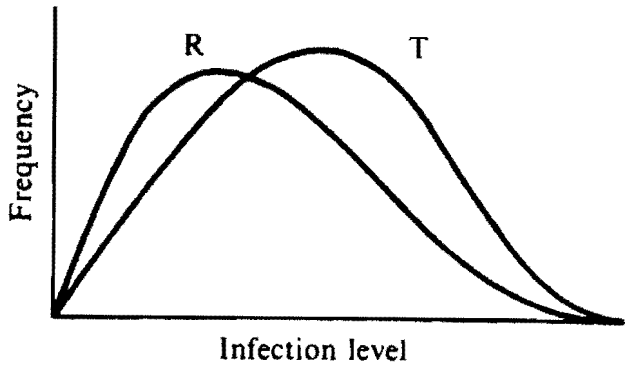

Figure 1. Distribution of infection levels in a resistant (R) and a test (T) groundnut genotype.

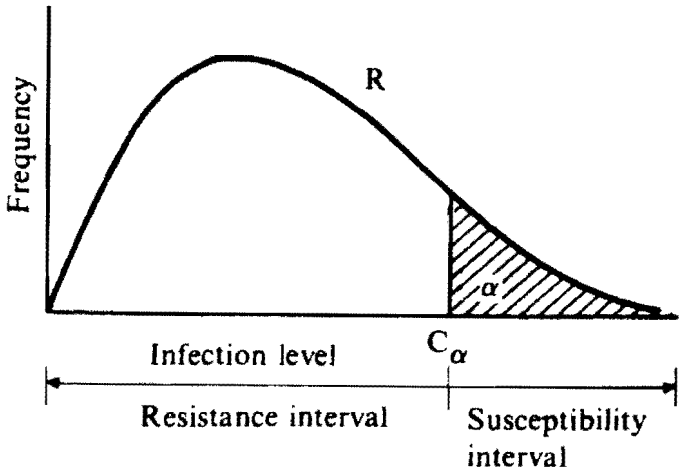

Figure 2. Distribution of infection level in a resistant (R) groundnut genotype and critical point (C_α) used to select resistant genotypes.

where x is the seed infection level. The test genotype will be resistant relative to the resistant control genotype if the hypothesis

H_0 : $F_T(x) = F_R(x)$ for all positive x, is not rejected against

H_1 : Shift to right side in the distribution of T.

If the distribution form is known, a parametric test can be applied to the sample observations or a non-parametric test if the distribution is not known, to test the significance of the difference between the two distributions. But this approach appears to be appropriate only for situations with very few genotypes, since the genotypes to be screened will have to be tested in large numbers of plots to examine their distribution functions.

Confidence Interval Method

Test genotypes can be screened by growing them in blocks with a resistant control genotype systematically grown across the field trial. The probability distribution of a resistant control genotype can be calculated based on its seed infection levels across the trial as shown in Figure 2. One can compute the upper 100α percent critical level C_α and define resistance and susceptibility intervals separated by C_α. The quantity C_α can be estimated empirically or by using the estimates of parameters of a confirmed distribution to which the sample may belong.

Let the probability distribution of seed infection levels of a resistant control genotype be normal N (μ, σ^2) where the mean μ, and variance can be estimated from sample mean $\bar{x}$, and variance s^2. In this case, a test genotype (T) with a mean $\bar{t}$ (calculated from r replicates) can be considered resistant relative to R if it falls in the resistant region defined by :

$$\bar{t} < C_\alpha$$

where $C_\alpha = \bar{x} + t_{\alpha, e} s/r^{1/2}$

and $t_{\alpha, e}$ is the upper 100α percentage point of t-distribution, with e degrees of freedom.

Any genotype with a mean seed infection level exceeding the critical boundary point C_α will be susceptible.

Data from the 1986 rainy-season trial at ICRISAT Center were subjected to the above

analysis to select genotypes resistant to seed infection by *A. flavus* in relation to the resistant control genotype J 11.

Confidence Interval and Clustering Methods

Various genotypes including a resistant control are usually grown in an experimental design. Each genotype will be observed in r plots (r = number of replications). As r is generally small, one can not accurately obtain the distribution of R (a resistant control genotype). In this situation one can apply the analysis of variance to the observations to estimate error variance and means. Such a data set can be used to select genotypes similar to R in one of the following ways:

Using confidence intervals

A genotype (T) can be regarded as resistant if its mean seed infection level ($\bar{t}$) does not differ significantly from the mean ($\bar{r}$) of R using upper tail t-test as in situation (2), i.e., when

$$\bar{t} - \bar{r} < t_{\alpha,e}\, \hat{SE}\,(\bar{t} - \bar{r})$$

or

$\bar{t}$ lies in the 100 $(1-\alpha)$% one-sided confidence interval $(0, \bar{r} + t_{\alpha,e}\, \hat{SE}(\bar{t}-\bar{r}))$.

Where $t_{\alpha,e}$ is upper 100α% point of the t-distribution with degrees of freedom e used to estimate standard error SE $(\bar{t} - \bar{r})$ of difference $\bar{t} - \bar{r}$, by $\hat{SE}(\bar{t} - \bar{r})$.

In cases where the standard error varies with extreme levels of seed infection, some modification may be required to obtain more precise estimates of error variance associated with the genotypes close to R. This can be done by splitting the genotypes into two groups—one group with genotypes close to R and the standard error can be computed for this group alone, while the other group may contain the rest of the genotypes with a different standard error.

Clustering

The replicate-wise data on genotypes can be used in the form of r-variate information to cluster genotypes based on their similarity in reaction to *A. flavus* infection as assessed in individual replicate plots. The numerical and graphical results in clustering methods can be obtained by using such statistical packages as GENSTAT and SAS. Without these packages, it is also easy to determine the genotypes that fall in a similar cluster with a resistant control at a specified similarity level as follows:

Let Z_{Rj} and Z_{Tj} be the infection levels of the resistant (R) and test (T) genotypes in j-th block.

The distance between R and T is

$$D(R,T) = \left(\sum_{j=1}^{r} d\,(R,T,j)\right)^{1/2}$$

where

$$d(R,T,j) = (Z_{Tj} - Z_{Rj})^2.$$

Further, the following modification in computing distance will be required so that a genotype which outperforms the resistant control in the block(s), is not rejected.
Thus, the difference $d(R,T,j) = 0$ if $Z_{Tj} < Z_{Rj}$.

The similarity would then be proportional to the negative of D(R,T). The range of similarity computed for all the genotypes in this manner can be set on a 0 to 100 scale. The cluster of genotypes at a specified level consists of those genotypes for which the similarity percentage is less than, or equal to, that level.

The comparison of the two methods may be rather difficult, as there is no obvious link between the confidence coefficient $(1-\alpha)$ of the confidence interval method and the percentage similarity level in the clustering technique. The confidence interval method is very sensitive to the estimator of experimental errors. This method has been grouping more genotypes (with higher susceptibility level, in some of our examples) at $\alpha = 0.05$ compared to the cluster method at 95% similarity level (see Table 1). Furthermore, the clustering method is able to pick up differences between test and control infection levels within each block, and hence may reject genotypes for susceptibility more often than does the confidence interval method where these differences (between R and T) across a block may average very close to zero. This feature would appear to be more useful when resistance screening is done across diverse environments, because the genotype × environment (g × e) interaction is taken into account by the clustering technique.

The confidence interval method and clustering technique can be illustrated using data on the percentage of seed infected by *A. flavus* from the following at ICRISAT Center.

Table 1. Confidence interval and clustering methods for groundnut genotypes similar to J 11, ICRISAT Center, rainy season 1985.

Clustering method:					
Similarity (%)	No. of genotypes	Cluster unit J 11[2]	Mean	SD	Range
> 94	6	GNP104, ICG 3700, ICG 4106 ICG 3660, ICG 2359, ICG 1326	0.5	0.18	0.33-0.67
89-93	10	ICGS(E) 119, ICG7 101[1], ICG 8666, ICG 7633	0.73	0.35	0.33-1.33
< 89	61	Many[1]	2.10	1.12	0.33-4.33
Confidence interval:					
Confidence coefficient	No. of genotypes	Resistant group of J 11	Mean	SD	Range
95 (at $P = 0.05$ one sided)	27	ICG 1323, ICG 1436, ICG 1720, ICG 1811, ICG 2359, ICG 3241, ICG 3251, ICG 3478, ICG 3499, ICG 3660, ICG 4106, ICG 6321, ICG 1684, ICG 3700, ICG 4749, ICG 7633, ICG 4502, ICG 4681, ICG 7101, ICG 3263, ICG 7886, ICG 8631, ICG 8666, ICG 8991, GNP 104, GNP 1020, ICG S(E)-119	1.0	0.395	0.33-1.67

1. In addition to genotypes of above group.
2. Mean (J 11) = 0.67±0.39

1986 rainy-season trial

At ICRISAT Center 196 genotypes were grown in a triple lattice design with J 11 as a standard resistant control and JL 24 as a susceptible control genotype systematically sown after every 7th test entry, and appearing thrice in each block. In order to explain the confidence interval method, we estimated the following parameters on the distribution of infection level in J 11.
mean ($\hat{\mu}$) = 1.71, standard deviation ($\hat{\sigma}$) = 0.99
Coefficient of skewness ($\hat{\beta}_1$) = 0.45 ± 0.22
Coefficient of kurtosis ($\hat{\beta}_2$–3) = 0.19 ± 0.43

In view of the low values of $\hat{\beta}_1$ and $\hat{\beta}_2$, it is reasonable to represent the distribution of J 11 as a normal distribution. At α = 0.05 (or 95% confidence coefficient), $t_{\alpha,e}$ = 1.645 (e is large) and confidence interval is {0, 2.65}.
The following genotypes fall in this interval; ICG 1910, ICG 9820, ICG 10021, ICG 10927, and ICG 10147
with mean = 1.73, standard deviation = 0.35, and range = 1.33 - 2.33.

1985 rainy-season trial

At ICRISAT Center 100 genotypes were grown in a triple lattice design with J 11, the standard resistant control as one of the entries. The lattice blocks did not show any better control of variation, so data were handled as if observed from a randomized complete block design. A set of 61 genotypes were found to cluster together around a 90% similarity level (with infection levels varying up to 4.33), while others had quite high levels of susceptiblity. For the analysis of variance to estimate experimental error variances, this group of 61 entries were separately analyzed, and the remaining entries were not included. The previously confidence interval and clustering methods were applied, and the results are presented in Table 1 for a 95% confidence interval, and >90 percent similarity level. We used the GENSTAT package (Lawes Agricultural Trust 1986) for cluster analysis modified as required for the similarity (or distance).

Distribution of *A. flavus* Seed Infection in Spanish and Valencia Genotypes

To study the nature of distributions of *A. flavus* infection levels in both spanish and valencia groundnut genotypes, the Kolmogorov-Smirnov single sample test (Pearson and Hartley 1976) was applied on original and log-transformed values. Mean *A. flavus* infection levels for trials in the 1986 rainy and 1985/86 postrainy seasons were separately used for this analysis. The distributions of genotypes in the two groups were also compared using the Kolmogorov-Smirnov two-sample test. The Kolmogorov-Smirnov single-sample test was also performed on seed infection levels in the two systematic control cultivars J 11, and JL 24 tested in the 1986 rainy season. The plot of the infection levels prompted us to look into the distribution of normal and log normal types.

1986 rainy-season trial

Out of 196 genotypes cited, 98 were valencia and 98 spanish type. The values of some basic statistics on the distribution for both types are presented in Table 2 for original as well as log

Table 2. Parameters of distribution of *Aspergillus flavus* infection levels in 98 valencia and 98 spanish groundnut genotypes, ICRISAT Center, rainy season 1986.

	Valencia		Spanish	
	Original	Log transformed	Original	Log transformed
Mean	10.19	2.17	9.23	2.08
SD	5.45	0.57	5.70	0.54
Skewnes	0.71	-0.45	2.28	0.07
SE	±0.24	±0.24	±0.24	±0.24
Kurtosis	-0.78	0.81	0.72	0.90
SE	±0.48	±0.48	±0.48	±0.48
Dmax	1.99	1.01	1.90	1.02
Prob	0.001	0.26	0.001	0.25

transformed observations. The low coefficient of skewness and kurtosis and high value of probability level (Prob) for Kolmogorov-Simirnov statistics (Dmax) for log transformed data indicate that the level of infection is log normally distributed. The mean infection level was found to be similar in the two groups (see also Fig. 3).

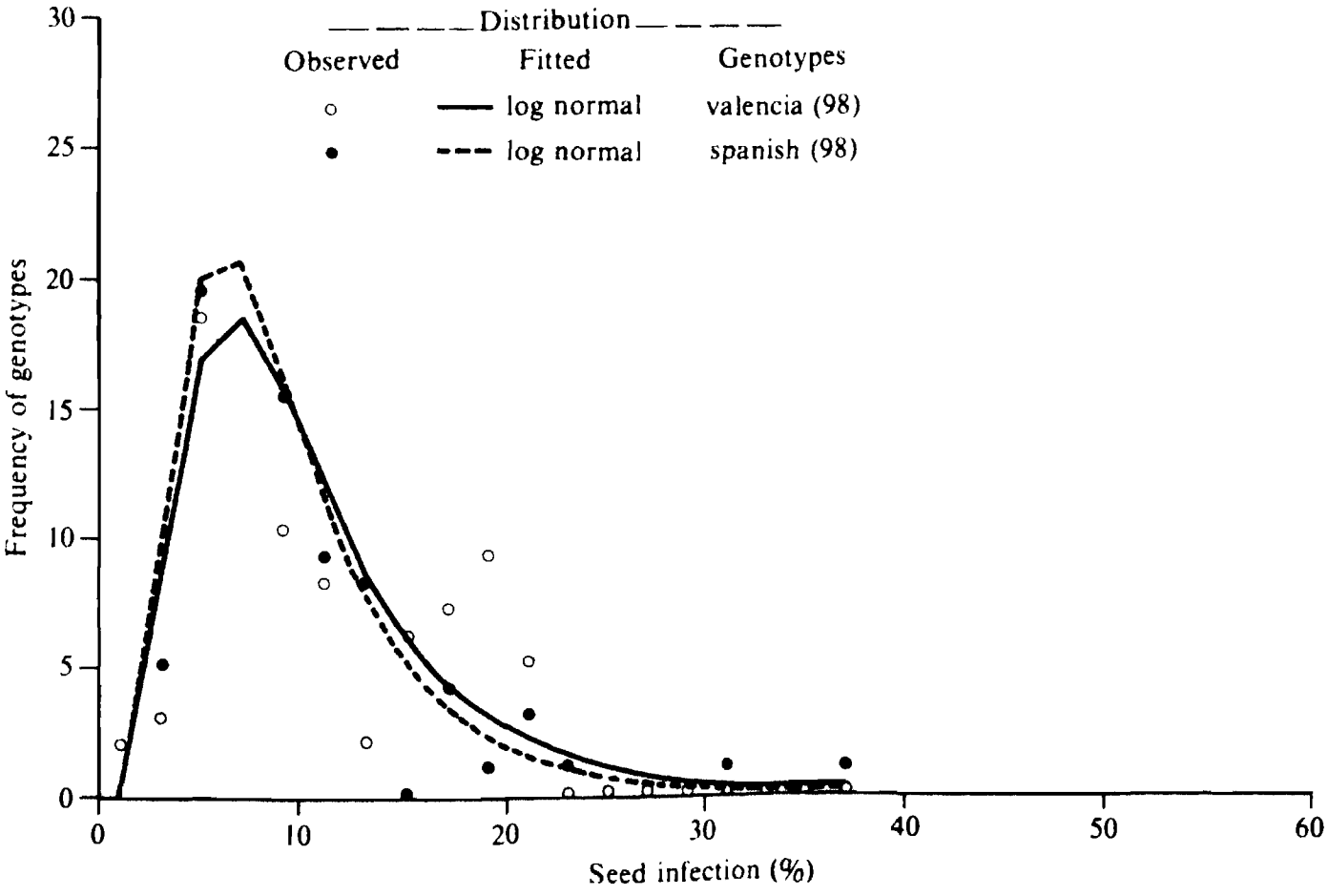

Figure 3. Observed and fitted log normal distributions of seed infection in 98 valencia and 98 spanish groundnut genotypes in Trial 2, ICRISAT Center, rainy season 1986.

Table 3. Parameters of distribution of *Aspergillus flavus* infection levels of 269 spanish and 163 valencia groundnut genotypes, ICRISAT Center, postrainy season trials 1985/86.

	Valencia		Spanish	
	Original	Log transformed	Original	Log transformed
Mean	24.55	3.07	18.42	2.76
SD	11.97	0.56	9.31	0.60
Skewness	0.69	-0.98	0.75	-1.04
SE	±0.19	±0.19	±0.15	±0.15
Kurtosis	-0.17	2.35	0.91	2.13
SE	±0.37	±0.37	±0.30	±0.30
Dmax	1.41	0.85	1.04	1.46
Prob	0.037	0.47	0.23	0.028

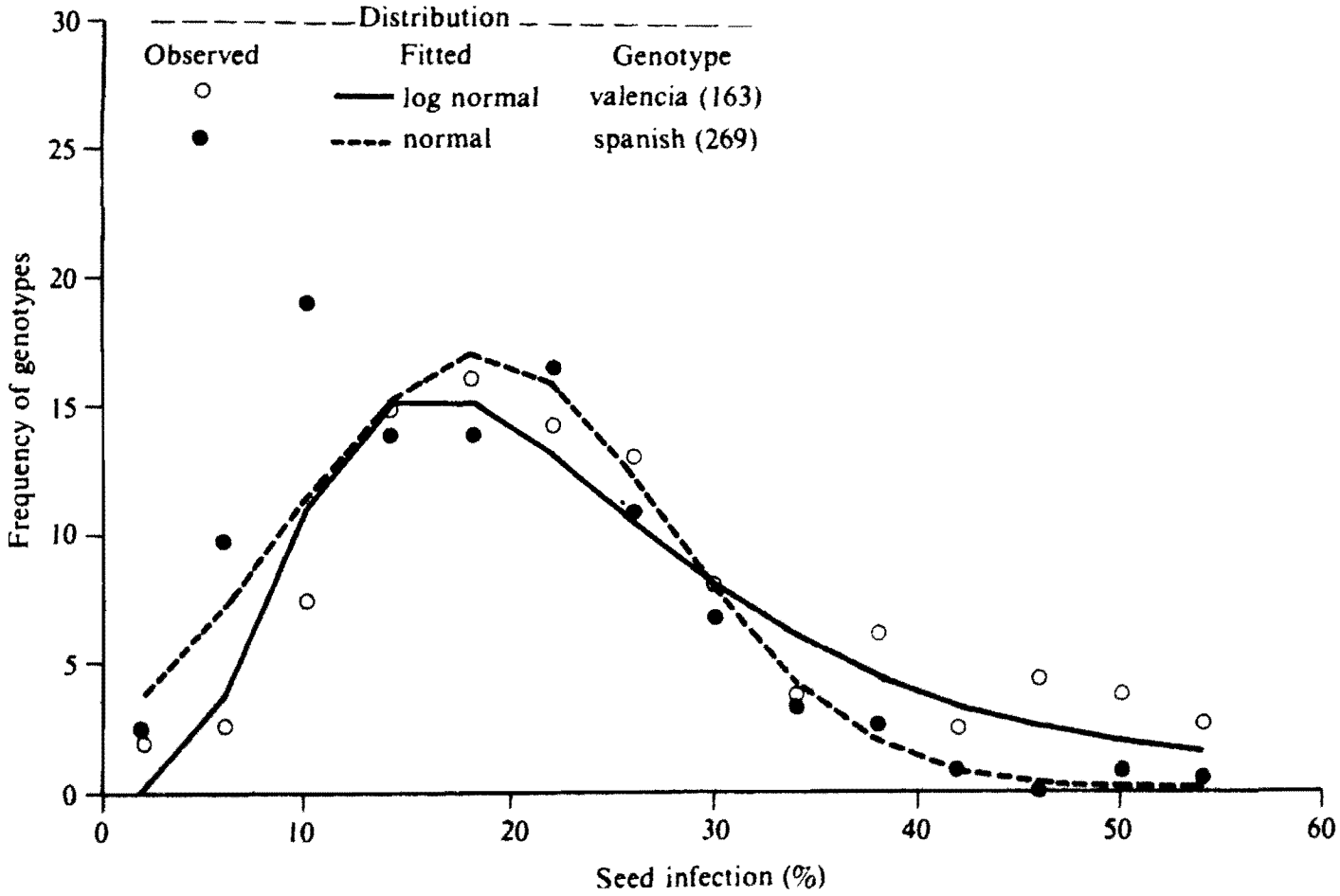

Figure 4. Observed distributions and fitted distributions of seed infection in 163 valencia (log normal distribution) and 269 spanish (normal distribution) groundnut genotypes in Trial 3, ICRISAT Center, postrainy season 1985/86.

1985/86 postrainy-season trial

Three sets each of 144 genotypes were sown in randomized blocks in the same field at ICRISAT Center. Of the 432 genotypes, 163 were of valencia, and 269 of spanish type. The basic statistics for on infection levels for the two groups are presented in Table 3, while the graphical presentation of the distributions (observed and fitted) are shown in Figure 4. Infection levels in the spanish types followed a normal distribution unlike those for the 1986 rainy-season trial and the two botanical groups appear to possess varying potential to provide genotypes with seed resistance to *A. flavus*.

Discussion

The selection of genotypes on the basis of their performance relative to a standard resistant control genotype allows for the flexibility/variability in infection levels that might result from variation in the environmental conditions under field experimentation. The comparison of distribution functions requires large numbers of plots and hence cannot be used to select several entries. While using the confidence method, the experimental error variance requires precise estimation or it may group susceptible entries along with resistant ones. In preparing similarities for cluster methods, one-sided distances should only be considered, since genotypes with infection levels below that of the resistant control are always desirable. Cluster analysis separates susceptible genotypes using differences within blocks while the confidence interval method may not. The application of the clustering method to data from International Cooperative trials would be more sound because genotype × environmental interactions would be successfully reflected in the form of distances (than differences in means if the confidence interval method is applied).

For most situations there appear to be no problems of discontinuous distribution of inoculum and associated levels of seed infection by *A. flavus*. Within trials levels of infection for specific genotypes showed little variation between replicate samples. If a situation occurred where inoculum pressure showed greater variation, it would be worth following the design and analysis approach recommended by Gilliver et al (1985) for sorghum resistance screening against *Striga* since this involves control with cultivars in close juxtaposition to test lines.

It is useful to compare distributions when examining the level of resistance in various botanical groups.

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Aspergillus flavus Resistance Breeding in Groundnut: Progress made at ICRISAT Center

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Abstract

Progress worldwide in breeding groundnuts resistant to seed colonization by Aspergillus flavus *and aflatoxin contamination is summarized, and research at ICRISAT described. Resistance to* A. flavus *infection may occur at various levels, but efforts to breed for resistance have concentrated on the utilization of the resistance in the testae of mature seeds. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), genotypes identified as resistant to* in vitro *seed colonization by* A. flavus *have been crossed with susceptible cultivars of good agronomic character, and several breeding lines with stable resistance to seed colonization and with acceptable yield and quality have been produced. The genetics of inheritance of testa resistance is discussed. It is important that when breeding for resistance to* A. flavus *and aflatoxin production, breeders incorporate other resistance traits.*

Résumé

Sélection pour la résistance à *Aspergillus flavus* chez l'arachide—résultats obtenus au Centre ICRISAT : *Les acquis au niveau international dans la sélection d'arachides résistantes à la colonisation des graines par* Aspergillus flavus *et à la contamination par les aflatoxines sont rappelés. La recherche menée à l'ICRISAT est décrite. La résistance se produit à divers niveaux, mais les travaux de sélection sont axés sur la résistance des téguments des graines mûres. A l'ICRISAT, les génotypes identifiés comme résistants à la colonisation in vitro des graines par* Aspergillus flavus *ont été croisés avec des cultivars sensibles ayant de bonnes caractéristiques agronomiques; ainsi, plusieurs lignées à résistance stable, à bon rendement et de bonne qualité ont été créées. L'hérédité de la résistance du tégument est étudiée. Les sélectionneurs devraient incorporer d'autres caractères de résistance.*

Resúmene

La selección para lograr resistencia a *Aspergillus flavus* en el cacahuate: Avances logrados en el centro ICRISAT : *Los avances logrados a nivel internacional en la selección de cacahuate resistente a la colonización de sus semillas por* Aspergillus flavus *y a la contaminación con*

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aflatoxinas, se presentan en forma resumida, y se describen las investigaciones realizadas en el ICRISAT. La resistencia a A. flavus *puede presentarse a diferentes niveles, pero los trabajos para lograr la resistencia se han concentrado en la utilización de la resistencia de las testas de las semillas maduras. En el Instituto Internacional de Investigación sobre Cultivos en los Trópicos Semiáridos (ICRISAT), los genotipos identificados como resistentes a la colonización* in vitro *de los granos por* Aspergillus flavus, *se han cruzado con cultivares susceptibles con buenas características agronómicas, y se han obtenido varias líneas genéticas de cacahuate con resistencia estable a la colonización de la semilla, y con rendimiento y calidad de semilla aceptables. Se examina la genética de la herencia de la resistencia en las testas. Es importante que cuando realicen la selección para lograr resistencia a* A. flavus, *los fitomejoradores incorporen simultáneamente otras características de resistencia.*

Introduction

Of the several control strategies for *Aspergillus flavus* Link ex Fries in groundnut, breeding for resistance is credited to be a sound, long-term approach to aflatoxin prevention in groundnut (Sanders 1983, Cole 1981, Diener et al. 1982, Mixon 1981, Mehan and McDonald 1984). In this paper, we review the general progress made in this area and describe in detail the progress made at ICRISAT Center. The problems and prospects for developing commercially acceptable cultivars of groundnut with resistance to *A. flavus*, are discussed and future research priorities are considered.

