Aflatoxin Contamination Problems in Groundnut in Asia

International Crops Research Institute for the Semi-Arid Tropics

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Abstract

The current status of research on aflatoxin contamination problems in groundnut in Asia is reviewed. Particular emphasis is given to recent advances in aflatoxin management technologies including genetic resistance to *Aspergillus flavus* infection and aflatoxin production, and analytical and immunochemical methods for the analysis of aflatoxins. The publication includes country papers summarizing the status of the groundnut aflatoxin problem in Bangladesh, China, India, Malaysia, the Philippines, Thailand, and Vietnam. Recommendations are made for collaborative research, and the need to interest potential donors in promoting the Group's activities is stressed.

Résumé

La contamination de l'arachide par les aflatoxines en Asie: comptes rendus de la Première réunion du Groupe de travail, 27–29 mai 1996, Ministère de l'Agriculture et du Développement rural, Hanoi, Vietnam. Cet ouvrage fait le point des travaux de recherche sur la contamination de l'arachide par les aflatoxines en Asie. Sont traités en particulier, les nouvelles technologies de gestion des aflatoxines, notamment la résistance génétique à l'infection par *Aspergillus flavus* et la production des aflatoxines, ainsi que les méthodes analytique et immunochimique. Cette publication comprend également les rapports sur le problème des aflatoxines en Chine, en Inde, en Malaisie, aux Philippines, en Thaïlande et au Vietnam. Des recommandations sont proposées pour la collaboration en la recherche, en soulignant le besoin de faire intervenir des bailleurs de fonds potentiels afin de soutenir les activités du Groupe.

Aflatoxin Contamination Problems in Groundnut in Asia

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Edited by

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ICRISAT
International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India

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Co-sponsors

Ministry of Agriculture and Rural Development (MARD), Vietnam
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

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Preface

Aflatoxin contamination of groundnut is a serious problem in several Asian countries. In response to the perceived need for regional cooperation to address this problem, an Asian Groundnut Aflatoxin Working Group was formed in 1996 to coordinate research efforts by various national and international research institutions. This first collaborative research planning meeting of the Asia Working Group was co-sponsored by the Ministry of Agriculture and Rural Development (MARD), Vietnam, and the Cereals and Legumes Asia Network (CLAN)/ICRISAT. The meeting was hosted by MARD, from 27 to 29 May 1996, at Hanoi.

Participants at the meeting included scientists from six countries and ICRISAT. Reports on the status of groundnut aflatoxin research worldwide and accounts of current research on the problem by national institutions were presented. Recent advances made in analytical and immunochemical methods for aflatoxin analysis, and aflatoxin risks in various agroecological regions were discussed. A great deal of progress has been made in recent years, and prospects of collaborative research into the management of the problem are encouraging.

We hope that these proceedings will provide a useful guide to the present status of the groundnut aflatoxin problem in Asia, and stimulate more coordinated research on this problem.

V K Mehan
C L L Gowda

Inaugural Session

Inaugural Address

Ngo The Dan[1]

Chairman, Dr Renard, distinguished guests, ladies and gentlemen. First of all, please allow me, on behalf of the Ministry of Agriculture and Rural Development (MARD), to warmly welcome our distinguished guests and participants to this meeting.

It is an honor for MARD to co-sponsor this 3-day meeting. This is the first meeting of the Asia Working Group on Groundnut Aflatoxin Management. I am glad to see that it is being attended by scientists representing six countries and an international institute.

In Vietnam, groundnut is the most important legume crop with a high export potential. Groundnut products are used locally in various forms of food and feed. In recent years, groundnut production in Vietnam has increased significantly. The annual area under groundnut cultivation has increased from 200 000 ha (in 1990) to 250000 ha (in 1995), and it is expected to increase further in the near future. Groundnut yield has reached 1.2 t ha^{-1} with a total annual production of 300 000 t. Improved cultivation technologies widely applied in various groundnut-growing areas are expected to substantially contribute to enhanced groundnut production in the coming years. The role of groundnut in Vietnam's national economy will be more important because the demand for feed sources for the development of animal husbandry is rapidly increasing.

