

# 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 avus* 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.*

## Resúmen

**El método del ICRISAT en sus investigaciones sobre el problema de las aflatoxinas en el cacahuete :** *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 cacahuete 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<sup>1</sup> 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 toxin contamination based on cultivars being inefficient substrates for aflatoxin production.

Nevertheless, 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 post-rainy- season irrigated crop (Nov–Apr). Access to two very different soils (Alfisol: 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 mycoflora. 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.

**That.** 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.

**J. Mc.Donald.** Agreed that FAO and other organizations are doing much to awaken interest at the government 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.