Resistance Traits and Their Possible Exploitation

Resistance to *A. flavus* in groundnut may operate at three sites in the plant—the pod, the seed coat, and the cotyledons. Zambettakis (1975) observed that the varieties Shulamit and Darou IV had lower levels of pod infection by *A. flavus* than other varieties tested in Senegal and attributed this to differences in pod-shell structure. Other workers have attributed resistance to the action of antagonistic microflora in the shell (Kushalappa et al. 1976), or to presence of thick-walled parenchyma cells (Pettit et al. 1977). After initial interest in the early 1970s only limited research on pod resistance has been reported.

Mixon and Rogers (1973) identified seed-coat resistance to *A. flavus* in the germplasm lines PI 337409 and PI 337394F by screening sound mature seeds of groundnut by artificial inoculation with *A. flavus* conidia in an environment favorable to *A. flavus* development. Subsequent reports confirmed seed-coat resistance in these lines and added several new germplasm lines and commercial varieties to the list of resistant materials. Among the reported resistant lines, the resistance in J 11, UF 71513, PI 337394F, PI 337409, Ah 7223, Faizpur 1-5, and Var.27 has been confirmed by testing over locations and years, but the stability of resistance in other lines has not been confirmed by multilocational testing. The lines with confirmed resistance have been used as gene donors for this characteristic. It has also been shown that resistance in J 11, PI 337394F, and PI 337409 can be transferred to other genetic backgrounds (Mixon 1986).

Genotypic differences are also reported for the ability of groundnut seeds to support the production of aflatoxins. Certain claims by earlier workers that US 26 (= PI 246388 = Koboka) and 'Asiriya Mwitunde' were resistant to aflatoxin production were not substantiated. However, Mehan et al. (1986) after screening over 500 genotypes, reported the low aflatoxin-producing ability in U 4-7-5 and VRR 245. No efforts are reported in the literature of genetically improving low aflatoxin-producing genotypes or transferring this trait to other lines. However, the two

germplasm lines reported to be low aflatoxin producers, provide us an opportunity of improving upon this trait and combining it with other *A. flavus* resistance traits.

Genetic variability has also been reported for resistance in groundnut which prevents the penetration and colonization by *A. flavus* in the field. The genotypes J 11, Ah 7223, UF 71513, and U 4-47-7 have been reported to be resistant to preharvest seed infection in India, while 55-437, PI 337409, 73-30, and 73-33 were reported to be resistant to seed infection by *A. flavus* in Senegal (Zambettakis et al. 1981). There has been only limited breeding effort to use preharvest resistance to seed infection as a selection trait. However, some of the sources of resistance to seed infection that also have seed-coat resistance, have been used in breeding programs to incorporate seed-coat resistance into high yielding cultivars.

It is interesting that although several factors such as; low testa permeability, increased surface wax accumulation, uniform wax coating, thin testa with compact and tight cell structure, compact palisade-like layer, small hilum, presence of tannins and inhibitory compounds, and differences in amino acid composition have been reported to contribute towards *A. flavus* resistance, no efforts have been made to breed for these traits. This may be because information on the contribution of these mechanisms to resistance traits is not fully available and/or they are highly influenced by environmental variations. There are no standard screening techniques for these mechanisms. More work will be required before the resistance mechanisms are understood.

Breeding for *A. flavus* Resistance at ICRISAT Center

Research is in progress at ICRISAT Center to incorporate seed-coat resistance into high yielding and adapted groundnut cultivars, and to study the genetics of seed-coat resistance. We are also exploring the possibilities of combining seed-coat resistance with low aflatoxin-producing ability, and hope to study the inheritance of low aflatoxin production.

Sources of Resistance and Crossing Plan

Genotypes used as parents in this breeding project have been selected based on the strength of their seed-coat resistance, and the stability of their resistance (Table 1). These genotypes have been used extensively as gene donors for seed-coat resistance. In addition, we have recently received genotypes AR 1, AR 3, and GFA 2 to be used as new sources of resistance; these have been multiplied and initial observations made to confirm their resistance. We have made crosses between resistant and adapted lines from important groundnut-growing countries where *A. flavus* infection and aflatoxin contamination are serious problems. We have also made crosses among source lines to bring together genes to strengthen resistance, assuming that different source lines possess non-allelic resistant genes.

Selection for Yield and Seed-coat Resistance

At ICRISAT Center, we follow a mass pedigree scheme to select for pod yield. In the F_2 generation, selection is based on the numbers of mature pods per plant. Progenies are advanced as bulks, and in each generation, selection is made for yield and other agronomic traits. In the F_6–F_8 generations, bulks are separated based on the apparent uniformity for their plant and pod

Table 1. *Aspergillus flavus* seed colonization (%) and pod yield (kg ha^{-1}) of selected groundnut breeding lines in multilocational testing in India (1983–1986).

		Postrainy season 1983/84		Rainy season 1984[1]		1986[2]	
Identity	Pedigree	SC (%)	Pod yield	SC (%)	Pod yield	SC (%)	Pod yield
ICGV 86168	(J 11 × PI 337394F)	15.24	5870	12.3	2420	9.17	1833
ICGV 86169	(PI 337409 × UF 71513)	11.62	4951	10.6	2294	10.31	1735
ICGV 86170	(Ah 32 × PI 337409)	14.36	5062	14.8	2336	16.87	1571
ICGV 86171	(J 11 × PI 337394F)	6.47	5796	9.6	1999	9.36	1617
ICGV 86173	(Faizpur 1-5 × PI 337409)	13.43	5407	12.4	2181	23.87	1586
ICGV 86174	(UF 71513 × PI 337394F)	11.71	5139	12.4	2262	10.21	1587
ICGV 87937	(NC 17 × PI 337394F)	16.40	4824	15.3	2225	NT	NT
ICGV 86177	(MH 2 × PI 337394F)	12.38	5302	14.9	2108	18.13	1740
Controls							
J 11[3]		12.88	5580	11.3	2077	9.69	1552
UF 71513[3]		11.58	5250	9.5	2151	9.71	1424
JL 24[4]		22.55	5262	39.2	2004	41.61	2001
Kadiri 3[4]		33.38	5000	31.1	2080	47.44	1570
Mean		16.33	5250	20.12–24.70	707–3269	15.0–31.0	326–2732
SE		±1.61	±270.1	±4.25–6.01	± 133.5–446.6	±2.5–6.2	± 53.6–464.4
CV (%)		17.1	8.9	33.8–47.2	14.1–20.6	27.7–42.0	11.7–31.2

1. Data mean from four locations; ICRISAT Center (High Input), ICRISAT Center (Low Input), Bhavanisagar, and Hisar.
2. Data mean from seven locations; ICRISAT Center (High Input Aflisol), ICRISAT Center (Low Input, Alfisol), ICRISAT Center (Low Input, Vertisol), Hisar, Dharwad, Bhavanisagar, and Anantapur.
3. *A. flavus* resistant varieties.
4. *A. flavus* susceptible varieties.

characters. These bulks are checked in the following generation for true-breeding character and uniformity. They are then entered into replicated yield trials. Harvested samples from these trials are sent to the laboratory where their seed-coat resistance is determined using the procedure described by Mehan et al. (1981), a modification of the procedure first described by Mixon and Rogers (1973).

If sufficient seed is available, breeding lines are tested in multilocational trials to evaluate the stability of their resistance. We emphasize the identification of stable resistance because past findings have indicated that environmental factors can influence seed-coat resistance (Diener et al. 1982, Sanders 1983, Davidson et al. 1983, Mehan et al. 1983).

In 1984, we modified the mass pedigree breeding system to include a stage of progeny-row testing in the F_3 generation, based on plant-to-row progenies obtained from selected F_2 plants. F_3 single-plant progenies are handled as progeny bulks from F_4 onwards and mass selection is made within each bulk. We plan to use a similar scheme to combine low aflatoxin production with seed-coat resistance. Because natural seed infection could be a better indicator of *A. flavus* resistance in the field, we are now planning to test breeding progenies in the field for preharvest seed infection by *A. flavus*.

Progress in Breeding

We have tested several hundred breeding lines for yield and seed-coat resistance. Generally, very few lines with *A. flavus* resistance and high yield have been recovered; this may be because of the low heritability of seed-coat resistance.

We now have eight breeding lines (Table 1) with seed-coat resistance levels equal to those of the resistant source lines J 11 and UF 71513. The yield levels in the selected lines, though fluctuating over the years, have been better than those of the resistant source lines. In a few years and locations, the resistant breeding lines have outyielded such susceptible commercial control varieties as JL 24 and Kadiri 3. Five lines, ICGV 86168, ICGV 86169, ICGV 86171, ICGV 86174, and ICGV 86177 are being evaluated in larger plots for seed infection and aflatoxin contamination.

We also have 32 breeding lines that have been tested once for seed-coat resistance. Some of these have high yield potential in addition to seed-coat resistance.

The importance of the stability of seed-coat resistance has been stressed by many previous workers. We have studied the stability of the resistant breeding lines, that were tested in the rainy seasons at four locations in 1984, and seven locations in 1986 (Tables 2 and 3), using the regression approach of Eberhart and Russell (1966). The regression coefficient (B) for resistance plotted against the mean percentage seed colonization (Fig 1, a and b) indicated that in both years, the selected resistant breeding lines were as stable as the resistant source lines and had similar levels of seed colonization to the resistance source lines. The regression coefficient (B_i) for yield plotted against the mean yield over locations indicated that some of the resistant breeding lines were also responsive to the environment.

Table 2. Stability parameters of eight breeding lines obtained from four Indian locations, rainy season, 1984.

	Pod yield (kg ha^{-1})			Seed colonization (%)		
Identity	Mean	B_i	S^2d_i	Mean	B_i	S^2d_i
ICGV 86168	2420	1.088	376608	12.30	0.495	-17.8
ICGV 86169	2294	1.060	-50440	10.65	0.288	-21.5
ICGV 86170	2336	1.101	-22702	14.82	0.336	-20.2
ICGV 86171	1999	0.991	62199	9.65	0.168	-11.2
ICGV 86173	2181	1.138	46684	12.47	1.038	-5.4
ICGV 86174	2262	0.896	54896	12.40	0.266	-19.9
ICGV 87937	2225	0.910	54465	15.37	1.794	-22.6
ICGV 86177	2108	1.008	-18571	14.93	1.007	-17.8
Controls						
J 11[1]	2077	0.868	-36506	11.37	0.868	-26.1
UF 71513[1]	2151	0.862	14654	9.50	0.606	-13.7
JL 24[2]	2004	1.040	-27055	39.20	2.565	20.0
Kadiri 3[2]	2080	0.833	113586	31.13	0.900	48.9

1. Resistant control varieties.
2. Susceptible control varieties.

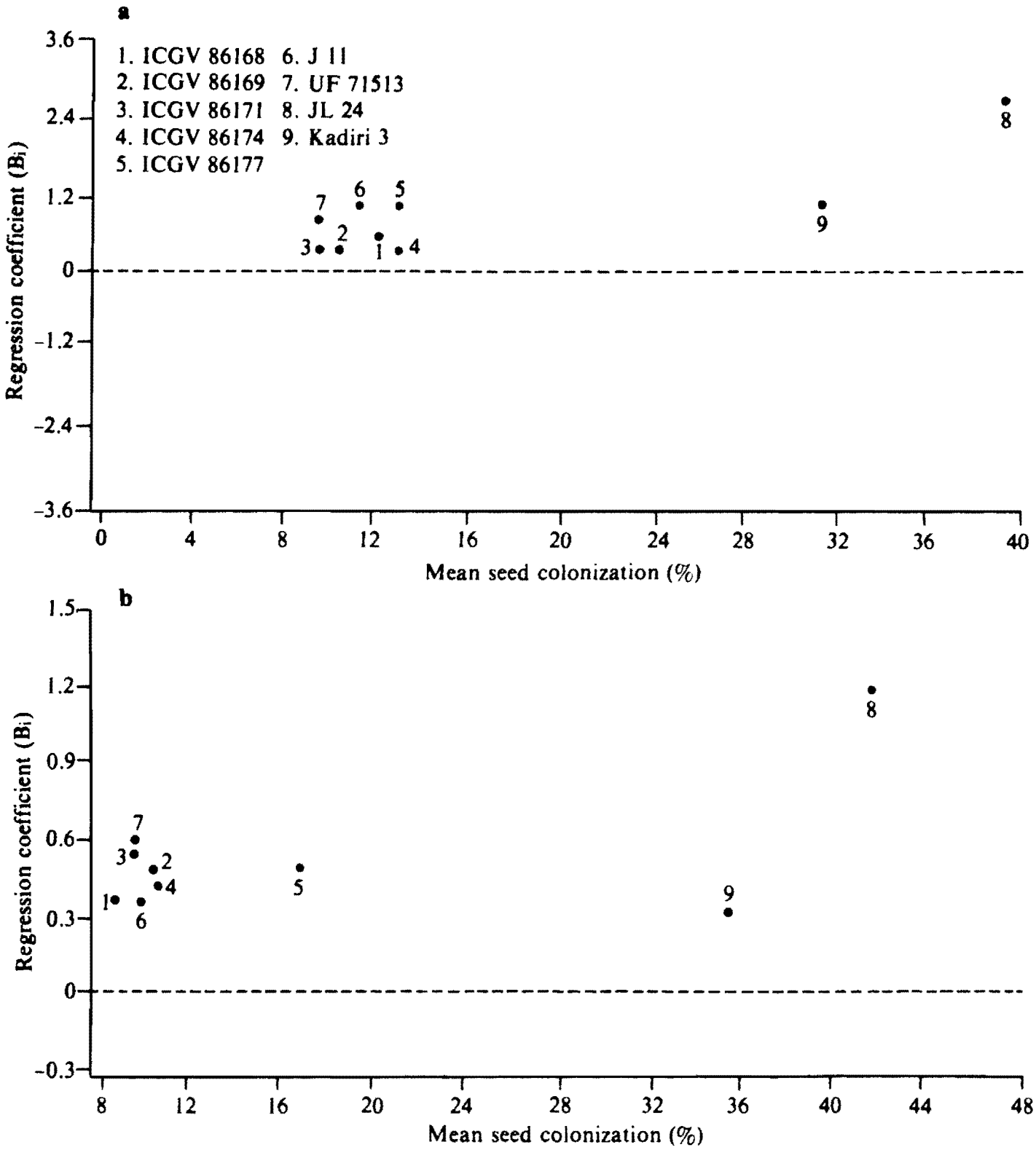

Figure 1. Stability of groundnut seedcoat resistance, ICRISAT Center. a. rainy season 1984, b. rainy season 1986.

Table 3. Stability parameters of six groundnut breeding lines obtained from seven Indian locations, rainy season 1986.

Identity	Pod yield (kg ha^{-1})			Seed colonization (%)		
	Mean	B_i	S^2d_i	Mean	B_i	S^2d_i
ICGV 86168	1833	1.173	76534	9.17	0.37	-1.74
ICGV 86169	1735	1.084	-3258	10.31	0.46	-3.65
ICGV 86170	1571	0.844	37960	16.87	0.50	38.26
ICGV 86171	1617	0.974	25471	9.36	0.56	-4.65
ICGV 86174	1587	0.783	187221	10.21	0.44	-2.73
ICGV 86177	1740	1.143	59424	18.13	0.83	11.93
Controls						
J 11[1]	1552	0.765	56973	9.69	0.36	-2.87
UF 71513[1]	1424	0.834	67347	9.71	0.59	-0.15
JL 24[2]	2001	1.229	-12013	41.61	1.21	23.01
Kadiri 3[2]	1570	0.810	115669	34.94	0.29	4.99

1. Resistant control varieties.
2. Susceptible control varieties.

Breeding for *A. flavus* Resistance at Other Locations

The group at Tifton, Georgia, USA lead by A.C. Mixon who first identified resistance in PI 337394F and PI 337409, have successfully transferred this resistance to other genetic backgrounds (Mixon 1983 a, 1983 b), producing the lines AR 1 to 4 which retain their resistance and yield more than their resistant parents in the USA. Breeding activities are in progress in Thailand (TCGIP 1985), and Senegal (Waliyar, Personal communication). At all the centers PI 337394F, PI 337409, UF 71513, and J 11 are common resistant parents in use as gene donors. In addition, scientists in Thailand have used AR 1 to 4 as new sources of resistance, and the variety 55-437 has been used in Senegal. A dry seed inoculation laboratory technique was used to screen selected lines in Thailand (Waranyuwat and Bhumibhaman 1985).

Genetics of Seed-coat Resistance

Of the different resistance traits, the genetics has been studied only for seed-coat resistance, and only one report (Mixon 1979) is available. This study, which evaluated the frequency distribution for percentage seed colonization from F_1 and F_2 plants of reciprocal crosses between PI 337409 (resistant) and PI 331326 (susceptible), indicated a broad sense heritability value of 78.5%. Subsequently, Mixon reported some more segregating population-evaluations to understand the genetics, but the conclusions were incomplete.

Investigations at ICRISAT Center have concentrated on understanding the combining abilities of the resistant parents. The main problem in the studies on inheritance of seed-coat resistance is the improper matching of the filial generations among testa, cotyledon, and embryo in the seed. In the seed of any of the filial generations, the testa belongs to the maternal generation while the cotyledons and embryo belong to the next generation. Thus, it has to be assumed that seed-coat resistance located in the testa is not influenced by the hybridity or

Table 4. General combining ability (GCA) effects for seven parental lines for seed-coat resistance in a F_0 line × tester study, ICRISAT Center.

Parent	GCA effect
Testers	
Kadiri 3	+ 0.97
ICGS(AF)78	+ 1.97
U 4-7-5	- 2.95
Lines	
UF 71513	- 10.57
Ah 7223	- 20.62
J 11	- 1.82
Var. 27	+ 33.02
SE (Line)	± 1.49
SE (Tester)	± 1.72

Table 5. General combining ability (GCA) effects for four groundnut lines for seed-coat resistance in a 4-parent F_0 and F_1 diallel, ICRISAT Center.

Parent	F_0 seed	F_1 seed
FESR-12-P_6-B_1-B_1	+27.04**	+26.85**
PI 337409	- 16.19**	- 8.84**
PI 337394F	- 6.88**	- 8.51**
UF 71513	- 2.61*	- 7.02**
Av. SE	± 1.03	± 1.13

Table 6. Reciprocal effects for six crosses for seed-coat resistance in a 4-parent F_0 and F_1 diallel, ICRISAT Center.

Cross[1]	F_0 seed	F_1 seed
P_1 × P_2	-[2]	+12.37**
P_1 × P_3	+ 0.41	+ 5.08*
P_1 × P_4	+34.92**	-[1]
P_2 × P_3	-0.45	-1.67
P_2 × P_4	+32.86**	+18.68**
P_3 × P_4	+ 5.08	-0.41
Av. SE	± 2.14	± 2.34

1. P_1 = FESR-12-P_6-B_1-B_1, P_2 = PI 337409, P_3 = PI 337394F, and P_4 = UF 71513.
2. Reciprocal cross missing.

heterosis exhibited by the cotyledon/embryo. Preliminary studies on combining ability using line × tester analysis on F_0 seed indicated that UF 71513 and Ah 7223 had significant negative GCA effects and therefore were good combiners for seed-coat resistance (Table 4). Variety J 11 registered a nonsignificant GCA effect. Var. 27 turned out to be a poor combiner. The F_0 and F_1 diallel study (Table 5) also indicated PI 337409, PI 337394F, and UF 71513 to be good combiners for seed-coat resistance. Significant reciprocal effects were noticed in some crosses both in F_0 and F_1 for seed-coat resistance (Table 6), perhaps because of the significant maternal influence on testa structure.

Problems in Breeding for *A. flavus* Resistance and Future Priorities

The Resistance Trait

At least three possibly interdependent resistance traits are known to operate but exact information on their relationships, interactions, and their possible contributions to reducing aflatoxin contamination of groundnut are not clearly established. Their relationships with preharvest natural seed infection and infections during postharvest handling and storage are not fully understood. Research is required to understand these traits and their interdependence, so that breeding activities can be properly focused.

Environmental Influences on Resistance Traits

Two questions arise concerning environmental influences on resistance traits; on the usefulness of breeding for these traits, and on the problem of the extensive sampling required to confirm the stability of resistance. Efforts are required to strengthen the sources of resistance by crossing lines with different resistance traits and bringing together the different resistance genes (assuming that the resistance genes are non-allelic).

Screening Techniques

Currently available screening techniques for low aflatoxin production are expensive. Cheaper and more reliable techniques are needed. Techniques to screen single plants for all the resistance traits should be developed.

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Discussion

B. Singh. How long does it take to identify a resistant line?

M.J. Vasudeva Rao. It may take a long time to identify a genotype with seed resistance to infection by *A. flavus* as this depends on the heritability of the trait, environmental influence, screening facilities available, etc. We do, however, know from work over the past 15-20 years that resistance does exist in some genotypes. To breed a cultivar with acceptable agronomic traits could take several years if there were significant deficiencies in the quality and yielding ability of the resistant source line.

B. Singh. The problem of aflatoxin contamination in groundnut is an urgent one in countries such as Senegal.

R.V. Bhat. It is very difficult to predict exactly how long it will take to identify resistant genotypes and to come up with resistant cultivars of good agronomic character. Investigations on possible varietal resistance commenced in the 1960s. There are problems due to environmental variation and to differences in the toxigenic fungi involved. However, there is no doubt that the production of a cultivar resistant to *A. flavus* infection of seeds and/or to aflatoxin production is the best approach to the problem.

K.J. Middleton. I am glad that this subject has been raised, as the use of *A. flavus*-resistant cultivars would be an ideal way to control the aflatoxin contamination problem. We know that it is not going to be easy to produce such cultivars, so in the meantime we have to put together a management package of all the possible ways of preventing and controlling the contamination.

M.J. Vasudeva Rao. I agree with you. We believe that a resistant variety should be a component of an integrated management package.

Polyphenols in Groundnut Genotypes Resistant and Susceptible to Seed Colonization by *Aspergillus flavus*

R. Jambunathan, V.K. Mehan, and Santosh Gurtu[1]

Abstract

Thirteen groundnut genotypes, eight resistant and five susceptible to in vitro seed colonization by Aspergillus flavus *were grown in replicated trials at three locations in Andhra Pradesh, India. Seed coats of these genotypes were analyzed for polyphenols using different methods. No significant correlation was observed between seed colonization and polyphenols content, which corroborates earlier observations on many genotypes using a single method for polyphenols estimation.*

Résumé

Les polyphénols dans les génotypes d'arachide résistants ou sensibles à la colonisation des graines par *Aspergillus flavus*: *Treize génotypes d'arachide, dont huit résistants et cinq sensibles à la colonisation in vitro des graines par* Aspergillus flavus *ont été étudiés dans le cadre d'essais répétés sur trois sites en Andhra Pradesh (Inde). Les téguments de ces génotypes ont été analysés par différentes méthodes pour la présence des polyphénols. On n'a observé aucune corrélation significative entre la colonisation et la teneur en polyphénols, ce qui corrobore une observation antérieure faite sur plusieurs génotypes en utilisant une seule méthode d'estimation des polyphénols.*

Resúmene

Los polifenoles presentes en los genotipos de cacahuate resistentes y susceptibles a la colonización de las semillas por *Aspergillus flavus*: *Trece genotipos de cacahuates, ocho resistentes y cinco susceptibles a la colonización de la semilla* in vitro *por* Aspergillus flavus, *se evaluaron en pruebas replicadas en tres localidades de Andhra Pradesh, India. Los tegumentos de las semillas de estos genotipos se analizaron para determinar su contenido de polifenoles, usando diferentes métodos. No se observó una correlación significativa entre la colonización de las semillas y su contenido de polifenoles, lo cual corrobora observaciones anteriores hechas en gran número de genotipos, usando un solo método de cuantificación de polifenoles.*

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Introduction

Tannins, which are polyphenols, may be defined as water-soluble phenolic metabolites of plants, with molecular weight of 500 or more, and with the ability to bind to and/or precipitate gelatin and/or other proteins from aqueous solutions (Butler In press). Tannins isolated from groundnut seed coat produce cyanidin during acid hydrolysis and therefore belong to the class of condensed procyanidins (Lansden 1982). Turner et al. (1975) isolated 5-7-dimethoxyisoflavone from groundnut and observed that it inhibited the growth of *Aspergillus flavus* Link ex Fries. Sanders and Mixcn (1978) reported a significant and negative correlation between percentage seed colonization by *Aspergillus parasiticus* Speare and tannin content in groundnuts. Amaya et al. (1980) reported a possible relationship between resistance to *A. flavus* and total soluble amino compounds and arabinose content in groundnut cultivars. Lansden (1982) isolated three fractions of tannins from groundnut testae and observed that the fractions inhibited the growth of *A. parasiticus*. Karchesy and Hemingway (1986) have identified various compounds that are present in groundnut seed coats and determined the structure of some of the isolated polyphenolic compounds. We examined polyphenol content of seed coats in relation to seed colonization by *A. flavus* in several groundnut genotypes.