In Vietnam, within the framework of the Vietnam/ICRISAT Collaborative Program, groundnut aflatoxin research is given high priority. ICRISAT scientists have helped Vietnam in the training of research staff, and have conducted surveys on aflatoxin contamination of groundnut in both northern and southern Vietnam. There is a strong need to strengthen these collaborative research efforts and intensify research on groundnut aflatoxin management in Vietnam. We welcome and highly appreciate the initiatives of the Cereals and Legumes Asia Network (CLAN) and ICRISAT in the coordination and organization of regional collaborative activities relating to research on groundnut aflatoxin management. MARD would be pleased to extend full support to such collaborative research efforts in Vietnam.

On this occasion, I would like to express our sincere thanks and appreciation to ICRISAT and CLAN for their help and collaboration in effective groundnut research and production improvement in Vietnam over the last few years.

Aflatoxin contamination is considered one of the most important groundnut food-quality problems in many countries, especially in tropical Asia. *Aspergillus* species produce aflatoxins that cause cancer in human beings and animals. Many importing countries have established strict maximum permissible limits for aflatoxin in groundnuts and groundnut products. Aflatoxin contamination is, therefore, a challenging

1. Vice Minister, Ministry of Agriculture and Rural Development, N2, Ngo ha, Ba dinh, Hanoi, Vietnam.

problem, and has attracted the attention of scientists in various disciplines worldwide.

We hope that this meeting will be a good opportunity for Vietnamese scientists to improve their knowledge and to learn from other countries' experiences in groundnut aflatoxin management.

I believe that the focus of this Working Group will be on international approaches towards improving the production and quality of groundnut. I am sure that the deliberations of this meeting will lead to some guidelines for coordinated international research and development work for the next 2-3 years.

Thank you all very much for coming to Hanoi, and I trust that you will have a very successful and enjoyable meeting.

I wish the meeting all success.

Welcome Address

C Renard[1]

Dr Ngo The Dan, Vice Minister, Ministry of Agriculture and Rural Development (MARD), Directors of the National Institute of Plant Protection, Vietnam Agricultural Science Institute, Institute of Agricultural Sciences, Oil Plant Institute, and Postharvest Technology Institute, and participants from China, India, Malaysia, the Philippines, Thailand, and Vietnam, on behalf of ICRISAT, I would like to welcome you to Hanoi and to the First Asia Working Group Meeting on Aflatoxin Contamination Problems in Groundnut in Asia. At the outset, I wish to express our thanks to MARD for agreeing to host the meeting, and to the local organizing committee for making all the arrangements, and providing the necessary logistic support.

Of the ICRISAT mandate crops, groundnut is grown in almost all the countries where we work. All other crops, except for groundnut, are subsistence crops. However, smallholders and large-scale farmers in the semi-arid tropics (SAT) and elsewhere trade extensively in groundnuts. The annual cropped area under groundnut worldwide (in 1993) was around 20.5 million ha, with a total production of 25 million t. Current world trade in groundnut is around 1.3 million t (excluding intra-country trade). Export of groundnut and its products (oil and cake) showed an increasing trend of 5.6% during the 1970s and the 1980s. However, there has been a decline in the export of groundnut from some of the groundnut-producing countries in Africa, Latin America, and Asia. This reduction in export is related to the awareness and concern among consumers in the importing countries (notably those in Europe and Japan) regarding aflatoxin contamination.

As you are aware, aflatoxins are highly toxic and carcinogenic substances produced by the fungus *Aspergillus flavus,* which infects several important oilseeds, legumes, and cereals. However, maize and groundnut are the most common food crops in which aflatoxin contamination can occur, and thus lead to health hazards for human beings and animals.

Many developed countries have placed limits on the levels of aflatoxin that range from zero to twenty parts per billion (μg kg^{-1}) in foodstuffs meant for human consumption. It would be good to have zero levels of aflatoxin in groundnuts and groundnut products. However, this is not always possible in nature, as groundnut is grown under varied conditions that often favor *A. flavus* infection and aflatoxin production. Apart from field-growing conditions, postharvest and storage practices can also lead to aflatoxin contamination. To sum up, increased levels of aflatoxin in groundnuts result in the loss of export earnings to many groundnut-producing countries.

Our aim of co-sponsoring this meeting with MARD, Hanoi, is to devise management practices that can reduce, if not eliminate, aflatoxin contamination in groundnut.

I wish you all a successful meeting.