Materials and Methods

Fifty groundnut genotypes grown at ICRISAT Center in the 1982 rainy season, and 13 grown in replicated trials at three locations (ICRISAT Center, Tirupati, and Anantapur) in Andhra Pradesh State, India, in the 1985 rainy season were used in this study. The genotypes were tested for their reactions to in vitro seed colonization by *A. flavus* (Mehan and McDonald 1980).

Sample Extracts

Seed coats of groundnut genotypes were ground to a fine powder in a PICA® mill (Pitchford Industries, Pittsburg, USA), and defatted with n-hexane in a Soxhlet apparatus. The samples were air dried and then further dried at 37°C for 2 h. Ground seed coat (100 mg) was extracted with 5 mL methanol for 1 h in a test-tube mixer (Stuart Tube Rotator TR-2®, UK). After centrifuging, the supernatant was saved and the residue was reextracted with 5 mL methanol for 30 min and the methanol extracts were pooled together. The residue was extracted with 5 mL methanol containing 1% concentrated hydrochloric acid (vol/vol) and after centrifuging, this procedure was repeated and the extracts were pooled together. Both the methanol and acidified methanol extracts were used for the estimation of polyphenols. Analyses were carried out on individual replicate seed-coat samples from two locations as well as on pooled samples from all three locations. Results obtained on pooled samples are reported here as the agreement between the values obtained on individual replicate and pooled data was excellent.

Polyphenol Estimation

Total phenols were estimated using the modified Prussian blue method (Butler 1982). Total phenols were also extracted independently from each seed coat sample using acidified methanol extract and then estimated by the Folin-Denis method (AOAC 1984). Protein-precipitable

phenols were estimated by using a modified procedure of Hagerman and Butler (1978) as described by Jambunathan et al (1986). The vanillin assay was carried out with glacial acetic acid (Butler et al. 1982). Leucoanthocyanidin and proanthocyanidin were estimated according to Walterson and Butler (1983). All the results are expressed as absorbance units g^{-1} of defatted seed coat except in the case of the Folin-Denis method where the results are expressed as mg g^{-1} of tannic acid equivalents.

Results and Discussion

The ranges and means of seed colonization and polyphenols contents in 50 genotypes are shown in Table 1. A significant and negative correlation ($r = 0.33$, $P < 0.05$) was found between seed colonization by *A. flavus* and polyphenols content estimated by the Folin-Denis method. However, when the groundnut genotype OG 43-4-1, which showed 97% seed colonization and had the lowest polyphenols content (2 mg g^{-1}), was excluded from the analysis, the correlation coefficient ($r = 0.06$) became insignificant.

Table 1. Seed colonization (%) by *Aspergillus flavus* and polyphenol content in groundnut genotypes[1].

Number of genotypes		Seed colonization (%)	Polyphenols[2], ($\mu g\ g^{-1}$)	Correlation coefficient (r)
50	Range	25.6-97.0	2-388	-0.33*
	Mean	40.4	267	
	SE	±1.7	±10.1	
49	Range	25.6-71.6	149-388	0.06
	Mean	39.2	272	
	SE	±1.3	±8.6	

1. Rainy season 1982.
2. Estimated by the Folin-Denis method on acidified methanol extract.
* Significant at $P < 0.05$.

The reactions of 13 groundnut genotypes to in vitro seed colonization by *A. flavus* are shown in Table 2. All the resistant lines had less than 15% seed colonized, except ICGS 58 at Tirupati (19.8%) and ICGS 78 (39.3%), ICGS 58 (36.8%), and PI 337394 F (15.8%) at Anantapur. All the five susceptible genotypes had more than 30% of their seed colonized across all three locations. The ranges and mean values of polyphenols estimated by the Folin-Denis method in the acidified methanol extract of seed coats and by Prussian blue method in the methanol extract are shown in Table 3. Variation between resistant and susceptible cultivars at any of the locations was not apparent from these values. The vanillin assay method has been used as one of the standard methods for the estimation of degree of polymerization of proanthocyanidin. The ranges and mean values obtained did not show a large variation between the resistant and susceptible genotypes at any location though the absorbance values g^{-1} of the seed coat were high for both the resistant and susceptible genotypes (Table 4). In contrast, protein-precipitable

Table 2. Reaction of groundnut genotypes from three Indian locations to in vitro seed colonization by *Aspergillus flavus*.

Genotypes	Reaction[1]	Seed colonized (%)		
		ICRISAT Center	Tirupati	Anantapur
Ah 7223	R	8.9	8.2	10.7
J 11	R	10.5	10.8	10.3
U 4-47-7	R	14.8	10.0	13.8
UF 71513	R	11.1	11.1	11.1
PI 337394F	R	11.5	14.0	15.8
Var 27	R	13.8	8.1	10.0
ICGS 58	R	13.2	19.8	36.8
ICGS 78	R	12.5	11.5	39.3
TMV 2	S	32.4	32.0	33.3
Faizpur 1-5 × NC Ac 17090	S	33.9	38.0	44.2
Gangapuri	S	37.4	34.0	34.6
NC Ac 17090	S	36.5	45.2	46.3
EC 76446(292)	S	38.0	44.9	41.1
SD		±12.1	±14.5	±14.7

1. R = Resistant; S = Susceptible.

Table 3. Polyphenol content in seed coats of groundnut genotypes grown at three Indian locations[1].

Number of genotypes	Folin-Denis (mg g^{-1})[2]			Prussian blue (A_{720} g^{-1})[3]		
	ICRISAT Center	Tirupati	Anantapur	ICRISAT Center	Tirupati	Anantapur
8 Resistant	224	221	215	262	265	343
	(171–271)	(124–255)	(167–236)	(146–422)	(96–438)	(232–440)
SD	±29.2	±41.3	±22.6	±81.8	±95.6	±68.5
5 Susceptible	220	238	223	279	302	343
	(161–286)	(163–285)	(175–272)	(149–453)	(100–467)	(246–507)
SD	±46.2	±45.9	±34.6	±110.0	±131.7	±105.3

1. Means and (ranges) are given.
2. Acidified methanol extract.
3. Methanol extract.

phenols estimated using bovine serum albumin showed much lower values in the resistant and susceptible genotypes though the susceptible genotypes had slightly higher values than resistant cultivars (Table 4). However, the differences were not large enough to distinguish between susceptible and resistant types. We observed that leucoanthocyanidin concentration in susceptible genotypes was higher at each of the locations than the resistant genotypes and the leucoanthocyanidin values were the lowest of all the polyphenols that were estimated in these samples (Table 5). However, when they were heated to measure proanthocyanidin, dramatic increases in the values were observed (Table 5).

Table 4. Polyphenol content (A510 g^{-1})[1] in seed coats of groundnut genotypes grown at three Indian locations[2].

Number of genotypes	Vanillin assay			Protein-precipitable phenols		
	ICRISAT Center	Tirupati	Anantapur	ICRISAT Center	Tirupati	Anantapur
8 Resistant	645	664	716	59.6	68.1	73.0
	(330-1020)	(196-1046)	(408-830)	(22.4-95.8)	(18.6-92.0)	(36.4-95.0)
SD	±199.4	±242.7	±141.0	±20.7	±22.9	±18.7
5 Susceptible	665	624	664	68.5	70.9	76.0
	(295-1102)	(190-984)	(388-1028)	(20.2-100.6)	(24.4-94.4)	(35.4-106.6)
SD	±288.2	±303.4	±248.2	±33.1	±26.8	±27.1

1. Absorbance at 510 nm calculated as units g^{-1}, methanol extract.
2. Means and ranges are given.

Table 5. Polyphenol content (A550 g^{-1})[1] in seed coats of groundnut genotypes grown at three Indian locations.

Number of genotypes	Leucoanthocyanidin			Proanthocyanidin		
	ICRISAT Center	Tirupati	Anantapur	ICRISAT Center	Tirupati	Anantapur
8 Resistant	15.1	9.8	11.0	659	585	742
	(11-19)	(4-16)	(9-12)	(230-1130)	(140-884)	(308-996)
SD	±3.1	±3.4	±0.8	±253.4	±216.7	±207.3
5 Susceptible	24.0	14.6	19.4	559	594	734
	(10-63)	(3-48)	(9-54)	(220-1185)	(112-926)	(324-1164)
SD	±22.8	±18.8	±19.5	±391.8	±296.7	±318.9

1. Absorbance at 550 nm calculated as units g^{-1}, methanol extract.

Although only the results obtained with the methanol extract have been discussed, the results obtained with the acidified methanol extract also did not show any large differences in the ranges or in the mean values between resistant and susceptible genotypes. Table 6 shows the correlation coefficients between the results obtained with polyphenol methods and the seed colonization data for groundnut genotypes grown at ICRISAT Center, Tirupati, and Anantapur. There was no significant correlation between any of the methods and seed colonization data. Also, no significant correlation was obtained between seed colonization data and the results of polyphenols obtained with acidified methanol extract of 8 resistant and 5 susceptible genotypes (Table 6). When the data for total polyphenols (polyphenols measured in methanol plus acidified methanol extracts) was compared with the seed colonization data from each of the locations, no significant correlation was obtained (Table 6).

Analysis of variance was carried out to examine the differences in resistant versus susceptible groups, genotypes within the groups, and their interaction with locations for seed colonization and various polyphenols estimations. Results showed (Table 7) significant interactions between the groups, and also between genotypes within the groups with locations for most of the

Table 6. Correlation coefficients between seed colonization by *Aspergillus flavus* and polyphenols content of seed coats of groundnut genotypes grown at three Indian locations.

	ICRISAT Center			Tirupati			Anantapur		
	M[1]	AM	MAM	M	AM	MAM	M	AM	MAM
Total phenols (Prussian blue)	0.11	-0.08	0.11	0.28	0.32	0.40	0.40	0.31	0.49
Protein-precipitable phenols	0.19	-0.29	0.15	0.23	-0.07	0.25	0.45	-0.35	0.44
Vanillin Assay	0.04	-0.25	0.01	0.03	-0.40	0.01	0.12	0.28	0.16
Leucoanthocyanidin	0.38	-0.09	0.38	0.34	0.11	0.30	0.33	0.29	0.32
Proanthocyanidin	-0.19	-0.19	-0.21	0.17	-0.06	0.15	0.34	-0.27	0.27
Folin Denis	-	-0.05	-0.05	-	0.32	0.32	-	0.46	0.46

1. M = Methanol extract; AM = Acidified methanol extract; MAM = Methanol plus acidified methanol extract.

Table 7. Analysis of variance (mean squares and probability levels).

		Variables						
Source	d.f.	SC[1]	PB	PP	VA	L	FP	FD
Location	1	17.9	13389.5	8204.1	615.0	3.2	41961.0	1295.1
Replicate/ Location	6	6.3	1718.7	833.8	10317.0	21.3	19202.0	22.9
Genotype	12	1376.4	67234.3	4658.3	403630.3	1298.3	540923.0	9574.8
Resistant (R) vs Susceptible (S)	1	15776.9	1380.0	4166.0	7939.0	796.3	28613.0	232.3
Resistant	7	42.1	60533.9	3410.0	337743.0	23.8	409652.0	7009.2
Susceptible	4	111.3	95423.6	6964.6	617855.0	3655.2	898725.0	16400.3
Genotype × location	12	39.0	4471.6	329.7	41741.3	83.2	31494.0	681.3
(R vs S) × location	1	71.5	2700.9	354.6	16089.0	447.2	7920.0	61.3
Resistant × location	7	31.6	5195.8	195.0	49639.0	61.1	17054.0	467.2
Susceptible × location	4	46.2	3646.9	559.0	34333.0	30.8	62656.0	1210.8
Error	72	5.1[2]	360.0	73.8	4263.0	4.04	5065.0	73.8

1. SC : Seed colonization; PB : Prussian blue; PP : Protein-precipitable phenols; VA : Vanillin acetic acid; L : Leucoanthocyanidin; FP : Proanthocyanidin; and FD : Folin Denis.
2. Error d.f. for SC is 64 due to 8 missing observations.

** $P < 0.01$

variables (as denoted by asterisks). Sanders and Mixon (1978), using the Folin-Denis method, reported a negative and significant correlation (r = 0.76) between seed color and tannin content in 16 groundnut genotypes. The tannin content in seed coats of these samples ranged from 33.5 mg g^{-1} in the highly susceptible to 344 mg g^{-1} in the resistant genotypes. In our 13 genotypes, the lowest value was 161 mg g^{-1} obtained in the susceptible genotypes grown at ICRISAT Center, and the highest value was again obtained in the susceptible genotype grown at ICRISAT Center that had 286 mg g^{-1}. In the 49 genotypes that were grown in the 1982 rainy season, the lowest value was 149 mg g^{-1} and the highest value was 388 mg g^{-1}. Thus our values in the susceptible cultivars were much higher than those reported by Sanders and Mixon (1978). However, the seed colonization data of Sanders and Mixon (1978) showed a range between 10.3 and 91.3%

while our seed colonization data on 13 genotypes ranged from 8.1 to 46.3%. In our data obtained on 50 genotypes, if we eliminated one genotype that showed 97.0% seed colonization, the correlation was not significant. It is true that the absolute quantity of polyphenols reported from laboratories varies widely because of the nature of the compound measured, the methodologies involved, and the influence of such other factors as environment and laboratory variations. However, we observed no significant correlation between seed colonization data and polyphenols content in methanol and acidified methanol extract using a variety of methods.

We made an attempt to find out if any of the various methods of polyphenols estimation could be useful to aid pathologists and plant breeders in evaluating and screening resistant and susceptible groundnut genotypes. Although we observed no significant correlation between seed colonization data and polyphenols, it is necessary to determine whether any of the various individual polyphenolic compounds could be involved in resistant characteristics of groundnut genotypes. However, from the data obtained so far, we can conclude that total polyphenols estimation in groundnut seed coat is not a reliable indicator when screening resistant genotypes for seed colonization by *Aspergillus flavus*.

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Discussion

R.E. Pettit. I understand that what you are reporting here are total phenols, if you look at the individual phenol compounds you might get a better correlation.
R. Jambunathan. I agree; and we plan to do this when we have found suitable methods.

Geocarposphere Mycoflora and Resistance of Groundnut to *Aspergillus flavus*

S. Nahdi[1]

Abstract

Four groundnut genotypes, two resistant and two susceptible to in vitro seed colonization by Aspergillus flavus *(IVSCAF), were grown in field trials at ICRISAT Center in the 1984 and 1985 rainy seasons. Geocarposphere mycoflorae were examined and significant quantitative and qualitative differences were observed between genotypes. Populations of* A. flavus *were higher in the geocarpospheres of the IVSCAF-susceptible genotypes than in those of the IVSCAF-resistant genotypes.*

Genotypes were also evaluated at the time of harvest for levels of seed infection by A. flavus. *The IVSCAF-susceptible genotypes had higher levels of infection in seed from nondamaged mature pods than had seed from IVSCAF-resistant genotypes.*

In a greenhouse experiment, exudates were collected from pods of the four genotypes. Exudates from the two IVSCAF-resistant genotypes inhibited in vitro germination of A. flavus *spores to a greater degree than did exudates from pods of IVSCAF-susceptible genotypes. The implications of these findings are discussed.*

Résumé

Microflore géocarposphérique et résistance des cultivars d'arachide à l'invasion par *Aspergillus flavus*: *Quatre génotypes d'arachide, dont deux résistants et deux sensibles à la colonisation in vitro des graines par* Aspergillus flavus *(IVSCAF), ont été étudiés dans le cadre d'essais aux champs au Centre ICRISAT pendant les saisons pluviales de 1984 et 1985. L'examen de la microflore géocarposphérique a révélé des différences quantitatives et qualitatives significatives entre les génotypes. La population d'*Aspergillus flavus *est plus élevée dans les géocarposphères des génotypes sensibles par rapport aux génotypes résistants.*

Les niveaux d'infection des graines par A. flavus *ont été également étudiés pour différents génotypes à la récolte. Les génotypes sensibles au test IVSCAF montrent des taux d'infection plus élevés, dans les graines provenant de gousses mûres et intactes, que les génotypes résistants au test IVSCAF.*

*Dans un essai conduit en serre, les exsudats des gousses de quatre génotypes ont été ramassés. Les exsudats prélevés de deux génotypes résistants à IVSCAF ont inhibé la germination in vitro des spores d'*A. flavus *plus que les exsudats des gousses des génotypes sensibles à IVSCAF. Les conséquences de ces résultats sont examinées.*

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Resúmene

La micoflora geocarposférica y la resistencia del cacahuate a *Aspergillus flavus* : *Cuatro genotipos de cacahuate, dos resistentes y dos susceptibles a la colonización de las semillas* in vitro *por* Aspergillus flavus *(CSIVAF) se evaluaron en pruebas de campo en el Centro ICRISAT, durante las temporadas lluviosas de 1984 y 1985. Se examinó la micoflora geocarposférica encontrándose diferencias cualitativas y cuantitativas entre los genotipos estudiados. Las poblaciones de* A. flavus *fueron mayores en las geocarpósferas de los genotipos susceptibles a CSIVAF, que en los genotipos resistentes a la misma.*

Los genotipos también se evaluaron en el momento de la cosecha para cuantificar los niveles de infección de las semillas por A. flavus. *Los genotipos sensibles en la prueba CSIVAF tenían niveles más altos de infección en las semillas procedentes de vainas maduras intactas, que las semillas procedentes de genotipos resistentes en la prueba CSIVAF.*

En un experimento de invernadero, se recolectaron los exudados de las vainas de cuatro genotipos. Los exudados procedentes de los dos genotipos resistentes en la prueba CSIVAF inhibieron la germinación in vitro *de las esporas de* A. flavus *en mayor grado que los exudados de las vainas de los genotipos susceptibles en la prueba CSIVAF.*

Introduction

It is established that the developing groundnut pod influences the constitution of microbial populations in the soil around it (McDonald 1969, Griffin 1972, Subrahmanyam and Rao 1977). Though quantitative differences have been found in geocarposphere mycoflorae of groundnuts grown in different parts of the world, qualitatively they have been remarkably similar. Such fungi as *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Fusarium oxysporum, Fusarium solani, Penicillium funiculosum*, and *Penicillium citrinum* have been commonly isolated from geocarposphere, shells, and seeds of groundnut (Diener et al. 1965, Joffee 1966, Jackson 1965, 1968). Of these, *A. flavus* is the most important as some strains produce the highly toxic and carcinogenic substances known as aflatoxins. Earlier studies on the groundnut geocarposphere were restricted to a few commercial cultivars that were all susceptible to pod and seed invasion by *A. flavus* (McDonald 1969, Subrahmanyam and Rao 1977).

In recent years, genetic resistance to invasion of seeds by *A. flavus* has been identified in some groundnut genotypes. Mixon and Roger (1973) and Mehan and McDonald (1983) reported varietal differences in reaction of groundnuts to in vitro seed colonization by *A. flavus*. Resistance was based in the testa and was lost if the testa was damaged.

Several genotypes resistant to in vitro seed colonization by *A. flavus* have shown good resistance to seed infection by *A. flavus* in the field, but so have some genotypes that do not have high resistance to *A. flavus* in laboratory inoculation tests. This study aimed to identify any quantitative or qualitative differences between the geocarposphere mycoflorae of genotypes resistant and susceptible to preharvest seed invasion by *A. flavus*, as it was thought that such differences could possibly influence pod invasion by *A. flavus* and other fungi.

Materials and Methods

Field Trials

All trials were conducted on Alfisols under rainfed conditions at ICRISAT Center farm,

Patancheru, India, in the 1984 and 1985 rainy seasons. Four groundnut genotypes of similar duration, TMV 2, EC 76446(292), PI 337394F and J 11 were grown in split-plot designs with three replications. J 11 and PI 337394F are resistant to seed invasion by *A. flavus*,and TMV 2 and EC 76446(292) are susceptible. Two sowing dates were used each year (14 and 29 Jun in 1984; 24 Jun and 15 Jul in 1985) to ensure different environmental conditions during the growing season. Plots were 9-m long and 4.5-m wide, and seeds were sown singly at 10-cm spacing along rows that were 75 cm apart.

Examination of geocarposphere soil

The geocarposphere mycoflorae were examined at different stages of maturity, (1) at 30 days before normal harvest, (2) at normal harvest, and (3) 10 days after normal harvest. To obtain geocarposphere soil two plants were taken from each row of each plot (i.e., 12 plants plot^{-1}). The plants were shaken to remove loosely adhering soil, and the most mature pods detached. The pods taken from each plot were bulked, and random samples of 10 undamaged pods were then taken. Each lot of 10 pods was placed in a sterile flask containing 50 mL of sterile distilled water. Flasks were then shaken for 2 min at 250 oscillations min^{-1}. Before the soil suspension had settled 1-mL samples were drawn off and sets of serial dilutions made. The dilution plate technique was used to obtain both qualitative and quantitative assessment of the geocarposphere mycoflora. Three petri dishes were used for each sample. One mL of the selected soil suspension was added to a sterile 9-cm diameter petri dish and then 20 mL of cooled, molten Czapek Dox-Rose Bengal-Streptomycin agar was poured into the plate which was swirled gently to mix the soil suspension throughout the medium. The plate was then incubated at 25°C for 7 days, and fungal colonies that had developed were then counted and identified. Each colony was considered to have originated from a single fungal propagule.

The quantity of soil present on each lot of 10 pods was determined in the following way. The pods were removed from the flasks, and any soil suspension still adhering to them was washed back into the flask with a stream of distilled water. The water in the flask was evaporated by heating and the mass of dry soil remaining in the flask determined. Numbers of fungal propagules g^{-1} of dry soil were calculated using the formula:

$$\text{Fungal population g}^{-1}\text{ of geocarposphere soil} = \frac{\text{Number of fungal colonies} \times \text{dilution taken}}{\text{Mass of dry geocarposphere soil}}$$

Fungi were also expressed as percentages of the total mycoflora using the formula:

$$\text{Percentage of a specific fungus} = \frac{\text{Number of specific fungal colonies} \times 100}{\text{Total number of fungal colonies}}$$

Testing for seed infection by fungi

At each harvest, samples of the most mature pods were also taken to estimate seed infection by *A. flavus*. Pods were hand shelled and 200 seeds from each plot were surface-sterilized by immersion in a 0.1% aqueous solution of mercuric chloride, rinsed in 3 changes of sterile water, then aseptically plated onto Czapek Dox-Rose Bengal-Streptomycin agar medium in 9-cm diameter petri dishes, 5 seeds to each plate. Plates were incubated at 25°C for 6 days, then any fungi growing out from the seeds were identified and recorded.

Greenhouse Trials

Collection of pod exudates and testing for effects on germination and growth of *A. flavus*

The same four genotypes (TMV 2, EC 76446(292), PI 337394F, and J 11) were grown in a greenhouse trial in specially designed plastic pots that enabled the roots and pegs/pods to develop in separate compartments (Fig. 1). One seedling was raised in each pot, with the roots in field soil and the pegs/pods developing in prewashed, sterilized coarse white sand (Fig. 2). Before pegs entered the fruiting medium, it was watered with 1000 mL $CaSO_4$ (gypsum) solution containing 1 gm Ca_2 $(1000\ mL)^{-1}$ deionized water. Taking 125 days after sowing as the normal maturity time, pods were lifted from the fruiting medium, without causing any damage to them, at 30 days before normal maturity, at normal maturity, and at 10 days after normal maturity. Around 10-15 mature, healthy, undamaged pods were taken from each plant and fully immersed in a beaker of glass-deionized distilled water. After 8 h, the pods were removed and all the pod exudates from one plant were pooled together. The fruiting medium (sand) in which the pods had developed was leached with water after the pods were removed. The pod exudate samples and leachates from the fruiting medium were filtered through two layers of Whatman No.1 filter paper, two to three times, and then freeze-dried in flasks. Flasks were sealed, cooled, and stored at 5°C until required for analyses.

Figure 1. Single groundnut plant growing in specially designed pots with rooting compartment (a) separated from fruiting compartment (b) greenhouse trial, ICRISAT Center, 1984.

Figure 2. Groundnut plant showing pegs and pods developing in fruiting medium of prewashed, sterilized sand, greenhouse trial, ICRISAT Center, 1984.

Effects of pod exudates on growth of *Aspergillus flavus*

During analyses, freeze-dried pod exudate samples were dissolved in 10 mL sterile glass-deionized distilled water and sterilized by Millipore filtration (pore size 0.22 μm).

Plates were prepared by pouring 1 mL of a spore suspension (4×10^8 spores mL^{-1} prepared from a 7-day-old culture) of *A. flavus* into a 9-cm diameter sterilized petri dish and adding 20 mL of cooled molten Czapek Dox-Rose Bengal-Streptomycin agar medium. A 6.25-mm diameter BBL disc loaded with 1 mL of pod exudate was placed on the cooled *A. flavus*-seeded plate, incubated at 25 to 27°C for 5 days, and then examined for any evidence of inhibition or stimulation of growth of the fungus. Five replicate plates were used for each sample.

Effects of pod exudates on germination of *Aspergillus flavus* spores

The hanging drop method was followed to study the effects of pod exudates on *A. flavus* spore germination. A spore suspension of *A. flavus* was prepared from 7-day-old cultures maintained on Czapek yeast extract medium and diluted to give 500–700 spores 0.1 mL^{-1} drop.

Sterilized pod exudate samples were shaken for 2 min on a vortex rotator and 0.2 mL of the sample was transferred into a sterilized vial containing 0.2 mL of *A. flavus* spore suspension. The sample and spore suspension were mixed thoroughly on a vortex rotator. Two drops of this mixture (exudate solution and spore suspension) were taken on sterilized slides and placed in a sterilized moist chamber. For each sample, five replicate slides were prepared. Moist chambers were kept in the semi-dark at 25–27°C. The germination of the spores and lengths of germ tubes were observed after 15–20 h of incubation. For all treatments a control was also included, 2 drops from a mixture of 0.2 mL of spore suspension and 0.2 mL of sterilized glass-deionized distilled water taken on a slide.

Statistical Analysis

Using arcsine transformed values, analyses of variance were done for seed infection levels by *A. flavus* and other fungi separately for two sowings of each field trial from the 1984 and 1985 rainy seasons. Analyses of variance were done separately for populations and also percentages of *A. flavus, A. niger, Penicillium* spp, and several other fungi in geocarposphere soil of four genotypes for two sowings of each trial of 1984 and 1985 rainy seasons. Using arcsine-transformed values, analyses of variances were also done for spore germination and germ tube lengths of *A. flavus* in pod exudates of four genotypes from the greenhouse trial.

Results and Discussion

Geocarposphere Mycoflorae

In both years, the total fungal populations in geocarposphere soil of the four genotypes increased with pod maturity. The second sowings showed higher total fungal populations in the geocarposphere than did the first sowings. Genotypes did not show significant differences for total fungal populations.

Populations of *A. flavus* increased as pods matured (Fig. 3). Significant differences were seen between genotypes for *A. flavus* populations in geocarposphere soil. Genotypes J 11 and PI

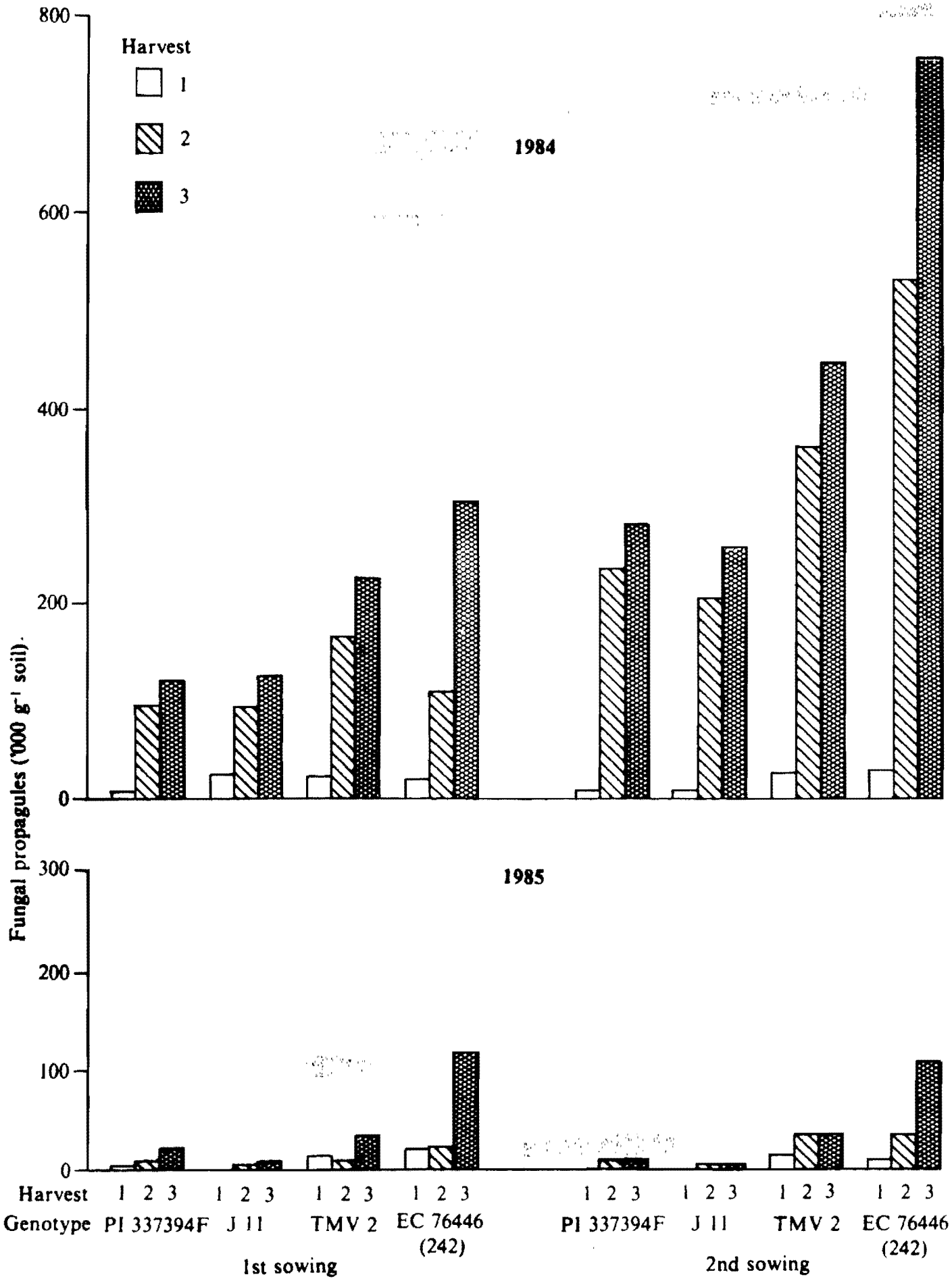


Figure 3. Populations of *Aspergillus flavus* in the geocarposphere soil, ICRISAT Center, 1984 and 1985.

337394F had significantly lower populations of *A. flavus* than had TMV 2 and EC 76446(292). Populations of *A. flavus* in the geocarposphere were quite low in 1985 for both sowings, presumably due to the severe drought (Fig. 4). The 1985 crop received less rainfall throughout the growing season. In 1985, the first sowing did not receive rainfall during the last 33 days, and the second sowing during the last 48 days of the crop cycle. The second sowing of 1984 lacked rainfall in the last 19 days of the crop cycle. Rainfall was regular throughout the crop growing cycle of the first sowing in 1984. Because of prolonged drought in 1985, the moisture content in geocarposphere zone might have fallen too low for the activity of *A. flavus*. The geocarposphere zone is affected before the shell and seed.

Significant genotypic differences were also observed for *A. niger* and *Penicillium* spp populations in geocarposphere soil. J 11 had significantly higher populations of *A. niger* than TMV 2 and EC 76446(292) in both sowings of 1984 and 1985 (Fig.5). PI 337394F had significantly higher populations of *Penicillium* spp (Fig.6). Among three *Penicillium* spp isolated, *P. funiculosum* was dominant. *Fusarium* spp also showed an increase with pod maturity but no significant differences between genotypes were observed.

There were significant differences among genotypes in the percentage of different fungi in the total fungal populations in geocarposphere soil (Fig.7). The *A. flavus* percentage was significantly lower in J 11 and PI 337394F than TMV2 and EC 76446(292), *A. niger* was higher in J 11 and *Penicillium* spp higher in PI 337394F genotype geocarpospheres. Joffe (1969) reported *A. niger* and *P. funiculosum* as antagonists to *A. flavus*. The antagonism between these fungi and interaction with other fungi may have resulted in low percentages of *A. flavus* in the geocarpospheres of J 11 and PI 337394F.

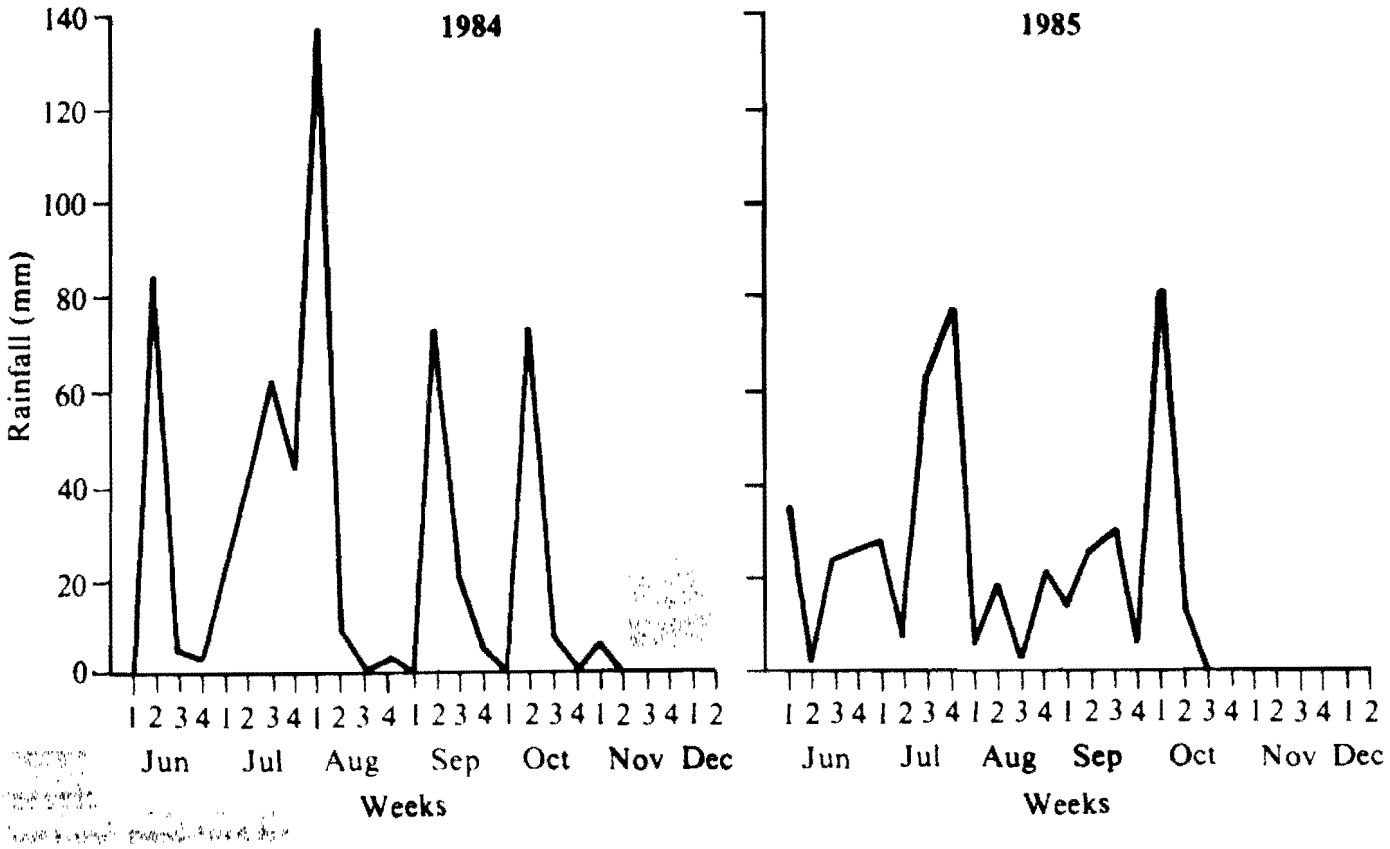


Figure 4. Weekly rainfall ICRISAT Center, Jun-Dec 1984 and 1985.

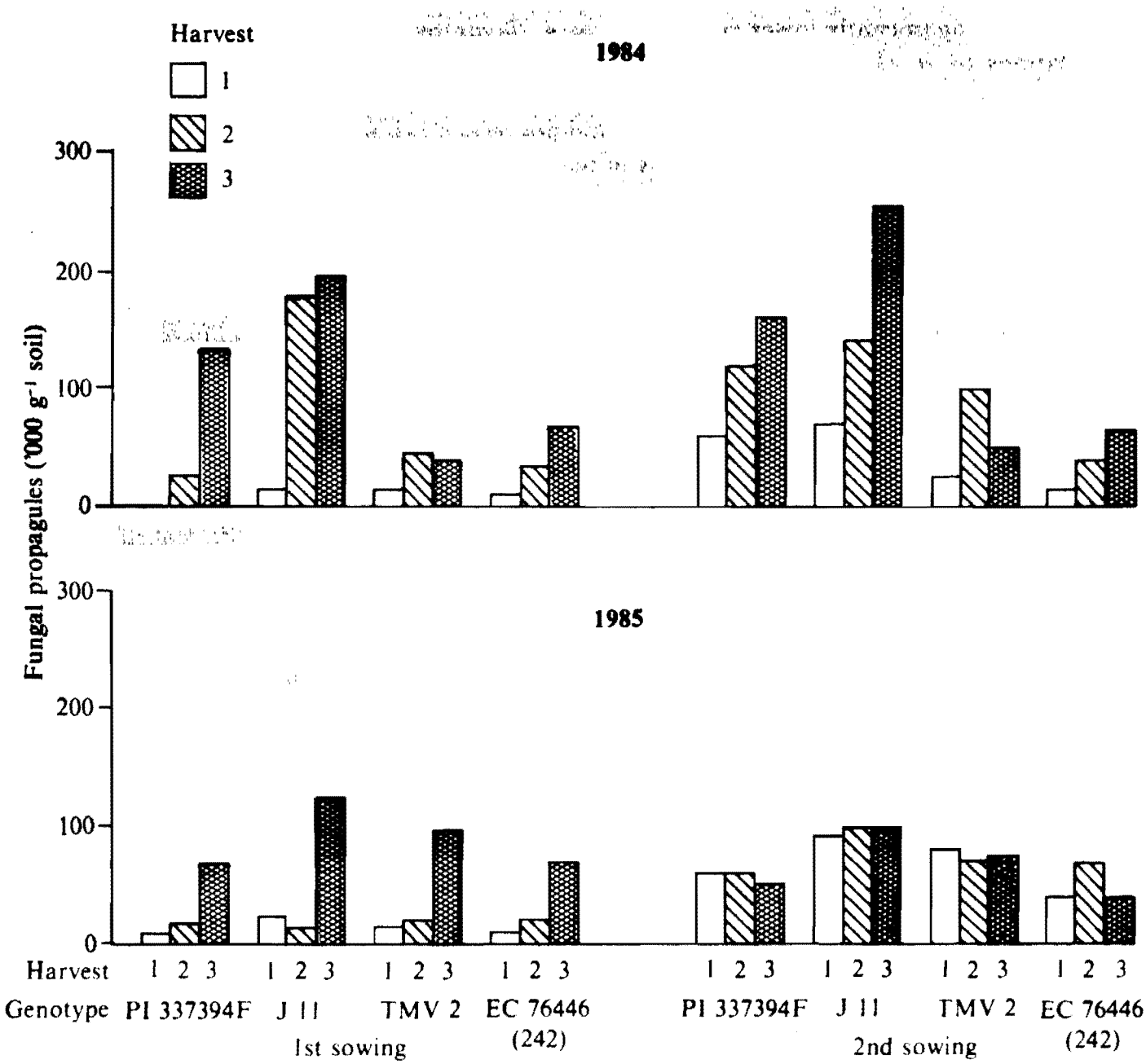


Figure 5. Populations of *Aspergillus niger* in the geocarposphere soil, ICRISAT Center, 1984 and 1985.

Natural Seed Infection by *A. flavus*

Results of seed infection by *A. flavus* of four genotypes in 1984 and 1985 are presented in Tables 1 and 2. Levels of seed infection in all genotypes increased with pod maturity. Genotypes differed significantly for *A. flavus* seed infection. EC 76446(292) and TMV 2 had significantly higher levels of infection than J 11 and PI 337394F. Infection levels were higher in the second sowing than the first sowing because the former crop was exposed to drought. The high levels of *A. flavus* infection in 1985 were possibly due to the severe drought experienced by the 1985 crop, as compared with the 1984 crop. Drought stress may eliminate microbial competitors of *A. flavus* by increasing the geocarposphere soil temperature. At the same time, it may increase susceptibility to fungal invasion by decreasing the moisture content of the pod and seed, and by reducing their physiological activity (Cole et al. 1985).

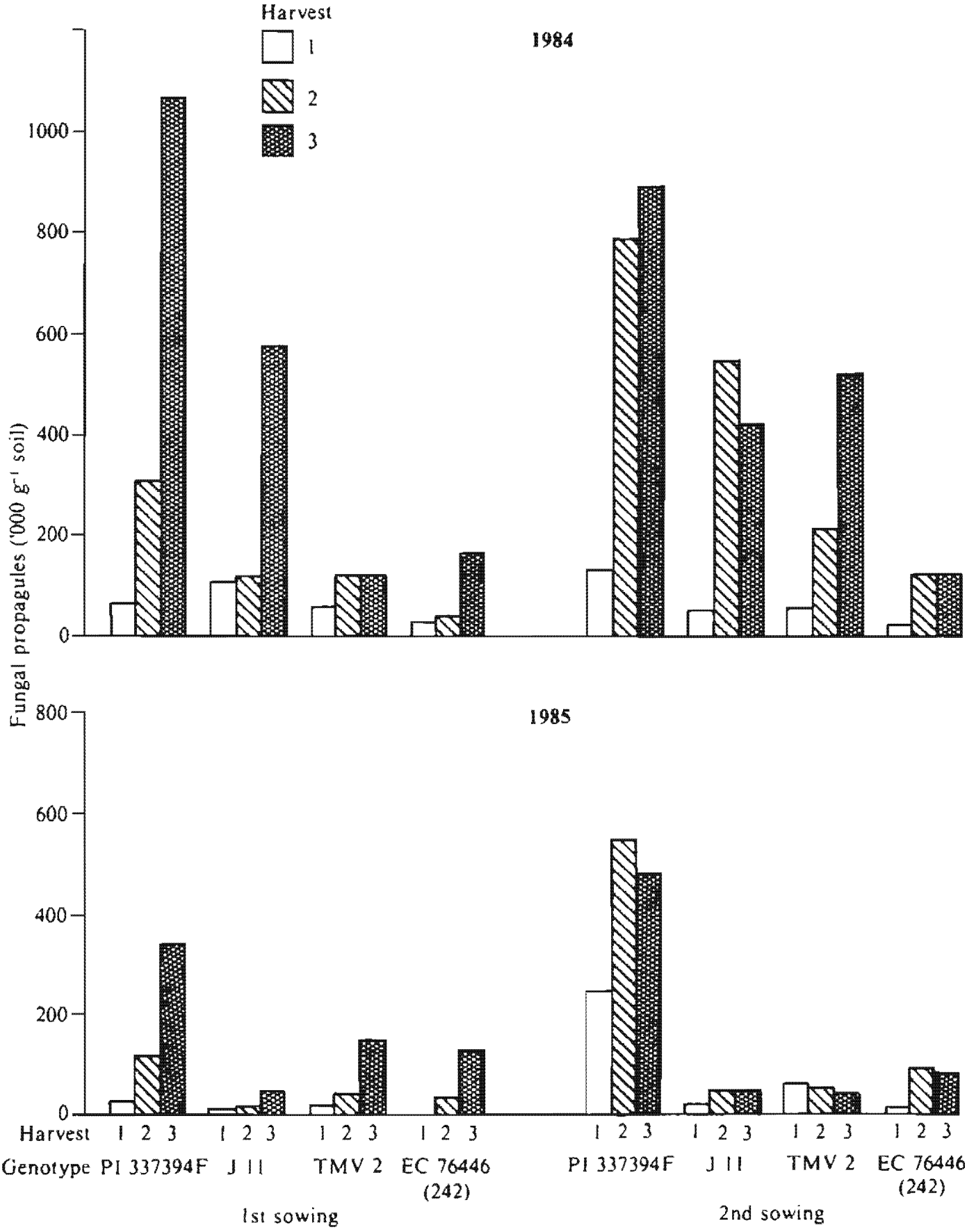


Figure 6. Populations of *Penicillium* spp in the geocarposphere soil, ICRISAT Center, 1984 and 1985.

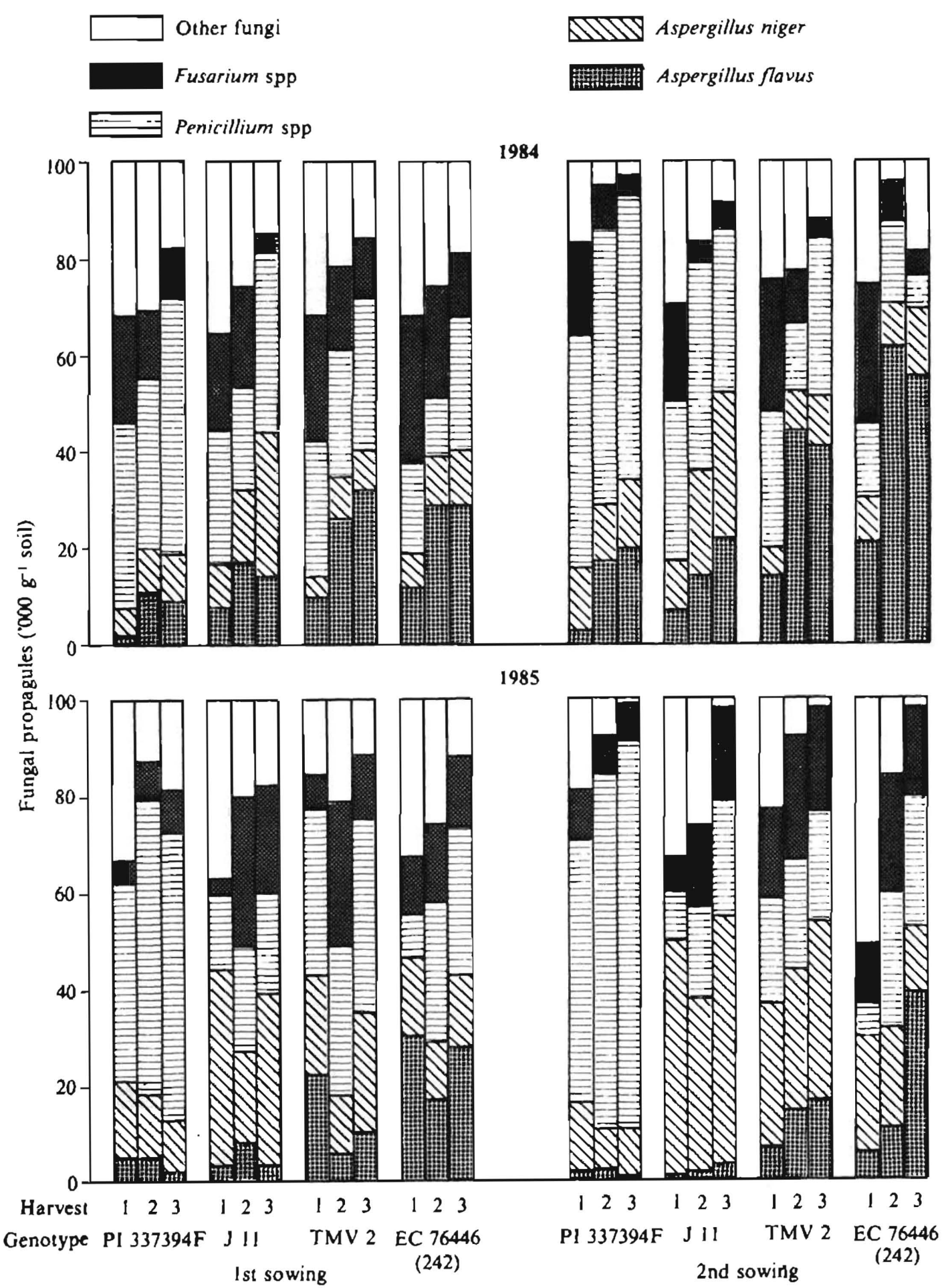


Figure 7. Different fungi as percentage of total fungal population in geocarposphere soil, ICRISAT Center, 1984 and 1985.