1. Executive Director, ICRISAT Asia Region, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.

Asia Working Group on Groundnut Aflatoxin Management

Asia Working Group on Groundnut Aflatoxin Management: Background, Objectives, and Goals

C L L Gowda and A Ramakrishna[1]

Groundnut is a major food legume and an important oilseed crop in many Asian countries. It is a major source of income to both small- and large-scale farmers. Groundnut also contributes significantly to the export earnings of several countries. Aflatoxin contamination is a major problem in many groundnut-producing countries. Aflatoxins are highly toxic, cancer-causing substances produced by the fungus *Aspergillus flavus,* which often infects groundnut pods and seeds. The presence of such toxic and carcinogenic substances in groundnut foods and feeds has a considerable impact on the utilization of, and trade in, groundnuts and groundnut products. Some groundnut-producing countries are losing export earnings because they are not able to achieve the maximum permissible limits of aflatoxin set by importing countries. Although many national and international research institutions are working on various aspects of the groundnut aflatoxin problem, a coordinated effort is needed to hasten the development of integrated aflatoxin management strategies. The International Groundnut Workshop held at ICRISAT in 1991 recommended the formation of a Working Group on Aflatoxin Management. This paper describes the concept and operation of a Working Group, and its role in assisting and enhancing research collaboration and outcomes.

Cereals and Legumes Asia Network (CLAN)

CLAN was established in April 1992 by amalgamating the erstwhile Asian Grain Legumes Network (AGLN) and the Cooperative Cereals Research Network (CCRN). CLAN serves as a single-window network in Asia, for research and technology exchange involving sorghum, pearl millet, chickpea, pigeonpea, and groundnut.

CLAN comprises of scientists and administrators in Asian countries who have indicated their interest and willingness to commit resources to undertake collaborative research, participate in network activities, and share results and technology.

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ICRISAT Conference Paper no. CP 1100.

Gowda, C.L.L., and Ramakrishna, A. 1997. Asia Working Group on groundnut aflatoxin management: background, objectives, and goals. Pages 9-12 *in* Aflatoxin contamination problems in groundnut in Asia: proceedings of the First Asia Working Group Meeting, 27-29 May 1996, Ministry of Agriculture and Rural Development, Hanoi, Vietnam (Mehan, V.K., and Gowda, C.L.L., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Membership includes staff from more than 15 Asian countries, regional and international institutions primarily in Asia, and ICRISAT. Currently, the Coordination Unit is located at, and supported by, the ICRISAT Asia Center.

The specific objectives of CLAN are to:

- Strengthen linkages and enhance exchange of germplasm, breeding material, technical information, and technology options among members,
- Facilitate collaborative research among members to address and solve high-priority production constraints, paying attention to poverty and equity issues as per the needs and priorities of member countries,
- Assist in improving the research and extension capability of member countries through human resource development,
- Enhance coordination of regional research on sorghum, pearl millet, chickpea, pigeonpea, and groundnut, and
- Contribute to the development of stable and sustainable production systems through a responsive research capability in member countries.

The overall objective of CLAN is to support, coordinate, and facilitate technology exchange involving CLAN priority crops and their resource management among Asian scientists. The ultimate goal is to improve the well-being of Asian farmers by improving the production and productivity of crops in a sustainable manner.

Working Groups

Individual laboratories and/or institutions are unable to take up comprehensive studies due to the scarcity of funds, facilities, and expertise. Therefore, it is not surprising that scientists should endeavor to join hands to address, and find solutions to, important regional problems for increased and sustainable food production. A Working Group (WG) can be defined as a group of committed scientists sharing a common interest in addressing and finding solutions to a high-priority regional problem. Working Groups are also called subnetworks, working parties, or consortia. International and regional Working Groups coordinate and stimulate cooperative research by bringing together experts from developed and developing countries, international agricultural research centers, and specialized research laboratories and institutions, to work together on a common platform as equal partners.

The concept of Working Groups is not new; scientists around the world have been pooling their resources and sharing the results either formally or informally. Many countries have collaborative ventures for sharing resources and research responsibilities. In the international arena, collaborative agricultural research networks are becoming increasingly popular as a means of utilizing funds, facilities, and staff more efficiently and effectively. For example, Working Groups on Asia-Pacific Groundnut Viruses and Bacterial Wilt of Groundnut have been successful in generating new research information, creating research partnerships, and disseminating research results, information and technologies.