Table 1. *Aspergillus flavus* seed infection in four groundnut genotypes ICRISAT Center, rainy season 1984.

Genotype	Seed infected (%)					
	1st sowing			2nd sowing		
	H_1[1]	H_2	H_3	H_1	H_2	H_3
PI 337394F	1.3 (6.5)[2]	2.3 (8.7)	3.3 (10.5)	0.7 (2.7)	1.3 (6.5)	3.3 (10.3)
J 11	0.3 (1.9)	1.3 (6.5)	2.0 (8.0)	0.3 (1.9)	1.3 (6.5)	2.3 (8.7)
TMV 2	1.7 (7.3)	4.7 (12.5)	8.3 (16.7)	2.3 (8.7)	4.7 (12.4)	6.7 (14.9)
EC 76446 (292)	-	6.0 (14.1)	17.3 (24.5)	4.3 (11.9)	13.3 (21.4)	19.7 (25.7)
SE			(±1.2)			
CV (%)			(19.1)			

1. H_1 = Before maturity, H_2 = Normal harvest, H_3=Late harvest.
2. Figures in parentheses are arcsine-transformed values.

Table 2. *Aspergillus flavus* seed infection in four groundnut genotypes, ICRISAT Center, rainy season 1985.

Genotype	Seed infected (%)					
	1st sowing			2nd sowing		
	H_1[1]	H_2	H_3	H_1	H_2	H_3
PI 337394F	0.0 (0.0)[2]	0.7 (3.8)	2.3 (8.7)	1.3 (6.5)	2.7 (9.4)	3.0 (9.9)
J 11	0.0 (0.0)	0.3 (1.9)	1.0 (5.7)	0.7 (3.8)	1.0 (5.7)	1.7 (7.3)
TMV 2	1.3 (6.5)	2.7 (9.3)	4.3 (11.9)	5.3 (13.3)	9.3 (17.7)	10.0 (18.4)
EC 76446 (292)	4.0 (11.3)	7.0 (15.3)	11.3 (19.6)	10.3 (18.3)	18.3 (25.3)	22.0 (27.9)
SE			(±1.21)			
CV (%)			(17.3)			

1. H_1 = Before maturity; H_2 = Normal harvest; H_3 = Late harvest.
2. Figures in parentheses are arcsine-transformed values.

Influence of Pod Exudates on Germination of Spores and Growth of *A. flavus*

Samples of pod exudates of PI 337394F and J 11 collected at normal maturity and at 10 days after maturity produced small but clear inhibition zones around impregnated discs placed on petri dish cultures of *A. flavus* (Fig. 8a and b). Discs impregnated with exudates from pods of EC 76446 (292) were overgrown by *A. flavus* (Fig. 8c).

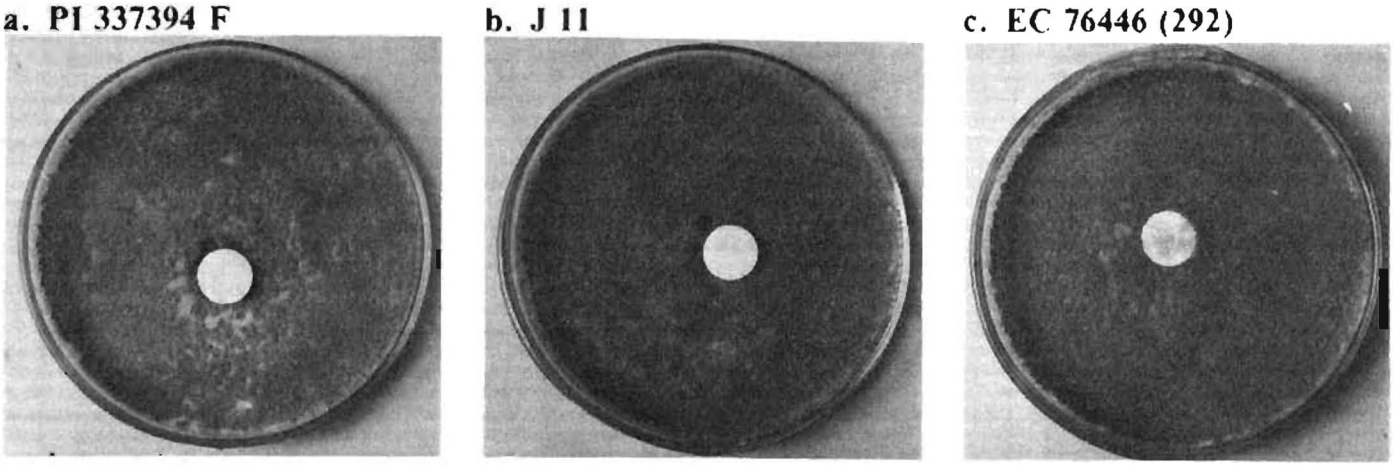


Figure 8. Growth inhibition of *Aspergillus flavus* around discs impregnated with pod exudates of groundnut genotypes; a. PI 337394 F, b. J 11, and c. EC 76446 (292)

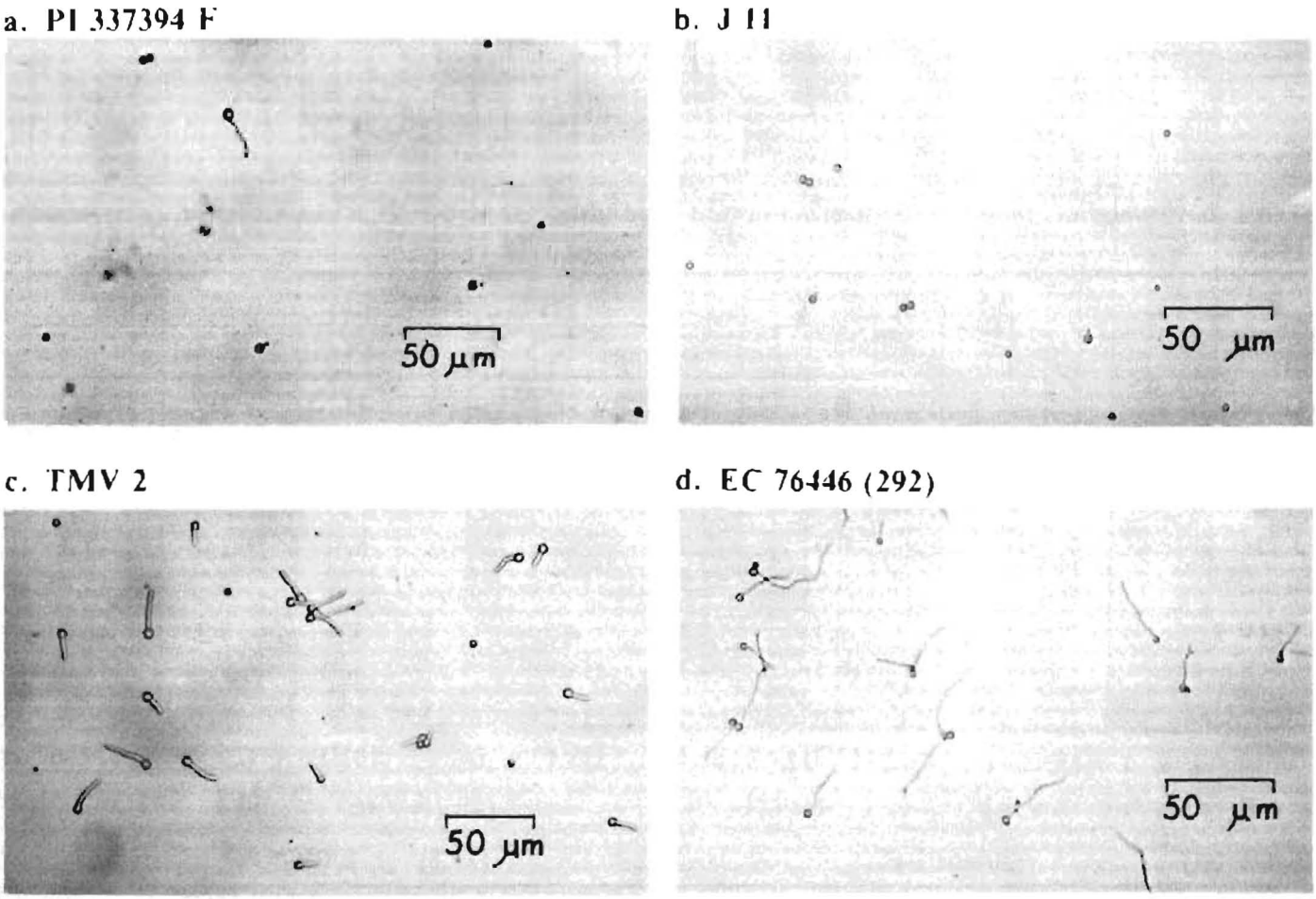


Figure 9. Spore germination of *Aspergillus flavus* in pod exudates of four groundnut genotypes; a. PI 337 394 F, b. J 11, c. TMV 2, and d. EC 76446 (292).

Effects of pod exudates on germination of *A. flavus* spores

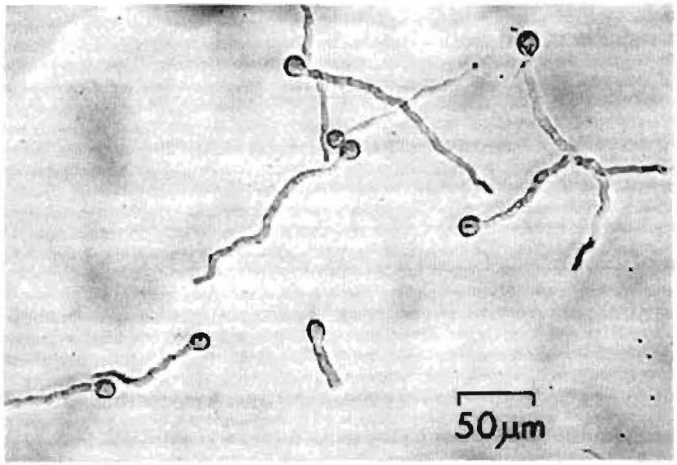
Spore germination in pod exudates of PI 337394F and J 11 samples collected at normal maturity was significantly lower than in exudates from pods of TMV 2 and EC 76446(292) (Fig. 9 a-d). Exudates collected 30 days before normal maturity showed no significant difference between cultivars.

Effect of pod exudates on germ tube length

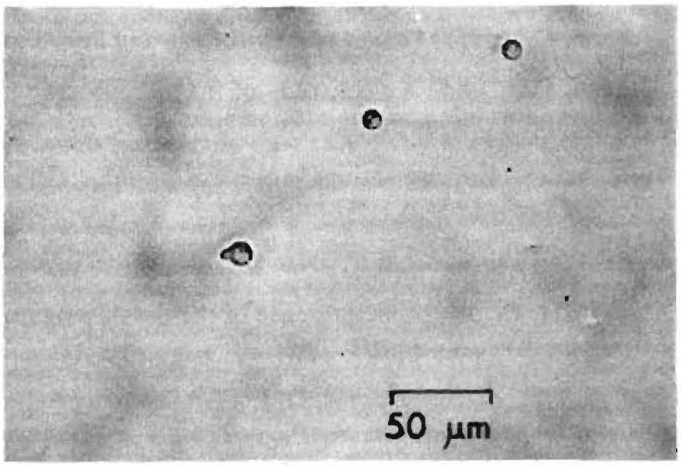
In exudates of cultivars TMV 2 and EC 76446(292) spore germ tubes were significantly longer than spore germ tubes in exudates of J 11 and PI 337394F (Fig. 10 a-c).

Hale and Griffin (1976) reported that injury to pods significantly stimulates *A. flavus* spore germination in the geocarposphere. Pass and Griffin (1972) found that among the sugars exuded from groundnut roots glucose was the most effective in stimulating the germination of conidia of *A. flavus*. Fructose and sucrose were much less effective. But the work of Hale (1978) shows that among sugars exuded from pegs and fruits of plants in axenic culture sucrose was present in the largest amount, fructose was second in quantity, and glucose was present in the least amount except in the pod stage. This shows that glucose is not the only carbon source for germination of

a. EC 76446 (292)



b. J 11



c. PI 337394 F

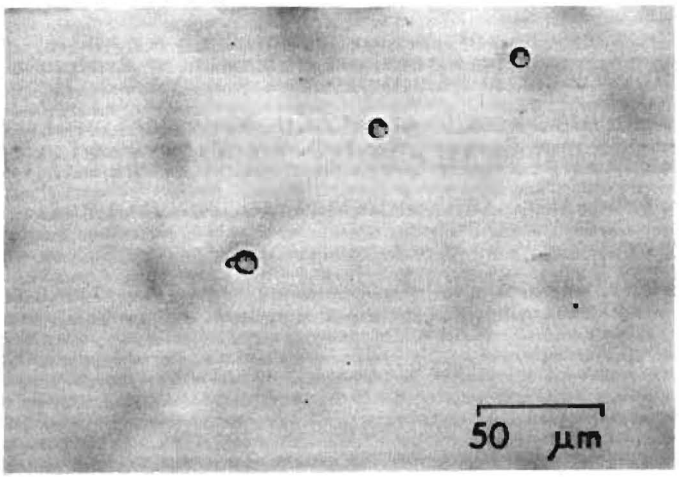


Figure 10. Germ tube length of conidia of *Aspergillus flavus* in pod exudates of groundnut genotypes; a. EC 76446 (292), b. J 11, c. PI 337394 F.

A. flavus conidia. Pass and Griffin (1972) reported that an amino acid mixture containing glucose supported the highest spore germination.

Pod exudates produce different types of sugars, amino acids, and many other compounds. Possibly some of these compounds are produced by certain cultivar(s) in specific quantity and quality, thus influencing the growth and germination of fungal spores.

Results of this experiment show that pod exudates may increase or inhibit growth and germination of *A. flavus*. It is the pod exudates, interaction of different fungi, the inherent characteristics of fungus and genotype, and some other factors that influence the mycoflora in the geocarpospheres around seeds of different genotypes.

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The Semi-Arid Tropical Crops Information Service (SATCRIS) and the Aflatoxin Database

L.J. Haravu[1]

Abstract

A description of the SATCRIS Project at ICRISAT Center, the characteristics of the SATCRIS database, and its information retrieval and dissemination services for groundnut, and the specialized database on aflatoxin.

Résumé

Les ressources et services du Semi-Arid Tropical Crops Information Service (SATCRIS) et la base des données sur les aflatoxines : *L'activité principale du projet SATCRIS au Centre ICRISAT, les caractéristiques de la base des données SATCRIS, ses services de recherche et de diffusion des données sur l'arachide ainsi que la base de données spécialiséee sur les aflatoxines sont decrits.*

Resúmene

El Servicio de Información sobre Cultivos del Trópico Semiárido (SATCRIS) y la organización electrónica de datos e información sobre las aflatoxinas : *Se presenta una descripción del proyecto SATCRIS del Centro ICRISAT, las características de la organización electrónica de datos e información del proyecto y los servicios de recuperación y distribución de información sobre el cacahuate, así como la organización electrónica de datos e información sobre aflatoxinas.*

Background

ICRISAT's capacity to provide information has been considered important not just to keep its own scientists abreast of the latest developments in their fields of interest but also to satisfy information requirements of many others who are working on its mandate crops in laboratories and fields located in areas where information resources are either nonexistent or meager. It is against this background that ICRISAT established a specialized information center, the

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Sorghum and Millets Information Center (SMIC) in 1976 as a part of its Library and Documentation Services to collect, collate, and disseminate information on sorghum and millets to research workers throughout the semi-arid tropics (SAT). The financial support to this project was partially provided by the International Development Research Centre (IDRC), Canada. Information products and services provided by SMIC included comprehensive bibliographies, selective dissemination of information (SDI), literature searches, an information analysis service, and a document delivery service. It is also published a newsletter thrice a year in English and French to provide current awareness to researchers in the SAT.

A project proposal to expand the information resources, products, and services of SMIC to cover all five ICRISAT mandate crops was made in 1986 to ICRISAT's management and to IDRC. The project, known as the Semi-Arid Tropical Crops Information Service, or SATCRIS for short, was approved in November 1986 and is funded in part by IDRC.

A major departure of SATCRIS from its predecessor SMIC is its emphasis on the exploitation of machine-readable products of global database producers such as the Commonwealth Agricultural Bureau International (CABI) and the Food and Agricultural Organization of the United Nations (FAO) for inhouse database development, and on the provision of information retrieval and dissemination services. The emphasis is also on closer collaboration with database producers, both for input provision, and in utilization of their expertise and infrastructure for the regular and timely delivery of comprehensive information dissemination services to users throughout the SAT. An important goal of SATCRIS is to establish a SATCRIS subcenter at the ICRISAT Sahelian Center (ISC) in Niamey. This subcenter will ensure the capture of nonconventional literature especially of West African origin and the input such data into the SATCRIS database. It will also provide document delivery services from its collection and liaise with the center in India for other services.

SATCRIS Objectives

Given these above broadly stated goals, SATCRIS has set for itself several tasks and objectives. These are:

- To convert the existing database of SMIC into computer-readable form for interactive use at ICRISAT.
- To provide input to global systems such as AGRIS and CABI of both conventional and non-conventional information generated within ICRISAT.
- To create a machine-readable database of SATCRIS using subsets of CABI and AGRIS databases and to integrate such data with locally generated input.
- To provide SDI, bibliographical, and literature search services using inhouse and external databases to scientists and others in the SAT working on ICRISAT mandate crops.
- To develop a subcenter of SATCRIS at ISC in Niamey, Niger.
- To hold a series of workshops in eastern and southern Africa to promote SATCRIS among potential users, and to build contacts with librarians and documentalists, and,
- To undertake well-defined information analysis projects in collaboration with scientists.

User Community

The primary users of SATCRIS information products and services are scientists and researchers at ICRISAT Center, ISC, in western, eastern, and southern Africa, and in the national, regional,

and international programs collaborating with ICRISAT. Scientists, academics, and extension personnel in other national and regional institutions will also be provided service on request. The user community of SATCRIS comprises plant breeders, entomologists, plant pathologists, botanists, microbiologists, biochemists, agronomists, agricultural engineers, land and water management specialists, agroclimatologists, soil physicists, soil chemists, economists, social anthropologists, and extension personnel. The output and services of SATCRIS, it is envisaged, will go a long way to providing the necessary information support to the stated user community in the SAT, particularly in Africa where library and information systems are still far from adequate.

Outputs

The following information products and services will be generated:

- monthly SDI output to users throughout the SAT,
- on-demand literature search outputs,
- abstracts, journals, and annual bibliographies in collaboration with CABI,
- information analysis products such as literature reviews,
- a newsletter, and
- interactive access for scientists at ICRISAT to the SATCRIS database.

SATCRIS Database

The central resource of SATCRIS is its machine-readable database, that has been developed by obtaining monthly subsets of the CABI and AGRIS databases in machine-readable form. The two databases have been chosen for CABI's excellent coverage of primary journal literature, and AGRIS's coverage of nonconventional literature.

A comprehensive profile of SATCRIS interests has been communicated to CABI so that they can generate a relevant monthly subset of their database.

A versatile software package called BASIS is used to derive data from the two databases. This data is integrated with locally generated input to create a single, multidisciplinary database on ICRISAT mandate crops. The existing SMIC database will also be integrated into the SATCRIS database using the BASIS software.

SDI Service

The manual SDI service of SMIC has been replaced by a fully automated service, i.e., one in which the selection of items to be output to a given individual is based on a carefully constructed and tailored user profile. The emphasis is on meeting as specifically as possible, the current awareness needs of individual scientists. A two-level service is provided. The first is based on macro-profiles which may serve a small research station, and the second provides highly specific and personalized profiles. The service will be based on the monthly inputs received from CABI and AGRIS. BASIS modules will be used to store the user profiles and for the production of SDI outputs. This system will allow us to offer the service to many more users than was possible using manual selection.

SDI service outputs are backed up by document delivery services. SATCRIS uses its own resources as well as those of others (e.g., NAL, BLLD) including the faster Dialorder services wherever necessary.

Literature Search Services

SMIC responded to more than 300 requests for literature searches from 1984 to 1987. SATCRIS continues to promote this on-demand search service, using the SATCRIS database wherever possible. Where access to a broad spectrum of information is required, searches are done using external databases in an online mode, using the DIALOG system in the USA.

Experience has shown that searches done for a given individual are often found useful by others. Dissemination of information on completed searches e.g., through the ICRISAT Research and Development Leaflet series distributed with our newsletter At ICRISAT, and through items in our other newsletters has made searches available to many others who might otherwise have not used the results.

At SATCRIS, search outputs are considered to be bibliographic items in their own right; they are input to the SATCRIS database and can be retrieved in response to interactive searches.

SATCRIS input to CABI and AGRIS databases

Since SATCRIS builds its database using CABI and AGRIS, it is in its interest to contribute to both of them. SATCRIS inputs ICRISAT-generated conventional and nonconventional literature to both AGRIS and CABI, and also provides input of nonconventional literature captured at ICRISAT Center and ISC to CABI.

Collaboration with CABI

An important objective of SATCRIS is to collaborate with CABI in the production of specialist abstract journals on ICRISAT's mandate crops. These CABI abstract journals are available free of cost to key libraries and individuals in SAT countries. It is believed that such collaboration with a major database producer ensures the regular dissemination of information on our mandate crops to scientists and others in national, regional, and international programs of the SAT. Also, the information thus disseminated is more comprehensive and current than that provided from local resources. The advantages of using the expertise and infrastructure of a major disseminator of biblographical information such as CABI more than compensates the costs involved.

CABI already produces the Sorghum and Millets Abstracts and will produce two new abstracts services, one for chickpea and pigeonpea and another for groundnuts. SATCRIS has identified individuals and libraries in the SAT who will receive these free abstract services directly from CABI.

Information Analysis Services

Scientists at ICRISAT will be involved in the choice of topics and subjects for information analysis that will produce reviews and critical evaluations of the literature on specific topics. In choosing topics, the emphasis will be on the usefulness of the repackaged information to scientists, researchers, and others working in Africa, since they do not have the benefit of regular interaction with the expertise available in a center such as ICRISAT. They will be written by subject specialists, whose services will be contracted from a few weeks to several months depending on the nature and scope of the subject. The project provides for short-term sabbaticals to scientists for this purpose. Information scientists of SATCRIS will provide literature search services, and document delivery. They will also attempt to identify key papers in the chosen topic using a citation-based approach.

Microcomputer Databases

An important objective of SATCRIS is to strengthen the information handling capabilities at ISC. In the long run, it is hoped that SATCRIS will be involved also in strengthening information handling capabilities in other national and regional agricultural research systems in the SAT.

One approach that SATCRIS is exploring is the development of specialized databases for decentralised use on microcomputers. The idea is that SATCRIS will create and maintain useful specialized databases and make available such databases to national and regional centers in the SAT. SATCRIS will also provide appropriate software, and wherever necessary, training to national and regional centers to enable them to utilize the databases. SATCRIS will of course periodically provide database updates on diskettes to centers in the SAT. In addition, SATCRIS will offer information retrieval and dissemination services in the subject areas of the specialized databases.

We believe that such an approach will serve two purposes:

- deliver useful information to SAT centers in a form that is easily accessible.
- introduce relevant information technology into national and regional centers.

Two specialized databases on which some work has already begun are: Aflatoxins and *Striga*. Information on a variety of primary documents, both conventional and nonconventional is being input to the databases. A public-domain software called Micro CDS/ISIS is being used to create and maintain the databases. The CDS/ISIS is a versatile software package and enables the definition of databases, data entry and updating, multidimensional searching, and provision of a variety of output products. Most importantly, CDS/ISIS supports variable length records, a vital requirement in bibliographic information systems.

Once such databases are developed SATCRIS will offer them to interested agricultural research centers in the SAT.

SATCRIS Activities in Africa

The SATCRIS subcenter at ISC will monitor sources of information in French, and provide reference and document delivery services to scientists at ISC and others in national and regional programs in Francophone West Africa. It will liaise with SATCRIS in India for search, bibliographic, and SDI services, will acquire relevant conventional and nonconventional literature of African origin, especially from Francophone sources. Such literature will be input to the SATCRIS and CABI databases.

Useful primary literature (journal articles, conference papers, etc.) and nonconventional literature already collected at SMIC and the ICRISAT library will be deposited in microfiche form at ISC. The collection will be kept up-to-date with regular input from the Center in India. A microfiche reader and reader-printer will also be provided at ISC to enable the use of this collection.

An informative leaflet in English and French describing the microfiche project and its objectives will be printed and distributed to institutions and individuals in Africa. The leaflet will describe the nature of the collection and the services that users may expect from the SATCRIS subcenter at ISC.

It is proposed to hold a number of workshops in eastern and southern Africa to improve the awareness of scientists to the resources and services of SATCRIS. The countries to be covered will be in the Southern African Development Coordination Conference (SADCC) region and

eastern Africa. The workshops will promote SATCRIS services using audio-visual aids, interpersonal discussions, display of information products, and enrolment of potential users to its services. The workshops will also attempt to establish stronger rapport with end-users and with librarians and documentalists leading to a better exchange of information, and increased utilization of the resources and services of SATCRIS.

Discussion

K.J. Middleton. Does the microcomputer database run only on IBM?
L.J. Haravu. Yes. At the moment it will run only on IBM PC, XT, AT and the Vax. It will very soon run on the Micro-vax family. I am hoping to soon become a distributor on behalf of UNESCO for this particular software package which comes on two floppy diskettes, to non-profit organizations.
R.V. Bhat. Are you proposing to link SATCRIS with Medline and national documentation organizations?
L.J. Haravu. At the moment we have not thought of interaction with Medline although for aflatoxin perhaps Medline is as relevant as an agricultural database. We will be concentrating more on the agricultural science databases. That is the Commonwealth Agriculture Bureau's mandate. Although this database does capture some biomedical literature related to agricultural products, for the other aspects we have to go to Medline or food science databases if we want to be totally comprehensive. We are definetely going to take care of national inputs particularly from ICAR institutions and so on.
P.D. Blankenship. We would like to suggest that you contact the Coastal Plain Experiment Station of the University of Georgia. They have a library with a comprehensive facility of peanut periodicals. It is available on the same kind of system you are talking about, but one point which makes it unique is that this library has access to all postgraduate theses that were done in the US.
L.J. Haravu. Thank you, we will do that.
H. Amra. Is this only for semi-arid tropics? Which crops are covered?
L.J. Haravu. It is intended for anyone who is actually working on the five ICRISAT mandate crops, and is not exclusive to the semi-arid tropics. We were thinking of microcomputers because we felt that, for instance, in Africa, not many institutions can afford to buy big computers, but they could probably afford a microcomputer. If you could develop a small database on a microcomputer then it will be easier for such institutions to use.

Session IX

Group Discussion Reports

I. Evaluation and Monitoring of Aflatoxin Contamination of Groundnuts and Groundnut Products

Participants

Name	Institution	Country
D. McDonald (Chairman)	ICRISAT	India
P. Subrahmanyam (Cochairman)	ICRISAT	India
A. Bockelée-Morvan	IRHO	France
B. Coulibaly	AGC	Nigeria
R.D. Coker	ODNRI	UK
S. Nahdi	ICRISAT/NIN	India
J. Kannaiyan	Msekera RRS	Zambia
C.T. Kisyombe	Chitedze ARS	Malawi
A. Pollet	ORSTOM	France
R. Quitco	NAP HIRE	The Philippines
P.S. Reddy	NRCG	India
M. Sabino	Instituto Adolfo Lutz	Brazil
B. Singh	Peanut CRSP	USA

The group was concerned largely with the country approach to the aflatoxin problem in groundnut. Participants agreed that there was a definite need in many countries to alert producers, processors, and consumers of groundnuts and groundnut products of the hazards to livestock, and the likely hazards to humans from ingesting aflatoxin-contaminated groundnuts and groundnut products.

Groundnuts are rarely consumed as an independent item in human diets or in animal feeds, and it was felt that agriculturalists and others concerned with such crops as maize, sorghum, and cotton should take some responsibility for publicizing the harmful effects of aflatoxin-contaminated foods and feeds.

It was recommended that groundnut-producing countries set up working groups comprising representatives of:

Agricultural research and extension institutions
Veterinary and animal production institutes
Medical research and public health institutes
Marketing organizations
Producers' associations
Processors' associations
Economists, etc.

Such a group could endeavor to establish a coordinated approach to the aflatoxin problem, inform policymakers, and send representatives to regional or international meetings on relevant topics.

The group felt that each country should develop a system to evaluate and continuously monitor the aflatoxin problem at all levels, including export-oriented and local consumption segments.

Well-proven, and statistically acceptable standardized sampling and aflatoxin assay methods should be selected on the basis of available technology and personnel and the degree of accuracy required. Some laboratories set up in the developing countries over the past 25 years have limited facilities, and are only able to carry out specific methods. The need to maintain these laboratories, upgrade their facilities, and train their staff in new techniques, was recognized. These laboratories are often the only facilities available in a country and they should not be discarded as obsolete until viable replacements are available.

Training in sampling methods and in aflatoxin analysis is a critical requirement. Regional training courses were advocated to facilitate this upgrading.

It was agreed that the most effective way to avoid contamination of produce was to prevent infection of the groundnuts and their products by *Aspergillus flavus* at all stages in production, storage, and processing.

It was recommended that emphasis be given to development of effective detoxification systems for (a) large-scale industrial plants, and (b) small-scale plants for village level operation, to cover both cake and oil production.

While it was agreed that there were reasonable prospects of prevention or removal of aflatoxin contamination from groundnuts produced for export markets, there was little optimism as to the likelihood of immediate improvements in the important local consumption segment.

It was noted that several speakers during the Workshop had highlighted the danger of contaminated nuts segregated from export produce being diverted into the local market. It was therefore advocated that those concerned with segregation should ensure that highly toxic material does not get back into the food chain. Where facilities exist, oil may be extracted from such material and detoxified, but the cake would presumably only be suitable for use as organic fertilizer.

The activities of such organizations as Peanut CRSP and ODNRI in carrying out studies of local systems of processing groundnuts for human consumption were commended. Advice on methods for such studies would enable socio-economists and home economists in developing countries to make recommendations to processors and consumers based on reliable and comprehensive data.

The idea of a literature database was welcomed by the group. The proposal to produce information bulletins or handbooks on aflatoxin management in groundnuts and groundnut products was strongly supported.

The need to have meetings on a regular basis to ensure information exchange and cooperation in research was expressed. This may be provided by holding special meetings as satellites to international and regional workshops as was done when a mycotoxin meeting was held in conjunction with the International Plant Pathology Congress in Australia.

II. Analytical Methods for Aflatoxins in Groundnuts and Groundnut Products

Participants

Name	Institution	Country
F.S.Chu (Chairman)	University of Wisconsin	USA
D.V.R. Reddy (Cochairman)	ICRISAT	India
R. J. Cole	USDA-ARS	USA
Xiao Daren	CAAS	China
T. Goto	NFRI	Japan
S. Moody	RSBS	Australia
J.D. Reed	ILCA	Ethiopia
I.A. Rana	NARC	Pakistan
A.H. Siwela	CSRI	Zimbabwe
M. Somabhi	FCRI	Thailand
R. Jambunathan	ICRISAT	India
V. Anjaiah	ICRISAT	India
S. Jayanthi	ICRISAT	India

The group considered that sample size and sampling techniques should be appropriate to the objectives of the study. For surveillance studies, and where cultivars are to be screened for resistance, etc., large samples are recommended. For purposes of quality control and regulation, official standardized sampling methods should be followed, such as the Overseas Development Natural Resources Institute (ODNRI) (adequate up to 30 $\mu g\ kg^{-1}$ level) and the USA sampling methods (preferred at the 20 $\mu g\ kg^{-1}$ level). Subsampling procedures and sample treatment are also important. Care should be taken to avoid recontamination after sampling by storing samples under cool and dry conditions.

Though several speakers and participants felt that while ELISA is a powerful tool to screen for aflatoxin in groundnuts, such well-established methods as the minicolumn should not be discarded. It was generally felt that ELISA could be adopted only after further collaborative study, and acceptance by the Association of Analytical Chemists (AOAC). However, others were of the opinion that rapid ELISA was a better approach than, for instance, the rapid ELISA (Quick-card) test.

It was noted that, while aflatoxin standards are commercially available, the purity of the materials should be checked by the thin layer chromatography (TLC) method and aflatoxin concentrations determined spectrophotometrically. It was suggested that aflatoxin standards be made available through "International" efforts. Groundnut samples containing specified amounts of aflatoxin (available from the European Economic Community, EEC) could be used as a control to check each laboratory's analytical capability and performance. Participation in the international mycotoxin check sample programme organized by the International Agency for Research against Cancer, Lyon, France was mentioned as a point of interaction. It was recommended that regional and national check sample programs be organized.

The group agreed that only those methods which had been subjected to a collaborative study

and have been adopted by a sponsoring agency such as the AOAC or the EEC, should be followed for quality control and regulatory measurement. For research purposes, other methods which have been shown to be comparable with the official methods could also be used.

The group agreed that ELISA is a simple, sensitive, and specific method for mycotoxin analysis, with the potential for use in quantitative analysis of aflatoxin in groundnuts, and as a screen test at lower detection limits of 5 to 10 $\mu g\,kg^{-1}$. It could be automated for screening a large number of samples. The high cost of commercially available ELISA kits as well as the availability and stability of reagents may limit its use in developing countries. Though studies have shown that results obtained from ELISA are comparable to TLC and high performance liquid chromatography (HPLC) methods, more comparative studies are needed. Currently, collaborative studies on two ELISA methods to screen aflatoxin in agricultural commodities including groundnuts are underway. If good results are obtained these two methods are likely to be adopted for use by the AOAC. It should be pointed out that collaborative studies on ELISA only evaluate ELISA protocols and serve to establish a set of standards for ELISA. The efficiency of each commercially available kit will have to be tested by the users. The group recommended that the following criteria be established for the evaluation of protocols in the collaborative studies:

1. standard range and limits of detection (sensitivity);
2. flexibility of using different extraction solvents;
3. limits for signal/noise ratio;
4. specificity (cross-relativity);
5. reproduciblity (cv); and
6. avoidance of interference of sample matrix.

The group strongly recommended that regional training workshops on analytical methodology be established. Such workshops should include both lectures and laboratory demonstrations on general analytical methodology as well as ELISA methods for mycotoxins. Trainees with relevant background and experience would be drawn from developing countries. It was recommended that an ad hoc committee be established for this purpose.

III. Research on On-farm Control of Aflatoxin Contamination

Participants

Name	Institution	Country
K.J. Middleton (Chairman)	QDPI	Australia
R.C. Nageswara Rao (Cochairman)	ICRISAT	India
M. Arora	University College, London	UK
V.Ramanatha Rao	ICRISAT	India
M.J. Freire	UEM/ICRISAT	Mozambique
J.I Pitt	CSIRO	Australia
M.J. Vasudeva Rao	ICRISAT	India
F. Waliyar	ICRISAT	India
S.N.Nigam	ICRISAT	India
P.D. Blankenship	USDA-ARS	USA
R.J. Cole	USDA-ARS	USA
V.K. Mehan	ICRISAT	India
R.S. Sandhu	FAO	Zambia
K.K. Shrestha	Department of Agriculture	Nepal
R.E. Pettit	Texas A&M University	USA
M.D. Raya	Department of Agriculture	Tanzania
J.H. Williams	ICRISAT	India

The group considered the on-farm control of aflatoxin contamination of groundnut under two main headings; firstly the development of packages of practices relevant for small farmers' use in the SAT, and secondly the identification of important deficiencies in the understanding of the factors that determine whether or not a groundnut is invaded by the toxigenic fungi with subsequent contamination with aflatoxin.

The group felt that there was already considerable information and advice available as to how the small farmers of the SAT could avoid, or at least greatly reduce, the risk of their groundnuts being contaminated with aflatoxins. The problem was considered to be lack of awareness on the part of farmers of the need to follow the recommended practices for control of fungal infection of seeds both before harvest and during postharvest curing and drying. Several countries have provided excellent aflatoxin control recommendations through their extension agencies and their example should be followed by others. Factors that should be stressed in preparing recommended practices include: the importance of late-season drought, because pods on plants that go into permanent wilt within the last 2-3 weeks of the crop maturation are very likely to contain aflatoxins; the role of soil insects in predisposing pods and seeds to invasion by the toxigenic fungi; the possible role of calcium deficiency in relation to fungal infection of pods; the importance of timely lifting of the crop to reduce the proportions of overmature and excessively immature pods in the harvested produce; avoidance of damage to pods during threshing/picking, and drying; and the importance of providing dry, well-ventilated, on-farm storage. Prospects of village level or on-farm detoxification of groundnut oil were also considered, the use of clays, sunlight, etc. being suggested. Inputs are required from health, nutrition, and home economics specialists in addition to agricultural extension staff if hazards of aflatoxin contamin-

ation are to be reduced at the farm and village levels. The need for training of such staff to better prepare them for the extension of aflatoxin control procedures was expressed, and the production of an information bulletin or handbook on control of aflatoxin in groundnuts was recommended.

Gaps in our knowledge of how the toxigenic *A. flavus* and *A. parasiticus* invade groundnut pods and seeds, and of environmental factors influencing this process, were considered. It is important to study the survival of the two fungi in the soil of groundnut fields and discover how soil type, cropping systems, and temperature and moisture conditions affect invasion. Production of sclerotia may be important. Seed transmission of *A. flavus* may be involved in building up the inoculum of the fungus in groundnut soils. It has been suggested that groundnuts found to contain moderately high levels of aflatoxin could be used for sowing. This was agreed to be a dangerous practice as such groundnuts could well contain viable mycelium of *A. flavus* and, given conditions unfavorable for germination, this could result in a complete emergence failure from aflaroot disease. A suggestion had been made that aflatoxin contaminated groundnuts or groundnut cake could be used as organic fertilizer. This may be feasible for cake where the heating during oil extraction would have killed any fungal mycelium present, but adding seeds that contain viable *A. flavus* to the soil could greatly boost the population of this fungus and exacerbate the contamination problem in succeeding groundnut crops. There is little definite information on the mechanisms of resistance in peg, pod, and seed to invasion by *A. flavus* and *A. parasiticus*. Further studies in resistant and susceptible cultivars should be carried out to examine possible infection and colonization of pods and seeds. Chemical resistance in the shell would be preferable to having such resistance in the seed in case protective chemicals have any antinutritional or toxic effects when ingested by humans or livestock. There was interest in research into phytoalexin production in groundnut seed, and it would be useful if a cultivar could be bred in which seeds retained the capacity to produce phytoalexins until they are mature.

Definitive information on the mechanisms of resistance to fungal invasion of pods and seeds would greatly facilitate the breeding of resistant cultivars. The contributions of different mechanisms to the overall resistance could be assessed and resistance screening techniques could be improved. Resistance breeding is an important longterm objective.

It was felt that a global survey of the occurrence of toxigenic and nontoxigenic strains of *A. flavus* and *A. parasiticus* would be of value. This would require precise identification of the two species and careful evaluation of their populations in the soil mycoflorae.

The need for training in resistance screening methods and in techniques for handling soil mycofloral analysis, etc. was expressed. Information on screening methods could be included in the proposed information bulletin.

IV. Research on Control of Aflatoxin Contamination with Reference to Storage, Transit, Processing, etc.

Participants

Name	Institution	Country
T.O.M. Nakayama (Chairman)	Peanut CRSP	USA
J.A. Wightman (Cochairman)	ICRISAT	India
R.V. Bhat	NIN	India
G. Chandrashekhar	IOPEA	India
C.L.L. Gowda	ICRISAT-AGLN	India
M. Machmud	BORIF	Indonesia
G.V. Ranga Rao	ICRISAT	India
M. Read	PMB	Australia
T. Shantha	CFTRI	India
D.B.T. Wijeratne	Directorate of Agriculture	Sri Lanka
H. Amra	NRC	Egypt
N.E. Ahmed	Gezira ARS	Sudan

The discussions covered problems of aflatoxin contamination during postharvest handling, storage, and processing of groundnuts. It was appreciated that growth of *Aspergillus flavus* and production of aflatoxin in groundnut produce could occur at all stages, and that with conidia of the fungus being almost universally present, the most important factor determining contamination was the moisture content of the groundnuts or groundnut products. The need to dry groundnuts to a safe level of seed moisture content was emphasized. Stored seed may acquire sufficient moisture to enable growth of *A. flavus* to occur if insect infestation of pods or seeds occurs, or if ambient conditions of high relative humidity prevail for several weeks. Methods of monitoring and controlling insect infestations are already well developed and are in common use in well-organized storage depots. Siting of groundnut stores in areas of high relative humidity should be avoided if possible. It was suggested that ICRISAT's Agroclimatology Unit could be asked to compile relative humidity data for major groundnut-growing regions of different countries during the months following harvesting of the crops. The point was made that where groundnuts were an export crop the produce is likely to be stored under very humid conditions in, or close to, seaports while awaiting shipment overseas. Problems of condensation in the holds of ships carrying produce from the tropics to temperate regions could also lead to wetting of groundnuts and growth of the toxigenic fungi. This supported the need for aflatoxin analysis of groundnut shipments on arrival at their destinations, and also indicated that detoxification should also be carried out in the area/country where the produce is to be processed unless suitable safeguards were instituted to avoid recontamination of the materials.

There was discussion on the efficiency of sampling of groundnuts and groundnut products and work at Overseas Development Natural Resources Institute (ODNRI) to refine the Tropical Production Institute (TPI) plan was described. This involves computer simulation and research on appropriate mathematical models to illustrate the distribution of aflatoxin in sample lots. The need to use reliable, standardized methods of aflatoxin analysis was also stressed.

The group strongly supported the need for further research into detoxification of groundnuts

and groundnut products. Contamination of lots of whole seeds may be reduced by removal of visible mold-damaged or discolored seeds. However, there may be a problem concerning the disposal of the rejected seeds. Such rejects are likely to have high levels of aflatoxin contamination and should not be used in foods or animal feeds. They could be crushed for oil, but the oil would probably have higher than usual levels of free fatty acids, and would probably also require special treatment for removal of aflatoxins. Detoxification of groundnut cake and meal was discussed in depth, with special reference to the use of ammonia. It was felt that further research was required to elucidate the possible toxicology of detoxified groundnut products. It was suggested that groundnut products could be detoxified at ports of discharge, there being legal provisions for warehousing and in-bond processing in importing countries.

The need for training in relation to postharvest handling of groundnuts, storage procedures including pest control and avoidance of wetting or hydration of produce, sampling and aflatoxin analysis, and detoxification processes, was agreed. It was suggested that ICRISAT could inform national programs and institutions of courses available worldwide on various aspects of the aflatoxin problem, and provide organizers of such courses with contacts in client countries to suggest names of potential participants. Specific mention was made of ODNRI's annual 13-week training course in aflatoxin analysis. The main objective of this course is to train potential trainers who can in turn impart training in their own countries. ICRISAT's proposal to produce an information bulletin on aflatoxin in groundnut giving up-to-date information on control procedures was strongly supported. Increasing awareness of the importance of aflatoxin could lead to increased demand for general and specialist training, necessitating effective collaboration among international and regional organizations concerned with the problem to meet such a demand.

Procès verbaux des discussions en groupes

I. Evaluation et contrôle de la contamination de l'arachide et des produits à base d'arachides par les aflatoxines

Participants

Nom	Organisme	Pays
D. McDonald (Président)	ICRISAT	Inde
P. Subrahmanyam (Co-président)	ICRISAT	Inde
A. Bockelée-Morvan	IRHO	France
B. Coulibaly	AGC	Nigéria
R.D. Coker	ODNRI	Royaume-Uni
S. Nahdi	ICRISAT/NIN	Inde
J. Kannaiyan	Mskera RRS	Zambie
C.T. Kisyombe	Chitedze ARS	Malawi
A. Pollet	ORSTOM	France
R. Quitco	NAP HIRE	Philippines
P.S. Reddy	NRCG	Inde
M. Sabino	Instituto Adolfo Lutz	Brésil
B. Singh	Peanut CRSP	Etats-Unis

Le groupe a considéré principalement comment le problème des aflatoxines chez l'arachide est abordé au niveau des pays. Les participants étaient tous de l'avis qu'il était nécessaire dans beaucoup de pays, d'avertir les producteurs, les transformateurs et les consommateurs d'arachides et de produits à base d'arachides du danger que représente l'ingestion d'arachides ou de produits à base d'arachides contaminés par les aflatoxines pour la santé du bétail et éventuellement pour la santé humaine.

Les arachides sont rarement consommées seules dans un régime alimentaire humain ou animal et il a donc été estimé que les agriculteurs et tous ceux intéressés par des cultures telles que le maïs, le sorgho et le coton devraient prendre une part de responsabilité dans l'information des effets nocifs des aliments contaminés par les aflatoxines et destinés à la consommation humaine et animale.

Il a été recommandé que les pays producteurs d'arachides mettent en place des groupes de travail composés de représentants des :

Organismes de recherches agricoles et de vulgarisation
Organismes vétérinaires et de production animale
Organismes de recherches médicales et de santé publique
Organisations de commercialisation
Associations de producteurs
Associations d'agents de transformation
Economistes, etc.

Un tel groupe pourrait tenter d'aborder le problème des aflatoxines de manière coordonnée,

informer les autorités et envoyer des représentants pour participer aux réunions régionales ou internationales traitant de thèmes pertinents.

Le groupe a été d'avis que chaque pays devrait mettre au point un système d'évaluation et de suivi continu du problème des aflatoxines à tous les niveaux, y compris les secteurs 'exportation' et 'consommation domestique'.

Des méthodes normalisées, éprouvées et statistiquement acceptables d'échantillonnage et de dosage des aflatoxines, doivent être adoptées selon la technologie et le personnel disponibles et en fonction du degré de précision exigée. Certains laboratoires installés dans les pays en développement depuis les 25 dernières années n'ont que des moyens limités et ne peuvent utiliser que certaines méthodes. Le besoin de maintenir ces laboratoires, d'améliorer leurs équipements et de former leurs personnels dans les nouvelles techniques a été reconnu. Ces laboratoires représentent souvent les seuls moyens d'un pays et il ne faut pas les abandonner avant de pouvoir les remplacer de façon viable.

La formation dans le domaine des méthodes d'échantillonnage et d'analyse des aflatoxines est un besoin critique. Des stages de formation régionaux ont été préconisés afin d'améliorer les connaissances dans ce domaine.

Il a été reconnu que le moyen le plus efficace d'éviter la contamination des produits par les aflatoxines était d'empêcher l'invasion des arachides et des produits à base d'arachide par *A. flavus* à chaque stade de la production, du stockage et de la transformation.

On a recommandé qu'une priorité soit accordée à la mise au point de systèmes efficaces de détoxification destinés (a) aux unités de production industrielles et (b) aux unités de production villageoises, afin de tenir compte à la fois de la production de tourteaux et de la production d'huile.

Si tout le monde a été d'accord sur les bonnes perspectives pour l'élimination des aflatoxines dans le cas des arachides produites pour l'exportation, on n'était guère optimiste en ce qui concerne les possibilités d'améliorations immédiates dans le secteur important des arachides destinées à la consommation domestique.

On a noté, au cours de cet Atelier, que plusieurs participants avaient souligné le danger d'un détournement des arachides contaminées et non exportables, vers le marché local. Il a donc été recommandé que ceux chargés de la ségrégation des arachides fassent bien attention que les arachides hautement toxiques ne se retrouvent pas dans la filière alimentaire. Si les moyens existent, l'huile peut être extraite de ce matériel et détoxifiée par la suite, mais les tourteaux ne seront vraisemblablement utilisables que sous forme d'engrais organique.

Les activités d'organisations telles que le Peanut CRSP et l'ODNRI dans la réalisation d'études sur les système locaux de transformation d'arachides destinées à la consommation humaine ont été approuvées par tous. Des conseils de méthodologie pour de telles études permettraient aux socio-économistes et aux experts d'économie domestique dans les pays en voie de développement de conseiller les agents de transformation et les consommateurs en s'appuyant sur des données fiables et complètes.

L'idée d'une base de données de documentation a été bien reçue par le groupe. La proposition de produire des bulletins d'information ou des manuels sur le contrôle de la contamination des arachides et des produits à base d'arachides par les aflatoxines a été fortement appuyée.

On a souligné le besoin de se réunir régulièrement afin d'échanger des informations et de coopérer dans le domaine des recherches. Ceci serait peut-être rendu possible par des réunions spéciales tenues en annexe des ateliers régionaux et internationaux, telle que la réunion sur les mycotoxines tenue conjointement avec l'International Plant Pathology Congress en Australie.