Advantages of Working Groups

Some of the advantages of Working Groups (WGs) are:

- Enhanced research partnerships in the region to address major production problems,
- Flexibility in operation to initiate and conclude research on specific problems,
- Cost-effectiveness and operational efficiency due to small size of WGs,
- Use of existing staff and facilities, avoidance of duplication, and saving of time and resources,
- Attractiveness of collective approach to donors for funding, and
- Support of overlapping activities of other WGs in areas such as training.

Organization and Structure of Working Groups

Working Groups consist of interested members from national, international, and regional programs/institutions. Each WG nominates a Technical Coordinator (TC) to be responsible for the liaison, coordination, and harmonizing of research. A TC is normally an expert in the subject, and can be from any collaborating institution. An example of a WG structure is given in Figure 1. Usually the TC of a WG is supported by a network or institution for the provision of necessary administrative and logistic support.

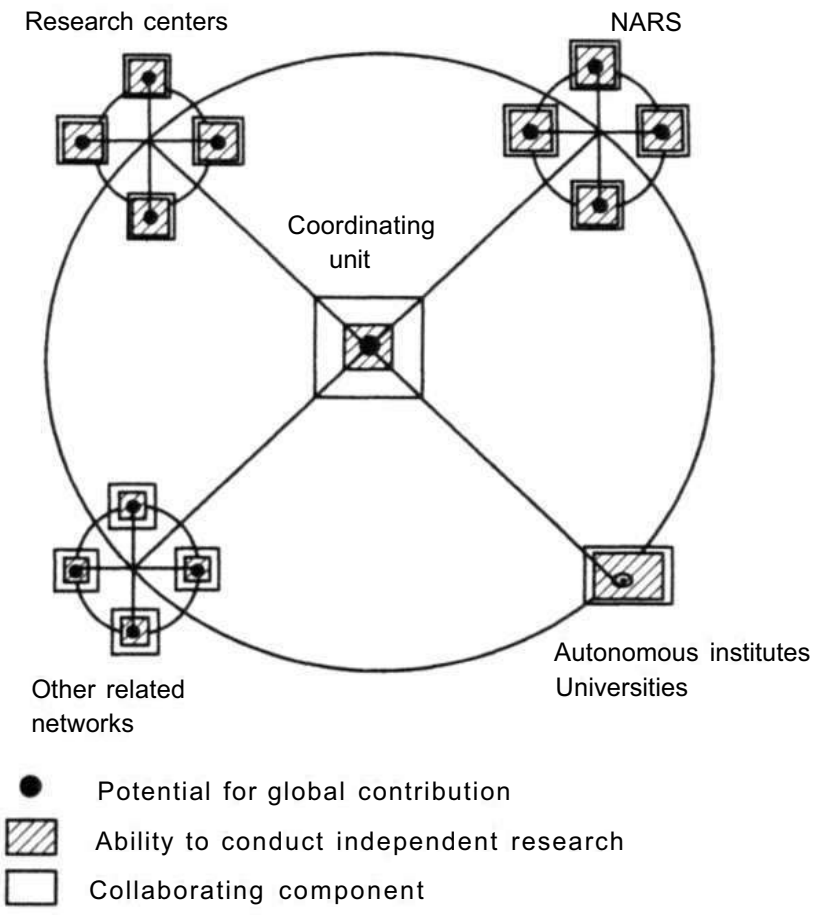

Figure 1. Structure of a Working Group,

Working Group on Groundnut Aflatoxin Management

A Working Group on Groundnut Aflatoxin Management is to be established, subject to approval at this meeting, to coordinate research on groundnut aflatoxin management.

The objectives of the meeting are to:

- Review the progress in recent years in analytical and immunochemical techniques for aflatoxin analysis,
- Document available knowledge on components of integrated management of groundnut aflatoxin problems, and
- Establish priorities for future research and collaboration.

It is hoped that the WG members will be able to share research responsibilities, depending on their capabilities and comparative advantages, and share and exchange research results and information. The goal is to provide management options to farmers for managing groundnut aflatoxin problems for better profits and the health of the human and animal populations of the world.

An Overview of Research on the Management of Aflatoxin Contamination of Groundnut

F Waliyar[1]

Aflatoxin contamination of groundnut is one of the most important constraints to groundnut production in many countries. It is also of significance in relation to public health and exports (Pettit et al. 1989, Waliyar 1978 and 1990, Wynne et al. 1991).

Most countries/institutions give high priority to research on the groundnut aflatoxin problem. Many national agricultural research systems (NARS) in Asia and Africa are faced with this problem because of the difficulty in reducing aflatoxin contamination in groundnuts and groundnut products to an acceptable level for export.

The concept of Aflatoxin Working Groups for Asia and Africa will help us to arrive at a better understanding of the actual research orientation of the activities of ICRISAT/NARS in Asia and Africa.

This paper gives an overview of aflatoxin research worldwide to allow for better planning of ICRISAT's future activities with NARS partners. A complete review and literature database on the groundnut aflatoxin problem is available at ICRISAT (Mehan et al. 1991).

Overview of Research on Aflatoxin Contamination

Aspergillus flavus infection of groundnuts occurs under both preharvest and postharvest conditions (Cole et al. 1989, Diener et al. 1987, Manzo and Misari 1989). Preharvest infection by *A. flavus* and consequent aflatoxin contamination are important in the semi-arid tropics (SAT), especially when end-of-season drought occurs (Azaizeh et al. 1989, Kisyombe et al. 1985). Drought stress may increase susceptibility to fungal invasion by decreasing the moisture content of the pod and seed, or by greatly lowering the physiological activity of the groundnut plant (Azaizeh et al. 1989, Kisyombe et al. 1985, Mehan et al 1988).

1. ICRISAT Western and Central Africa Region, B P 320, Bamako, Mali.

ICRISAT Conference Paper no. CP 1150.

Waliyar, F. 1997. An overview of research on the management of aflatoxin contamination of groundnut. Pages 13-17 *in* Aflatoxin contamination problems in groundnut in Asia: proceedings of the First Asia Working Group Meeting, 27-29 May 1996, Ministry of Agriculture and Rural Development, Hanoi, Vietnam (Mehan, V.K., and Gowda, C.L.L., eds). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Worldwide Progress in Aflatoxin Research

Research on the aflatoxin problem is not regularly carried out by all groundnut-producing countries. This is because of the lack of qualified personnel. Nevertheless some countries have been regularly monitoring groundnuts and groundnut products for aflatoxin at different stages (farm, storage etc.).

Before the 1980s, the aflatoxin problem was considered a postharvest problem. Therefore, research was focussed only on postharvest problems. However, severe preharvest aflatoxin contamination was reported in Australia, and in several countries in Asia and Africa.

Since the early 1980s, several national and international institutes, including ICRISAT, have carried out research on preharvest aflatoxin contamination. It is now well-established that aflatoxin contamination is also a preharvest problem in the SAT, particularly in areas where late-season drought is common. In the more humid tropics, it-is largely a postharvest problem. Investigations on the effects of climate, edaphic factors, and their interactions in the field and under controlled conditions have provided considerable information on pre- and postharvest infection by *A. flavus* and consequent aflatoxin production. Accordingly, a number of important recommendations were formulated for use by farmers and those concerned with purchase, storage, and processing of groundnuts and groundnut products (Dickens 1977, Mehan et al. 1991, Mehan 1992). These practices include:

- Avoiding damage to plants and pods from soilborne diseases and during cultivation,
- Avoiding late-season drought stress by manipulation of crop duration and supplementary irrigation,
- Lifting the crop at optimum maturity,
- Discarding damaged pods,
- Drying pods to below 8% moisture content,
- Storage under clean, dry, and insect-free conditions, and
- Avoiding re-wetting of pods/seed during storage.

Genetic Resistance

One of the possible means of reducing aflatoxin contamination of groundnut is the use of resistant cultivars. Several studies have established the presence of field resistance to seed infection by *A. flavus* in some cultivars. Resistance to preharvest field infection is particularly important in areas where late-season drought stress is a common occurrence (Mehan et al. 1987, Mehan et al. 1991, Mixon 1983, Waliyar et al. 1994, Zambettakis et al. 1981). Some cultivars such as J 11, 55-437, and PI 337394F have shown stable resistance to A *flavus* across locations. These sources among others have been used in breeding programs, and several lines have been reported to possess resistance and produce high yield. Several breeding lines from ICRISAT have been reported to be resistant to seed infection and colonization; these are ICGVs 87084, 87094, 87110, 91278, and 91284.

More resistant cultivars adapted to different production systems need to be developed to meet the requirements of producers and users.