II. Méthodes analytiques applicables aux aflatoxines présentes dans les arachides et dans les produits à base d'arachides

Participants

Nom	Organisme	Pays
F.S. Chen (Président)	Université de Wisconsin	Etats-Unis
D.V.R. Reddy (Co-président)	ICRISAT	Inde
R.J. Cole	USDA-ARS	Etats-Unis
Xiao Daren	CAAS	Chine
T. Goto	NFRI	Japon
S. Moody	RSBS	Australie
J.D. Reed	ILCA	Ethiopie
I.A. Rana	NARC	Pakistan
A.H. Siwela	CSRI	Zimbabwe
M. Somabhi	FCRI	Thaïlande
R. Jambunathan	ICRISAT	Inde
N. Anjaiah	ICRISAT	Inde
S. Jayanthi	ICRISAT	Inde

Le groupe a été d'avis que la taille de l'échantillon et les techniques de prélèvement doivent correspondre aux objectifs de l'étude. Pour des études de suivi, ou dans le cas d'un criblage des variétés pour leur résistance, par exemple, on préconise des échantillons de taille importante. Dans le cas des contrôles de qualité ou de la réglementation, il convient de suivre les méthodes d'échantillonnage normalisées officielles, telles que celles de l'Overseas Development Natural Resources Institute (ODNRI) (convient jusqu'au niveau de 30 $\mu g\ kg^{-1}$) et les méthodes d'échantillonnage des Etats-Unis (préférable au niveau de 20 $\mu g\ kg^{-1}$. Les procédés de sous-échantillonnage et de traitement des échantillons sont importants également. Il faut veiller à éviter la recontamination après l'échantillonnage en stockant les échantillons dans un endroit sec et frais.

Bien que plusieurs interlocuteurs et participants ont considéré que le test ELISA était un outil puissant de criblage des aflatoxines chez l'arachide, les méthodes bien établies, telles que la mini-colonne, ne devraient pas être abandonnées. Dans l'ensemble, on a considéré que le test ELISA ne pourrait être retenu qu'après une étude plus approfondie en collaboration et après avoir été accepté par l'Association of Analytical Chemists (AOAC). Néanmoins, d'autres pensent que le test Elisa rapide, par exemple, était une meilleure façon d'aborder le problème que le test ELISA (Quick-card).

Bien que des étalons d'aflatoxines existent dans le commerce, on a noté que la pureté de ces matériels doit être vérifiée par la chromatographie en couches minces (CCM) et qu'il faut déterminer leurs concentrations en aflatoxines par spectrophotométrie. Il a été proposé de rendre disponibles, par des efforts "internationaux", des étalons d'aflatoxines. Des échantillons d'arachides ayant des teneurs en aflatoxines spécifiques (fournis par la Communauté Economique Européenne, CEE) pourraient être utilisés comme contrôles afin de vérifier l'aptitude à l'analyse et la performance de chaque laboratoire. La participation au programme d'échantillons de contrôle organisé par l'Agence internationale pour la recherche contre le cancer à Lyon,

France, a été désignée comme un point d'interaction. On a recommandé la mise en place de programmes d'échantillons de contrôle au niveau régional et national.

Le groupe a conclu que seules les méthodes ayant fait l'objet d'une étude collaborative et ayant été retenues par une agence garantissant leur valeur, telle que l'AOAC ou la CEE, devraient être utilisées pour effectuer des contrôles de qualité ou des mesures officielles. D'autres méthodes comparables aux méthodes officielles pourront être utilisées dans le domaine de la recherche.

Le groupe a reconnu que le test ELISA est une méthode simple, sensible et spécifique d'analyse des mycotoxines, ayant un potentiel d'utilisation dans l'analyse quantitative des aflatoxines chez l'arachide en tant que test de criblage avec des limites de détection les plus basses, entre 5 et 10 $\mu g\ kg^{-1}$. Ce test pourrait être automatisé pour le criblage d'un grand nombre d'échantillons. Le coût élevé des kits ELISA dans le commerce, ainsi que la disponibilité et la stabilité des réactifs pourraient limiter son utilisation dans les pays en voie de développement. Bien que des études aient montré que les résultats obtenus par le test ELISA sont comparables à ceux obtenus par la chromatographie en couches minces (CCM) et par la chromatographie liquide à haute performance (HPLC), d'autres études comparatives restent néanmoins nécessaires. Des études sont actuellement en cours sur deux méthodes ELISA destinées au criblage des aflatoxines dans des matières premières agricoles, y compris les arachides. Si les résultats sont bons, ces deux méthodes sont susceptibles d'être retenues par l'AOAC. Il faut souligner que les études effectuées sur le test ELISA n'évaluent que les protocoles ELISA et servent à établir un ensemble de normes pour ELISA. En général, l'efficacité de chaque kit disponible dans le commerce devra être testée par les utilisateurs. Le groupe a recommandé que les critères suivants soient établis pour l'évaluation des protocoles des études en collaboration :

1. Plage standard et limites de détection (sensibilité);
2. Souplesse d'utilisation des divers solvants d'extraction;
3. Limites du rapport signal/bruit
4. Spécificité (relativité réciproque)
5. Reproductibilité (CV) et
6. Elimination des interférences dues à la matrice d'échantillonnage.

Le groupe a fortement recommandé la mise en place d'Ateliers régionaux sur la méthodologie d'analyse. De tels Ateliers devraient comporter des conférences, ainsi que des démonstrations au laboratoire, relatives à une méthodologie générale d'analyse, ainsi qu'aux méthodes ELISA appliquées aux mycotoxines. Des stagiaires provenant des pays en développement, présentant les qualifications et l'expérience appropriées, seraient invités à y participer. On a proposé qu'un comité spécial soit créé à cet effet.

III. Recherches sur les méthodes de lutte contre la contamination par aflatoxines au niveau de la ferme

Participants

Nom	Organisme	Pays
K.J. Middleton (Président)	QDPI	Australie
R.C. Nageswar (Co-président)	ICRISAT	Inde
M. Arara	University College, London	Royaume-Uni
V. Ramanatha Rao	ICRISAT	Inde
M.J. Freire	UEM/ICRISAT	Mozambique
J.I. Pitt	CSIRO	Australie
M.J. Vasudeva Rao	ICRISAT	Inde
F. Waliyar	ICRISAT	Inde
S.N. Nigam	ICRISAT	Inde
P.D. Blankenship	USDA-ARS	Etats-Unis
R.J. Cole	USDA-ARS	Etats-Unis
V.K. Mehan	ICRISAT	Inde
R.S. Sandhu	FAO	Zambie
K.K. Shrestha	Department of Agriculture	Népal
R.E. Pettit	Texas A&M University	Etats-Unis
M.D. Raya	Department of Agriculture	Tanzanie
J.H. Williams	ICRISAT	Inde

Le groupe a considéré deux aspects majeurs de la lutte contre la contamination de l'arachide par les aflatoxines au niveau de la ferme : la mise au point d'un ensemble de pratiques utilisables par les fermiers des zones tropicales semi-arides, et l'identification de carences importantes dans la compréhension des facteurs qui gouvernent l'invasion ou non de l'arachide par le champignon toxigène accompagnée par la suite d'une contamination par les aflatoxines.

Le groupe a constaté qu'il existe déjà une grande quantité d'informations et de conseils permettant aux fermiers des tropiques semi-arides d'éviter, ou au moins de réduire de manière importante, la contamination de leurs arachides par les aflatoxines. On a conclu que le problème principal était un manque de conscience de la part des fermiers de la nécessité de suivre les pratiques préconisées pour lutter contre l'infestation des graines avant la récolte ainsi que pendant la conservation et le séchage post-récolte. Plusieurs pays ont fait d'excellentes recommandations pour la lutte contre les aflatoxines, par l'intermédiaire de leurs services de vulgarisation et d'autres devraient suivre leur exemple. La préparation de pratiques recommandées nécessite que les facteurs suivants soient soulignés : l'importance de la sécheresse en fin de cycle, puisque les gousses sur les pieds qui se fanent de façon permanente au cours des 2-3 dernières semaines de maturation sont fortement susceptibles d'être contaminées par les aflatoxines; l'action des insectes dans le sol qui rend les gousses et les graines plus susceptibles à l'infestation par le champignon toxigène; le rôle possible d'une déficience en calcium vis à vis de l'infestation fongique des gousses; l'importance d'arracher les récoltes au bon moment, afin de réduire les proportions de gousses trop mûres ou trop immatures récoltées; la nécessité d'éviter d'abîmer les gousses pendant le battage/l'égoussage et le séchage, ainsi que le besoin d'assurer un lieu de

stockage sec et bien aéré au niveau de la ferme. On a aussi considéré les possibilités, au niveau du village ou de la ferme, de la détoxification de l'huile d'arachide par l'utilisation d'argiles, d'exposition au soleil etc. Une réduction des risques de la contamination par aflatoxines au niveau de la ferme ou du village nécessitera la participation d'experts dans les domaines de la santé, de la nutrition et de l'économie domestique, ainsi que celle des agents de vulgarisation. On a constaté un besoin de former de tels agents afin de mieux les préparer à la vulgarisation des méthodes de lutte contre la contamination par aflatoxines, et on a recommandé la création d'un bulletin d'informations ou d'un manuel sur la lutte contre les aflatoxines chez l'arachide.

Les lacunes dans nos connaissances de la façon dont a lieu l'infestation des gousses et des graines d'arachide par les champignons toxigènes *A. flavus* et *A. parasiticus*, ainsi que les facteurs de l'environnement qui influencent cette infestation, ont été considérés. Il est important d'étudier la survie de ces deux champignons dans le sol des champs d'arachides et de découvrir comment le type de sol, les pratiques culturales, la température et le taux d'humidité influencent l'infestation. La production de sclérotes pourrait être importante. La transmission d'*A. flavus* par les graines pourrait jouer un rôle dans l'accumulation de l'inoculum du champignon dans les sols plantés en arachides. Il avait été suggéré que les graines ayant une teneur moyenne en aflatoxines puissent être utilisées pour le semis. Tout le monde a été d'accord que ceci serait une pratique dangereuse, puisque de telles graines pourraient bien contenir un mycélium viable d'*A. flavus*, et qu'il existerait, dans des conditions défavorables à la germination, un risque d'échec total de la levée provoqué par la maladie d'aflaroot. Il avait également été suggéré que les arachides ou les tourteaux d'arachide puissent être utilisés sous forme d'engrais organique, ce qui serait éventuellement possible dans le cas des tourteaux, puisque la température élevée pendant l'extraction de l'huile aurait tué tout mycélium fongique présent; par contre, l'addition au sol de graines renfermant de l'*A.flavus* viable pourrait augmenter de manière importante la population de ce champignon et aggraver le problème de contamination dans les récoltes ultérieures. Il n'existe que peu d'informations sur la résistance des gynophores, des gousses et des graines à l'infestation par *A. flavus* et *A. parasiticus*. D'autres études sont nécessaires sur des variétés résistantes et sensibles, afin d'examiner l'infestation et la colonisation éventuelles des gousses et des graines. Une résistance chimique au niveau de la coque serait préférable à une telle résistance au niveau des graines, afin d'éviter tout danger anti-nutritionnel ou toxique suite à l'ingestion par l'homme ou par le bétail. On s'est intéressé aux recherches sur la production de phytoalexines dans les graines d'arachide, et il serait utile de pouvoir sélectionner une variété dont les graines gardent la capacité de produire des phytoalexines jusqu'à leur maturité.

Des informations déterminantes sur les mécanismes de résistance des gousses et des graines à l'infestation par les champignons faciliteraient beaucoup la sélection de variétés résistantes. La contribution des divers mécanismes à la résistance globale pourrait être évaluée et des techniques de détection de la résistance pourraient être améliorées. La sélection sur les caractères de résistance représente un but important à long terme.

Il a été convenu qu'une prospection globale de l'apparition des souches toxigènes et non toxigènes d'*A. flavus* et de *A. parasiticus* serait intéressante. Ceci nécessiterait une identification précise des deux espèces et une évaluation soigneuse de leurs populations dans la mycoflore du sol.

Le besoin de formation dans les méthodes de détection de la résistance, ainsi que dans l'analyse de la mycoflore du sol etc., a été mentionné. Des informations sur les méthodes de détection de la résistance pourraient être incluses dans les bulletins d'informations déjà proposés.

IV. Recherches sur des méthodes de lutte contre la contamination par les aflatoxines au niveau du stockage, du transport, et de la transformation, etc.

Participants

Nom	Organisme	Pays
T.O.M. Nakayama (Président)	Peanut CRSP	Etats-Unis
J.A. Wightman (Co-président)	ICRISAT	Inde
R.V. Bhat	NIN	Inde
G. Chandrashekhar	IOPEA	Inde
C.L.L. Gowda	ICRISAT-AGLN	Inde
M. Machmud	BORIF	Indonésie
G.V. Ranga Rao	ICRISAT	Inde
M. Read	PMB	Australie
T. Shantha	CFTRI	Inde
D.B.T. Wijeratne	Directorate of Agriculture	Sri Lanka
H. Amra	NRC	Egypte
N.E. Ahmed	Gezira ARS	Soudan

Les discussions ont porté sur les problèmes de la contamination par les aflatoxines pendant la manipulation post-récolte, le stockage et la transformation des arachides. Il a été admis que le développement d'*Aspergillus flavus* et la production d'aflatoxines dans les produits à base d'arachides peuvent intervenir à tout moment, et, les conidies du champignon étant présentes presqu'à tout instant, le facteur principal qui détermine la contamination est la teneur en eau des arachides ou des produits. Le besoin de sécher les arachides afin d'arriver à une teneur en eau qui ne présente pas de risque a été souligné. Les graines stockées sont susceptibles de reprendre une teneur en eau suffisante pour permettre le développement d'*Aspergillus flavus*, s'il y a infestation des graines ou des gousses par les insectes, ou si les conditions hygrométriques restent élevées pendant plusieurs semaines. Les méthodes de surveillance et de lutte contre les infestations par insectes ont déjà été mises au point et sont déjà souvent appliquées dans des dépôts de stockage bien organisés. Dans la mesure du possible, les lieux de stockage ne doivent pas être localisés dans des zones d'hygrométrie élevée. Il a été proposé de demander à l'Unité d'Agroclimatologie de l'ICRISAT d'établir les données hygrométriques pour le zones principales de culture des arachides dans les divers pays pendant les mois suivant les récoltes. On a constaté que les arachides destinées à l'exportation étaient susceptibles d'être stockées dans des conditions très humides, près du port ou dans le port en attendant l'embarquement. Le problème de condensation dans la soute des navires transportant des produits des tropiques vers des régions tempérées peut aussi augmenter la teneur en eau des arachides et provoquer le développement des champignons toxigènes, d'où le besoin d'analyser les envois une fois arrivés à leur destination, et d'effectuer une détoxification dans la région ou dans le pays de transformation si des mesures de protection contre la récontamination n'y étaient pas déjà mises en place.

L'efficacité des méthodes d'échantillonnage des arachides et des produits à base d'arachides a été discutée et les travaux effectués par l'Overseas Development Natural Resources Institute

(ODNRI) dans le but d'affiner le plan du Tropical Production Institute ont été décrits. Ces travaux comportent des simulations par ordinateur et des recherches sur des modèles mathématiques appropriés, afin d'illustrer la répartition des aflatoxines dans des lots échantillonnés. On a souligné la nécessité d'utiliser des méthodes fiables et normalisées d'analyse des aflatoxines.

Le groupe a fortement exprimé le souhait de voir davantage de recherches sur la détoxification des arachides et des produits à base d'arachides. La contamination de lots de graines entières peut être réduite en éliminant toutes les graines portant des traces visibles de moisissure ou de décoloration. Néanmoins, il pourrait y avoir un problème en ce qui concerne l'utilisation des graines refusées. De telles graines risquent d'avoir une teneur en aflatoxines importante et ne doivent pas être utilisées pour la consommation humaine ou animale. Elles pourraient éventuellement être utilisées pour l'extraction d'huile, mais cette huile aurait probablement une teneur en acides gras libres plus élevée que d'habitude et nécessiterait également, sans doute, un traitement spécial afin d'éliminer les aflatoxines. La détoxification des tourteaux et de la farine d'arachide a été discuté en détail, un intérêt particulièr étant accordé à l'utilisation de l'ammoniac. On a conclu que d'autres recherches étaient nécessaires pour clarifier la toxicologie éventuelle des produits détoxifiés. On a proposé que les produits soient détoxifiés au port de débarquement, vue l'existence de dispositions légales dans les pays importateurs rélatives au stockage et à la transformation de produits entreposés.

Le besoin de formation en ce qui concerne la manipulation post-récolte des arachides, les méthodes de stockage, y compris la lutte contre les ravageurs et la production contre une réhydratation des produits, l'échantillonnage et l'analyse des aflatoxines et les procédés de détoxification a été reconnu par tous. On a proposé que l'ICRISAT informe les programmes et les institutions nationaux des stages partout dans le monde traitant des divers aspects du problème des aflatoxines, et créé les contacts entre les organisateurs de ces stages et les pays concernés afin de proposer des participants potentiels. On a fait mention plus particulièrement du stage annuel de 13 semaines organisé pr l'ODNRI sur l'analyse des aflatoxines. Ce stage a pour but principal de former des formateurs potentiels, qui, par la suite, pourront réaliser la formation dans leurs propres pays. La proposition de l'ICRISAT de produire un bulletin d'information sur la contamination des arachides par les aflatoxines qui fournirait les renseignements les plus récents sur les procédés de contrôle a été très bien reçue. Une connaissance accrue de l'importance des aflatoxines pourrait créer une demande plus importante de formation générale et spécialisée, ce qui nécessiterait une collaboration efficace entre les organisations régionales et internationales concernées par ce problème afin de répondre à cette demande.

Informes de los grupos de trabajo

I. Evaluación y vigilancia continua de la contaminación con aflatoxinas del cacahuate y sus derivados

Participantes

Nombre	Institución	País
D. McDonald (Moderador)	ICRISAT	India
P. Subrahmanyam (Comoderador)	ICRISAT	India
A. Bockelée-Morvan	IRHO	Francia
B. Coulibaly	AGC	Nigeria
R.D. Coker	ODNRI	ReinoUnido
S. Nahdi	ICRISAT/NIN	India
J. Kannaiyan	Msekera RRS	Zambia
C.T. Kisyombe	Chitedze ARS	Malawi
A. Pollet	ORSTOM	Francia
R. Quitco	NAP HIRE	Filipinas
P.S. Reddy	NRCG	India
M. Sabino	Instituto A. Lutz	Brasil
B. Singh	Peanut CRSP	EUA

El interés primordial del grupo fue cómo enfocan los países el problema de las aflatoxinas en el cacahuate. Los participantes concordaron en que existe en muchos países una verdadera necesidad de poner sobre aviso a los productores, los procesadores y los consumidores de cacahuate y sus derivados, acerca de los peligros para el ganado y los peligros probables para los humanos que presenta la ingestión de cacahuates y productos derivados de éstos cuando están contaminados con aflatoxinas.

Los cacahuates raras veces se consumen como artículo independiente en las dietas humanas o en los alimentos para animales, y había el consenso que los agricultores y otras personas que intervienen en el manejo de cultivos, tales como maíz, sorgo y algodón, deberían asumir parte de la responsabilidad de comunicar al público los efectos dañinos que producen los alimentos humano y animal cuando se encuentran contaminados con aflatoxinas.

Se recomendó que los países productores de cacahuate establecieran grupos de trabajo integrados por representantes de:

Instituciones de investigación y extensión agrícola
Institutos veterinarios y de producción animal
Institutos de investigaciones médicas y de salud pública
Organizaciones de mercadeo
Asociaciones de productores
Asociaciones de procesadores
Economistas, etc.

Un grupo de esta índole podría promover la adopción de un enfoque coordinado del problema de las aflatoxinas, podría mantener informado a los sectores que definen las políticas y enviaría representantes a reuniones regionales o internacionales en las cuales se tratan aspectos pertinentes al problema.

El grupo de trabajo era del parecer que cada país debería desarrollar un sistema de evaluación y vigilancia continua del problema de las aflatoxinas a todos los niveles, incluyéndose en este, tanto la supervisión de los sectores orientados hacia la exportación de productos, como aquellos predominantemente de consumo local.

Deberán adoptarse métodos confiables y uniformes de muestreo y análisis de las aflatoxinas, que ya hayan sido probados ampliamente y que permitan la obtención de resultados estadísticamente aceptables, en base a la tecnología, infraestructura y personal existente, y el grado de exactitud requerido. Algunos de los laboratorios establecidos en los países en desarrollo en los últimos 25 años tienen facilidades limitadas, y solamente pueden llevar a cabo análisis con determinados métodos. Se reconoció la necesidad de rehabilitar estos laboratorios, de mejorar sus instalaciones y de capacitar a su personal en las nuevas técnicas analíticas. Estos laboratorios frecuentemente son las únicas instalaciones analíticas del país, y no deberán ser descartadas como obsoletas hasta que las nuevas instalaciones hayan sido terminadas.

La capacitación de personal en los métodos de muestreo y análisis de aflatoxinas es un requerimiento básico. Se recomendaron cursos regionales de capacitación para facilitar la mejoría en este rubro.

Se acordó que la manera más eficaz de librarse de la contaminación consistía en evitar la infección por *Aspergillus flavus* en todas las etapas de producción, almacenamiento y procesado de la cosecha.

Se recomendó que debe dedicarse atención al desarrollo de sistemas eficaces de eliminación de las toxinas, para uso en: a) plantas industriales de gran capacidad y en b) plantas de capacidad reducida que operan en los pequeños pueblos, a fin de proteger tanto la producción de aceite, como la de torta de cacahuate.

Si bien se concordó que existen perspectivas razonablemente aceptables de poder evitar o eliminar la contaminación con aflatoxinas de los cacahuates producidos para los mercados de exportación, había poco optimismo sobre la probabilidad de mejorías, en el futuro inmediato, en el importante sector de consumo local.

Se comentó que varios expositores en este taller han subrayado el peligro que existe de que los cacahuates contaminados que se eliminan de los productos exportados sean entregados a los mercados locales. Por lo tanto, se recomienda que aquellas personas o empresas que llevan a cabo la separación de los cacahuates contaminados, aseguren que el material altamente tóxico quede definitivamente eliminado de ser usado como alimento. Donde existen instalaciones idóneas, el aceite podrá extraerse de tales materiales y eliminarse las toxinas del mismo, pero la torta de cacahuate resultante solamente podría usarse como abono orgánico.

Las actividades de organismos, tales como el Peanut CRSP y ODNRI, que realizan estudios sobre los sistemas locales del procesado de cacahuates para el consumo humano, recibieron caluroso reconocimiento. El asesoramiento sobre los métodos empleados en tales estudios permitiría a los socioeconomistas y especialistas en economía doméstica de los países en desarrollo, hacer recomendaciones a los procesadores y consumidores, basadas en una información completa y enteramente confiable.

La idea de poder contar con un servicio de información bibliográfica, con organización electrónica de los datos, fue aceptado con gran interés por el grupo de trabajo. La propuesta de producir boletines informativos o manuales sobre el control de las aflatoxinas en los cacahuates y sus derivados fue apoyada vigorosamente.

Se expresó la necesidad de realizar reuniones periódicamente para aseguar el intercambio de información y fomentar la cooperación en las investigaciones. Esto puede lograrse mediante la realización de reuniones especiales, simultáneamente con los talleres regionales e internacionales, tal como se hizo en la reunión sobre micotoxinas, que se efectuó conjuntamente con el Congreso Internacional de Fitopatología, en Australia.

II. Métodos para analizar las aflatoxinas en el cacahuate y sus derivados

Participantes

Nombre	Institución	País
F.S. Chu (Moderador)	Universidad de Wisconsin	EUA
D.V.R. Reddy (Comoderador)	ICRISAT	India
R.J. Cole	USDA-ARS	EUA
Xiao Daren	CAAS	China
T. Goto	NFRI	Japón
S. Moody	RSBS	Australia
J.D. Reed	ILCA	Etiopía
I.A. Rana	NARC	Pakistán
A.H. Siwela	CSRI	Zimbabwe
M. Somabhi	FCRI	Tailandia
R. Jambunathan	ICRISAT	India
V. Anjaiah	ICRISAT	India
S. Jayanthi	ICRISAT	India

El grupo consideró que el tamaño de las muestras y las técnicas de muestreo deben ser apropiados a los objetivos de cada estudio. Para los estudios de vigilancia y supervisión, y cuando se seleccionan cultivares con base en su resistencia, etc., se recomienda el uso de muestras grandes. Para fines de control de calidad y cumplimiento de normas legales deberán seguirse los métodos oficiales de muestreo, tales como los del Instituto para el Desarrollo de Recursos Naturales en Ultramar (ODNRI) (adecuado hasta el nivel 30 g kg^{-1}) y de los Estados Unidos (que son preferidos al nivel de 20 g kg^{-1} de aflatoxinas). Los procedimientos de subdivisión de las muestras y de manejo de éstas también son importantes. Se debe ejercer cuidado para evitar recontaminaciones después de efectuado el muestreo, almacenándose las muestras bajo condiciones frescas y secas.