The relationship between different resistance mechanisms, and their interactions have not been clearly established. Therefore, there is a need to carry out research to elucidate the mechanisms of resistance to pod/seed infection by A *flavus* and aflatoxin production.

Biotechnological Research

Efforts have been made to develop aflatoxin-resistant transgenic groundnut plants. This can be an effective long-term genetic approach to the problem.

Biological Control

Several biocontrol agents have been reported to control aflatoxin in groundnut. Cotty (1990) has done considerable research on the use of nontoxigenic strains of *A. flavus* to control aflatoxin contamination. This approach is based on the substitution of aflatoxin-producing strains of *A. flavus* with nontoxigenic strains. As high levels of the inoculum of nontoxigenic strains are required, this may result in the increased incidence of aflaroot in the field, and increased seed infection can lead to the production of free fatty acids and the loss of seed quality for commercial processing.

Detoxification and Decontamination

Large-scale detoxification procedures, using ammonia under high pressure, have been developed; these are now operational in Senegal and in the Sudan. Detoxification techniques suitable for small groundnut processors are needed. In India, some simple approaches for the detoxification of groundnut oil have been developed. Detoxification of crude oil in binding aflatoxin in groundnut oil and cake was studied. Some of these procedures can be used at the small-scale industry or the household level (Mehan 1995). The use of red clays in West African countries has been found to be very effective in binding aflatoxin in contaminated groundnut cake.

In Senegal, it was found that exposure to sunlight for 18 to 24 h destroyed 100% of the toxin in contaminated oil (Kane 1996). The contaminated oil is kept in sunlight in transparent and translucent containers. This simple method is a very useful way of reducing aflatoxin levels, and can be used by oil processors at the village level.

Other methods such as use of electronic devices to remove infected seed from groundnut lots have been used. These methods are expensive and not suitable for farmers in the SAT.

Cultural Control

Several recommendations have been made for the control of aflatoxin by adopting certain cultural practices. Some cultural practices, such as adjustments of sowing and

harvesting dates, and application of gypsum, are effective in preventing aflatoxin contamination. The relationship between drought stress, termite population and seed contamination has been established. A period of drought at the end of the rainy season also favors aflatoxin contamination and increases the termite population.

There is a need for on-farm research to demonstrate the effectiveness of these cultural practices.

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Country Reports on the Status of Groundnut Aflatoxin Research

Status of the Groundnut Aflatoxin Problem and its Management in Bangladesh

Md. Ahsan Ullah[1]

The present annual production of groundnut in Bangladesh is about 40 000 t, the major part of which is consumed as roasted nuts. The rest is used in confectionery. Groundnuts are not used for oil extraction.

Kishoreganj, Noakhali, Dhaka, Faridpur, Sylhet, Comilla, Mymensingh, Rangpur, and Chittagong are the major areas where groundnut is grown in the postrainy season (Oct-Mar). In the Comilla, Rajshahi, Dhaka, and Kushtia areas, the crop is also grown in the rainy season (Apr-Sep), mainly as a seed-source for the postrainy-season crop. The major soil types in groundnut-growing areas are: acid basin clays; gray and non-calcareous dark gray flood plain soils; red brown terrace soil; calcareous alluvium soil; calcareous dark gray and brown flood plain soils; gray flood plain soil; non-calcareous dark gray flood plain soils; brown hill soils; and peat soils.

Status of the Aflatoxin Problem

In Bangladesh, studies on aflatoxin contamination in groundnuts and groundnut products are at the preliminary stage. No official report on the health hazards posed by aflatoxin contamination to human beings or animals in Bangladesh is available. The Bangladesh Council for Scientific and Industrial Research (BCSIR) has recently established a modern laboratory for the analysis of aflatoxin levels in groundnut, maize, and other products.

Technologies Available for Aflatoxin Management

Groundnuts with 8-9% moisture content were found to remain free of aflatoxin even after 7-8 months of storage in polythene-lined gunny bags or tin containers. Detailed studies are yet to be carried out to find suitable technologies for managing the aflatoxin problem, particularly during storage.

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Constraints to the Adoption of Aflatoxin Management Options

Producers, processors, and consumers of groundnuts and groundnut products are not aware of the health hazards posed to human beings and livestock from ingesting aflatoxin-contaminated foods and feeds. Proper storage practices are not followed.