Aunque varios expositores y participantes manifestaron que consideraban que el método de ELISA es una herramienta poderosa para la selección de los cacahuates por su resistencia a las aflatoxinas, pensaban que métodos tan bien establecidos, como el uso de minicolumnas, no deberían descartarse. En general, prevalecía el sentir que el método ELISA solamente se podría adoptar después de realizar estudios adicionales colaborativos y la aceptación del mismo por la Asociación de Químicos Analíticos (AOAC). Sin embargo, otros opinaban que el método ELISA rápido era una alternativa mejor que, por ejemplo, la prueba rápida con tarjeta, de ELISA (Quick-card test).

Se observó que, aunque se pueden obtener en el comercio estándares de aflatoxinas, la pureza de los materiales debe revisarse mediante el método de cromatografía de capa delgada (TLC) y las concentraciones de las mismas deben determinarse en forma espectrofotométrica. Se sugirió que patrones de aflatoxinas se pusieran a la disposición, a través de acciones "internacionales". Muestras patrones de cacahuates que contienen cantidades conocidas de aflatoxinas (que pueden obtenerse de la Comunidad Económica Europea, CEE), podrían usarse como muestras

testigo, para verificar la capacidad analítica y precisión de cada laboratorio. La participación en el programa internacional de muestras testigo de aflatoxinas ha sido organizado por la Agencia Internacional de Investigación contra el Cáncer, con oficinas en Lyon, Francia, fue mencionada como posible vía de interacción. Se recomendó que se organicen programas regionales y nacionales de análisis de muestras testigo. El grupo concordó en que solamente aquellos métodos que se hayan sometido a un estudio colaborativo y que hayan sido adoptados por un organismo patrocinador, tal como el AOAC o la EEC, deban usarse con fines de control de calidad y el cumplimiento de normas legales. Para fines de investigación, podrán usarse otros métodos que han demostrado ser comparables a los métodos oficiales.

El grupo concordó en que ELISA es un método sencillo, sensible y específico para el análisis de micotoxinas, con potencial de ser usado en el análisis cuantitativo de aflatoxinas en cacahuates y como prueba de selección en los límites inferiores de detección, de 5 a 10 g kg^{-1}. Podría automatizarse para la selección de un gran número de muestras. El alto costo de los equipos ELISA que existen en el mercado, así como la dificultad de obteción y variable estabilidad de los reactivos empleados, pueden limitar su uso en los países en desarrollo. Si bien algunos estudios han demostrado que los resultados obtenidos con ELISA son comparables a aquellos obtenidos con cromatografía de capa delgada (TLC) y cromatografía líquida de alto rendimiento (HPLC), se requieren aún más estudios comparativos. Actualmente, se llevan a cabo estudios colaborativos sobre dos métodos ELISA para la detección de aflatoxinas en productos agrícolas, incluyendo cacahuates. Si se obtienen buenos resultados, estos dos métodos probablemente serán aprobados por la AOAC. Debe recalcarse que los estudios colaborativos sobre ELISA solamente evalúan los protocolos ELISA y sirven para establecer un juego de patrones para ELISA. Los usuarios tendrán que verificar la eficacia de los equipos que se encuentran en el mercado. El grupo recomendó la adopción de los siguientes criterios para la evaluación de los protocolos en estudios colaborativos:

1. Alcance normal y límites de detección (sensibilidad);
2. Flexibilidad de uso con diferentes solventes extractores;
3. Límites de la relación "señal"/"estática" o "ruido de fondo";
4. Especificidad (probabilidad de reacciones indeseables);
5. Reproducibilidad (CV), y
6. Cómo evitar interferencias causadas por la matriz de la muestra.

El grupo recomienda vigorosamente que se establezcan talleres regionales de capacitación en metodología analítica. Tales talleres deberán incluir tanto clases formales y prácticas de laboratorio sobre metodologías analíticas en general, como los métodos ELISA para el análisis de micotoxinas. Los candidatos a recibir esta capacitación deberán tener la experiencia y antecedentes pertinentes, y serán seleccionados entre los países en desarrollo. Se recomendó que un comité sea establecido para implementar lo antes expuesto.

III. Investigaciones sobre el control de la contaminación con aflatoxinas en los predios rurales

Participantes

Nombres	Institución	País
K.J.Middleton (moderador)	QDPI	Australia
R.C. Nageswara Rao (Comoderador)	ICRISAT	India
M. Arora	University College, Londres	Reino Unido U.K.
V. Ramanatha Rao	ICRISAT	India
M.J. Freire	UEM/ICRISAT	Mozambique
J.I. Pitt	CSIRO	Australia
M.J. Vasudeva Rao	ICRISAT	India
F. Waliyar	ICRISAT	India
S.N. Nigam	ICRISAT	India
P.D. Blankenship	USDA-ARS	EUA
R.J. Cole	USDA-ARS	EUA
V.K. Mehan	ICRISAT	India
R.S. Sandhu	FAO	Zambia
K.K. Shrestha	Depto. de Agricultura	Nepal
R.E. Petit	Universidad Texas A & M.	EUA
M.D. Raya	Depto. de Agricultura	Tanzania
J.H. Williams	ICRISAT	India

El grupo consideró el control de la contaminación de los cacahuates en los predios rurales, bajo dos rubros principales: primero, el desarrollo de prácticas adecuadas para los pequeños agricultores en las regiones del trópico semiárido (TSA) y, segundo, la identificación de las deficiencias más importantes en el entendimiento de los factores que determinan si el cultivo del cacahuate es invadido, o no, por hongos toxigénicos, con la subsecuente contaminación con aflatoxinas.

El grupo sintió que ya había considerable cantidad de información y recomendaciones acerca de cómo podrían los pequeños agricultores del TSA evitar, o cuando menos reducir grandemente, el riesgo de que sus cacahuates se contaminen con aflatoxinas. Se considera que el problema consiste en que hay una falta de conocimiento de los agricultores, de la necesidad de seguir las prácticas recomendadas para el control de la infección fungosa de las semillas de cacahuate, tanto antes de la cosecha, como durante el período del arranque de las plantas, su secado y la trilla de las vainas. Varios países han proporcionado a sus agricultores excelentes recomendaciones para el control de las aflatoxinas, a través de sus oficinas de extensión agrícola y su ejemplo debe emularse por otros países. Los factores que deben subrayarse al elaborar las recomendaciones de las prácticas a seguir son: la importancia que tienen las sequías durante la última parte del ciclo de desarrollo del cultivo, porque las vainas en las plantas que sufren marchitamiento permanente durante las últimas dos o tres semanas de maduración de la cosecha, muy probablemente contendrán aflatoxinas; el papel de los insectos del suelo en

predisponer a las vainas y semillas a la invasión por hongos toxigénicos; el posible papel de una deficiencia de calcio en el suelo en relación con la infección fungosa de las vainas; la importancia de arrancar oportunamente las plantas, a fin de reducir las proporciones de vainas excesivamente maduras o excesivamente inmaduras en la cosecha; el evitar daños a las vainas en el arranque de las plantas, su secado y trilla; y la importancia de contar con un lugar seco y bien ventilado para el almacenamiento de la cosecha en el predio rural. Las perspectivas de eliminar las micotoxinas del aceite de cacahuate, tanto en el predio rural como en los pueblos pequeños, se discutieron, sugiriéndose el uso de arcillas, luz solar, etc. Se requiere de la participación de especialistas en salud pública, nutrición y economía doméstica, además del personal de extensión agrícola para que los peligros de la contaminación con aflatoxinas puedan reducirse al nivel de los predios rurales o poblados pequeños. Se señaló la necesidad de capacitar al personal mencionado, a fin de prepararlo para la difusión masiva de los procedimientos de control de aflatoxinas, y se recomendó la elaboración de un boletín informativo o manual sobre el control de las aflatoxinas en cacahute.

Se discutieron las lagunas existentes en nuestros conocimientos, acerca de la forma en la cual los hongos toxigénicos *A. flavus* y *A. parasiticus* invaden las vainas y semillas de cacahuate, y de los factores del medio ambiente que influyen en este proceso. Es importante estudiar la sobrevivencia de estos hongos en el suelo de los campos cacahuateros y descubrir en qué forma el tipo de suelo, sistema de manejo del cultivo, y las condiciones de temperatura y humedad influyen en las invasiones fungosas. La producción de esclerosis puede ser importante. La transmisión en la semilla de *A. flavus* puede influir en la acumulación de inóculo fungoso en los suelos cacahuateros. Se ha sugerido a veces que cuando los cacahuates contienen niveles moderadamente altos de aflatoxinas, pueden emplearse como semilla para siembras. Se sabe que esta es una práctica peligrosa, ya que tales cacahuates bien pudieran contener micelios viables de *A. flavus* y bajo condiciones desfavorables a la germinación de la semilla, dan origen a fallas completas en la emergencia, debido a la necrosis de las radículas, causadas por la presencia de aflatoxinas en las plántulas nacientes. Se ha sugerido que los cacahuates o torta de cacahuate contaminados con aflatoxinas podrían usarse como abonos orgánicos. Esto quizá sea factible donde el calentamiento durante el proceso de extracción del aceite mata los micelios fungosos presentes, pero el uso de semillas o torta de cacahuate que contienen *A. flavus* en forma viable, podría incrementar grandemente la población de este hongo en el suelo y exacerbar el problema de contaminación con aflatoxinas en las siembras subsecuentes de cacahuate. Hay poca información definitiva sobre los mecanismos de la resistencia en los ginóforos, vainas y semillas a la invasión por *A. flavus* y *A. parasiticus*. Estudios adicionales deben efectuarse con cultivares resistentes y susceptibles para examinar, en ambos casos, los procesos de infección y colonización de las vainas y las semillas. La resistencia química en la cáscara de las vainas es preferible a este tipo de resistencia en la semilla, porque los compuestos químicos protectores podrían tener efectos antinutricionales, o tóxicos, cuando fueran ingeridos por seres humanos o animales. Hubo interés en las investigaciones sobre la producción de fitoalexinas en las semillas de cacahuate, y se considera que sería muy útil que se pudiera obtener mediante el fitomejoramiento un cultivar cuyas semillas retuvieran la capacidad de producir fitoalexinas hasta que maduraran las plantas.

Contar con información definitiva sobre los mecanismos de resistencia de las vainas y semillas a la invasión fungosa, facilitaría grandemente los trabajos genéticos para la obtención de cultivares resistentes. Las contribuciones de diferentes mecanismos a la resistencia total podrían evaluarse, y las técnicas de selección por resistencia, mejorarse. El fitomejoramiento para lograr resistencia es una importante meta a largo plazo.

Se considera que un estudio global sobre la presencia de cepas toxigénicas y no toxigénicas de

A. flavus y *A. parasiticus* sería valioso. Esto requeriría la identificación precisa de las dos especies citadas y una evaluación cuidadosa de sus poblaciones en la micoflora del suelo.

Se expresó la necesidad de capacitar personal en los métodos de selección para lograr resistencia y en las técnicas empleadas en el análisis de la micoflora del suelo. La información existente sobre métodos de selección podría incluirse en el boletín informativo propuesto.

IV. Investigaciones sobre el control de la contaminación con aflatoxinas en el almacenamiento, transporte, procesado, etc.

Participantes

Nombre	Institución	País
T.O.M. Nakayama (Moderador)	Peanut CRSP	EUA
J.A. Wightman (Comoderador)	ICRISAT	India
R.V. Bhat	NIN	India
G. Chandrashekhar	IOPEA	India
C.L.L. Gowda	ICRISAT-AGLN	India
M. Machmud	BORIF	Indonesia
G.V. Ranga Rao	ICRISAT	India
M. Read	PMB	Australia
T. Shantha	CFTRI	India
D.B.T. Wijeratne	Dirección de Agricultura	Sri Lanka
H. Amra	NRC	Egipto
N.E. Ahmed	Gezira ARS	Sudán

Las discusiones cubrieron los problemas de contaminación con aflatoxinas durante el manejo postcosecha, el almacenamiento y el procesado de los cacahuates. Se concluyó que el desarrollo de *Aspergillus flavus* y la producción de aflatoxinas en los cacahuates o sus derivados, podría ocurrir en todas las etapas, y por estar las conidias del hongo presentes casi universalmente, el factor determinante más importante es el contenido de humedad presente en los cacahuates o sus derivados. Se subrayó la necesidad de secar las semillas hasta que su contenido de humedad no presente riesgos. La semilla almacenada puede absorber del aire humedad suficiente para permitir el desarrollo de *A. flavus*, si existe una infestación de insectos en las vainas o semillas, o si hay alta humedad relativa durante varias semanas. Los métodos de vigilancia constante y de control de las infestaciones de insectos están bien establecidas y son de uso común en depósitos y almacenes bien organizados. Debe evitarse, de ser posible, la ubicación de depósitos de cacahuates en áreas que tienen alta humedad relativa. Se sugirió que se pidiera a la unidad de agroclimatología del ICRISAT, que compilara la información existente sobre la humedad relativa de las principales regiones productoras de cacahuate en diferentes países, durante los meses subsecuentes a la cosecha de este cultivo. Se señaló, asimismo, que donde los cacahuates son un producto de exportación, probablemente serán almacenados bajo condiciones muy húmedas en las proximidades de algún puerto marítimo, mientras llega el momento de embarque para su transporte a ultramar. La condensación de la humedad presente en las bodegas de los barcos que transportan productos de los trópicos a las regiones templadas, puede también dar origen al humedecimiento de los cacahuates y al desarrollo de hongos toxigénicos. Lo antes expuesto fundamenta la necesidad de realizar análisis de aflatoxinas en los lotes de cacahuates, al llegar éstos a sus destinos, y también señala que el proceso de eliminación de toxinas debe realizarse en el área o país donde el producto va a ser procesado, a menos de que se

establezcan medidas de protección adecuadas que eviten la recontaminación de los materiales tratados.

Se trató ampliamente la eficiencia del muestreo de cacahuates y sus derivados y se describieron los trabajos que se desarrollan en el Instituto para el Desarrollo de Recursos Naturales en Ultramar (ODNRI) para refinar el plan del Instituto de Producción Tropical. Esto incluye la simulación en computadora e investigaciones para encontrar modelos matemáticos apropiados que representen la distribución de las aflatoxinas en las muestras. La necesidad de usar métodos confiables y debidamente calibrados en los análisis de las aflatoxinas también se recalcó.

El grupo apoya fuertemente la necesidad de realizar investigaciones adicionales sobre la eliminación de las toxinas de los cacahuates y sus productos derivados. La contaminación de lotes de semillas enteros puede reducirse mediante la eliminación de las semillas decoloradas o visiblemente dañadas por el ataque de hongos. Sin embargo, puede haber un problema en lo referente al uso subsecuente que se da a las semillas desechadas. Estas probablemente tienen altos niveles de contaminación con aflatoxinas y no deben ser usadas en el alimento humano o animal. Se podría extraer su aceite, pero éste probablemente tendría un nivel más elevado que lo normal de ácidos grasos libres y probablemente también requerirá un tratamiento especial para eliminar aflatoxinas. La eliminación de las toxinas de las tortas y granulados de torta de cacahuate se examinó con especial atención al uso del amoniaco. Es el parecer general que investigaciones adicionales son necesarias para esclarecer la posible toxicología de los productos derivados de cacahuate, a los cuales se hayan eliminado las toxinas. Se ha sugerido que a estos productos se les podría eliminar las toxinas en los puertos de desembarcación, dado que existen disposiciones legales sobre su almacenamiento y para su procesado bajo fianza, en los países importadores.

Se concordó en la necesidad de iniciar programas de capacitación en el manejo postcosecha de los cacahuates, en los procedimientos de almacenamiento (incluyendo el combate de plagas y la prevención del humedecimiento o hidratación de los granos), en el muestreo y análisis de aflatoxinas y en los procesos de eliminación de las toxinas. Se sugirió que el ICRISAT podría informar a los programas e instituciones nacionales acerca de cursos que se imparten en diferentes partes del mundo, sobre diversos aspectos del problema de las aflatoxinas y poner a los organizadores de tales cursos en contacto con las instituciones o personas idóneas, en países que estarían interesados en los mismos, a fin de que propusieran nombres de participantes. La propuesta del ICRISAT de producir un boletín informativo sobre aflatoxinas en el cacahuate con información actualizada acerca de los procedimientos para su control, fue apoyada en forma unánime. La creciente comprensión de la importancia de las aflatoxinas puede originar una gran demanda de programas de capacitación, tanto de índole general como especializada, lo cual requerirá del establecimiento de una estrecha colaboración entre las organizaciones regionales e internacionales responsables de combatir el problema de las aflatoxinas a fin de poder satisfacer la demanda mencionada.

Session X

Recommendations

Recommendations

The Workshop identified various areas of concern about aflatoxin contamination of groundnut and made recommendations that are covered in detail in the Group Discussions. The overall recommendations of the Workshop are as follows.

Information and Training

The Workshop emphasized the need to increase awareness of the dangers of aflatoxin contamination of groundnuts and groundnut products among international groups, national governments, the groundnut industry, the producers, and ultimately the consumers. Organizations such as FAO, WHO, the EEC, etc. could do more in this respect and could cover the problem of aflatoxins in all commodities. National governments are likely to take more notice of advice from FAO and WHO than from other organizations.

The need to make information on the groundnut aflatoxin problem more readily available was stressed. ICRISAT was encouraged to proceed with the preparation of a database on literature on the subject, and to organize the production of a handbook or information bulletins on sampling and analytical methods, and on management practices for control of aflatoxin in groundnut. It was also suggested that ICRISAT could act as a clearing house to inform all concerned with the aflatoxin problem of proposed training courses, workshops, etc.

Strategies

The concerned groups, AGC, EEC, FAO, and Codex Alimentarius, should continue to work towards a standard international legislation on regulatory levels of aflatoxin in groundnuts and groundnut products for human and animal consumption. At the national level, countries are recommended to set up interdisciplinary working groups to coordinate the evaluation of the aflatoxin problem in their country, identify a responsible agency, organize monitoring of aflatoxin levels in foods and feeds, and initiate and coordinate research with a view to preparing recommendations for control at all levels. Particular attention was directed to ensuring that control measures for reduction of aflatoxin levels in groundnuts destined for export should not further exacerbate the problem in groundnuts for local consumption.

Research Needs

Research needs should be clearly defined in the light of each country's problems and capabilities, and work should be carried out using the most appropriate technologies and by the most relevant organizations. Training of staff in new techniques will be required in many countries if rapid progress is to be made. The need for cooperation in training and research, both nationally and internationally, was recognized.

Recommandations

L'Atelier a identifié les divers domaines de préoccupation en ce qui concerne la contamination des arachides par les aflatoxines et a proposé des recommandations qui sont rapportées en détail dans les Discussions en Groupes. Les recommandations globales de l'Atelier sont les suivantes :

Information et formation

L'Atelier a souligné le besoin de rendre les groupes internationaux, les gouvernements nationaux, l'industrie arachidière, les producteurs et même les consommateurs plus conscients du danger de la contamination des arachides et des produits à base d'arachides par les aflatoxines. Des organisations telles que la FAO, l'OMS et la CEE, etc., pourraient faire plus dans ce domaine et pourraient s'intéresser au problème des aflatoxines dans toutes les matières premières agricoles. Les gouvernements nationaux sont plus susceptibles de tenir compte des conseils de la FAO et de l'OMS que ceux venant d'autres organismes.

Le besoin de rendre plus disponibles des informations sur le problème des aflatoxines a également été souligné. L'ICRISAT a été encouragé à poursuivre la mise en place d'une base de données relative à la documentation existant sur ce sujet et à organiser la production de manuels où de bulletins d'informations traitant des méthodes d'échantillonnage ou d'analyse et de la gestion des contrôle des aflatoxines chez l'arachide. On a également proposé que l'ICRISAT agisse en tant que centre de diffusion, fournissant à tous ceux concernés par le problème des aflatoxines des informations sur les stages de formation proposés, les Ateliers etc.

Stratégies

Les Groupes concernés, l'AGC, la CEE, la FAO et le Codex Alimentarius, doivent continuer leurs efforts pour établir une législation internationale standard en ce qui concerne les teneurs en aflatoxines légalement admissibles des arachides et des produits à base d'arachides destinés à la consommation humaine et animale. Au niveau national, on préconise que les pays mettent en place des groupes de travail pluridisciplinaires chargés de coordonner et d'évaluer le problème des aflatoxines dans le pays concerné, identifier un organisme compétent et organiser le suivi des teneurs en aflatoxines des aliments destinés à la consommation humaine et animale, et d'initier et coordonner des recherches visant la préparation de recommandations pour un contrôle à tous les niveaux. On doit veiller particulièrement à ce que les mesures de contrôle des teneurs en aflatoxines des arachides destinées à l'exportation n'aggravent pas le problème en ce qui concerne les arachides destinées à la consommation locale.

Besoins en recherches

Les besoins en recherches doivent être clairement définis en tenant compte des problèmes rencontrés par chaque pays et selon les moyens du pays concerné; le travail doit être réalisé en faisant appel aux technologies les plus adaptées et doit être effectué par les organismes les plus appropriés. Dans beaucoup de pays, il sera nécessaire de former le personnel dans les nouvelles techniques, afin d'assurer des progrès rapides. Le besoin de coopération dans le domaine de la formation et de la recherche, à un niveau national et international, a été reconnu par tous.

Recomendaciones

El taller identificó varias áreas que son motivo de preocupación, en relación con la contaminación del cacahuate con aflatoxinas, y formuló recomendaciones que se exponen en detalle en los informes de los grupos de trabajo. Las recomendaciones generales del taller son las siguientes.

Información y capacitación

El taller subrayó la necesidad de aumentar la comprensión de los peligros de la contaminación con aflatoxinas en los cacahuates y sus derivados, entre grupos internacionales, gobiernos nacionales, la industria cacahuatera, los productores y los consumidores. Las organizaciones tales como la FAO, OMS, CEE, etc., podrían hacer más en este respecto y podrían cubrir el problema de las aflatoxinas en todos los productos comerciales. Los gobiernos nacionales probablemente ponen mayor atención a los consejos recibidos de la FAO y OMS que las que reciben de otras organizaciones. La necesidad de lograr que la información sobre el problema de las aflatoxinas en cacahuate pueda obtenerse con mayor facilidad fue subrayada. Se alentó al ICRISAT a proceder a la organización electrónica de la bibliografía existente sobre aflatoxinas, y a organizar la elaboración de un manual o boletín informativo sobre muestreo y métodos analíticos, y sobre prácticas de manejo del cultivo para combatir las aflatoxinas en el cacahuate. También se sugirió que el ICRISAT podría funcionar como una bolsa informática, que haría del conocimiento de todos los organismos responsables del combate del problema de las aflatoxinas, los lugares y fechas en que se llevarán a cabo cursos de capacitación, talleres, etc., sobre diversos aspectos de interés para ellos.

Estrategias

Los grupos interesados, AGC, CEE, FAO y el Codex Alimentarius, deben continuar trabajando para lograr el establecimiento de legislación internacional uniforme, que reglamente los niveles aceptables de aflatoxinas en los cacahuates y sus derivados para el consumo humano o animal. A nivel nacional, se recomienda a los países que organicen grupos de trabajo interdisciplinarios, para que coordinen la evaluación del problema de las aflatoxinas en su país; que designen una agencia gubernamental responsable de darle seguimiento a las acciones requeridas; que organicen la vigilancia constante de los niveles de aflatoxinas presentes en los alimentos humano y animal e inicien y coordinen las investigaciones necesarias para la elaboración de recomendaciones para combatir las aflatoxinas a todos los niveles. Se pidió que se dedicara especial atención a asegurar que las medidas utilizadas para reducir los niveles de aflatoxinas en los cacahuates destinados a la exportación no agraven el problema existente en los cacahuates destinados al consumo local.

Investigación requerida

Debe quedar claramente definida la investigación requerida en función de los problemas existentes en cada país y de la infraestructura y personal que se dispone para desarrollar investigaciones sobre aflatoxinas. El programa debe desarrollarse con las tecnologías más

apropiadas y con la participación de las organizaciones más relevantes. La capacitación de personal en las nuevas técnicas será necesaria en muchos países, si se desea lograr progresos rápidos. Se reconoció la necesidad de cooperación nacional e internacional, tanto en la capacitación del personal, como en el desarrollo de las investigaciones requeridas.

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