Present Staffing and Resources Available for Research

At BCSIR, four scientists are engaged in research on aflatoxin contamination problems in groundnut and other crops. Biochemists and pathologists at the Oilseed Research Centre of the Bangladesh Agricultural Research Institute (BARI) are also involved in research on aflatoxin.

Future Plans

Priority will be given to research on:

- Genetic resistance to pod and seed invasion by *Aspergillus flavus,*
- Developing preharvest cultural practices that minimize damage to pods,
- Developing postharvest handling and storage technologies to control *A. flavus* infection, and
- Monitoring aflatoxin levels.

Status and Management of Aflatoxin Contamination in Groundnut in China

Xiao Daren and Wang Shengyu[1]

Aflatoxin contamination of food crops is a common and serious problem in China. Maize, groundnut, rice, wheat, and various legumes are often infected by the aflatoxin-producing fungus, *Aspergillus flavus,* and contaminated with aflatoxin in regions where climatic conditions are warm and humid. This paper gives a brief report on the status of the groundnut aflatoxin problem and its management in China.

Distribution and Levels of Aflatoxin in Foods

During 1972-80, a general survey of aflatoxin contamination in 18 kinds of foods was carried out in 24 provinces by the Cooperative Research Group on Controlling Aflatoxin Contamination in Food. Over 20 000 samples were analyzed for aflatoxin. Aflatoxin contamination levels were most serious in groundnut. Of the 1795 samples of groundnut and 3194 samples of groundnut oil analyzed, the percentages of samples containing the toxin were 41.3 for groundnuts and 76.7 for oil. Some samples were found contaminated with very high levels of aflatoxin (234 000 µg kg^{-1} in groundnuts and 8000 µ*g* kg^{-1} in oil).

The percentage of contaminated samples decreased with increase in latitude, being high in southern China, moderate in the Yangtze valley, and low in northern China.

Although *A. flavus* is widely distributed in nature, the percentages of aflatoxigenic strains (PAS) and the aflatoxin-producing ability (APA) are different in various regions. In general, the PAS and APA are higher in the southern, central, and eastern regions than in the northern and western regions of China. The percentages of aflatoxigenic strains were much higher (40.7-59.3%) in the Guangxi, Jiangxi, Guangdong, Hubei, and Jiangsu provinces than in the other provinces (7.4-27.9%). Interestingly, none of the 66 strains from the Gansu province was aflatoxigenic (Liu Xingjie et al. 1981). A positive correlation occurred between the PAS and percentages of positive samples in all the provinces ($r=0.7018$, $P<0.01$).

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Detoxification of Groundnuts and Groundnut Products

Removal of aflatoxin from groundnut oil. Since the 1970s, adsorption, photolysis, and alkali refining have often been used to detoxify groundnut oil. Of these, adsorption with white clay is extensively used in oil mills in southern China. Treatment of contaminated oil with 0.5-1.5% activated white clay at 60-80°C for 30 min can reduce aflatoxin levels from 50-800 to 5 µg kg^{-1} (Zhang Lisheng et al. 1982).

Removal of aflatoxin from groundnut seed and cake. The detoxification of contaminated seed and cake is not as easy as that of oil. Ammoniation and aromatic oil fuming are the main methods used to remove aflatoxin from groundnut seed and cake. Researchers at the Institute of Grain and Oil, Hunan, have reported a 99% reduction in aflatoxin levels by fumigating contaminated seed with an aromatic oil [from *Litsea cubeba* (Lour) Pers] for 155 days.

Screening for Resistance to Aflatoxin Contamination

The best approach to the aflatoxin contamination problem is to utilize genetic resistance to seed infection by *A. flavus* and/or aflatoxin production. Since the 1980s, resistance screening and breeding research have been carried out at several institutes in China, and some promising results have been obtained.

Screening for Resistance to Seed Colonization by A *flavus*

Li Siulin et al. (1992) evaluated 270 groundnut genotypes in the laboratory for resistance to seed colonization by *A. flavus.* Three genotypes (Xinhuixiaoli, Zhanqiu 48, and Meixianhongyi) showed the lowest level of colonization (3-14%), while six other genotypes exhibited a moderate level (17-25%). The results indicated that all the 10 varieties promoted in the Guangdong province were highly susceptible (90-100%) to seed colonization by *A. flavus.*

Gao Guoqing et al. (1995) reported four genotypes resistant to seed colonization from the screening of 853 groundnut genotypes. The mean colonization levels of these resistant genotypes (701, 702, Mini-1, and Mini-2) in four years were 13.6,13.7, 14.1, and 16.1% respectively.

Xiao Daren (1992a) evaluated 1576 genotypes for their reaction to seed colonization by *A. flavus.* Only two genotypes, Jinxianzhanyangzi and N 1049, showed low levels of seed colonization; the mean colonization levels in three years were 12.8% (range: 3-23.4%) for Jinxianzhanyangzi and 14.9% (range: 4-24%) for N 1049.

Screening for Resistance to Aflatoxin Production

Although some genotypes have been reported to have resistance to seed colonization by *A. flavus,* seed colonization can be significantly influenced by environmental fac-

tors. The levels of seed colonization vary considerably across testing years. Therefore, researchers are often more interested in screening for resistance to aflatoxin production than seed colonization by *A. flavus.*

Xiao Daren (1992a) evaluated 873 groundnut genotypes for their abilities to support aflatoxin production. Though all the genotypes supported aflatoxin production, significant differences in production levels were found. The levels of aflatoxin B_1 produced ranged from 18 to 496 µg g^{-1}. The two genotypes (91322 and 91211) consistently supported much lower levels of aflatoxin (8-26 µg g^{-1}) across four years (1991, 1992, 1994, 1995) compared with the susceptible control cultivar G 119 (496 µg g^{-1}).

Screening Methods for Resistance to Aflatoxin Production

To identify genotypes resistant to aflatoxin production, vast numbers of genotypes have to be rapidly and economically screened. Therefore, a simple and reliable method for the quantification of aflatoxin is needed.

Xiao Daren (1992b) described a tube fluorescence (TF) method for large-scale screening for resistance to aflatoxin production. The extract from 1 g of groundnut sample is added to 1 mL of $NH_4H_2PO_4$-$HgCl_2$ solution containing 0.005 g agar in a tube. The concentration of aflatoxin can be visually estimated by the TF method. The lowest amount that could be detected was 0.04 µg g^{-1} by visual observation under UV light. The aflatoxin amounts estimated by the TF method were slightly lower than those estimated by a thin-layer chromatography (TLC)-based method, but there was no significant difference between the methods. This method is convenient and rapid when a single plant of each genotype is available for screening.

Xiao Daren (1992b) also developed a half-seed method for screening for resistance to aflatoxin production in single seeds. This method needs only half of a single seed to estimate aflatoxin production. If the tested half shows resistance, the other half is grown for the next generation.

Future Plans

Since the 1980s, researchers in the Hubei, Guangdong, Guangxi, and Fujian provinces have identified several genotypes as resistant to seed colonization by *A. flavus* and/or aflatoxin production. But the performance of these resistant genotypes in different environments has not been confirmed.

Future research plans will be focused on:

- Evaluating a large number of genotypes for their ability to support aflatoxin production, and studying the inheritance of resistance to aflatoxin production,
- Breeding high-yielding cultivars with resistance to infection by *A. flavus* and/or aflatoxin production,
- Studying the role of chitinase in resistance to *A. flavus* invasion and colonization in groundnut, and

- Establishing a cooperative network of all institutes involved in aflatoxin research in China for the exchange of information.

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Aflatoxin Contamination of Groundnut and its Management in India

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Groundnuts are often infected with the aflatoxin-producing fungus, *Aspergillus flavus,* and consequently contaminated with aflatoxins, in the field, during storage, and in transit.

Status of the Aflatoxin Problem

Various surveys conducted in different parts of India (Ghewande et al. 1989, Sahay and Rajan 1990, Kolhe et al. 1994) have revealed that groundnuts and groundnut products are high-risk commodities for aflatoxin contamination. Levels of aflatoxin contamination (0.8 to 2200 µg kg^{-1} in groundnuts; traces to 200 µg kg^{-1} in edible flour; 786 µg kg^{-1} in unrefined oil; 27 to 1122 µg kg^{-1} in cake) varied from location to location depending upon the agroclimatic and storage conditions.

The rainy-season crop is often subjected to drought, particularly end-of-season drought, in most of the areas in the major groundnut-producing regions. This encourages *A. flavus* infection and aflatoxin contamination. Research carried out on the evaluation of groundnut-producing environments in terms of aflatoxin contamination during the 1993-95 rainy seasons at different locations (Chiplima, Orissa; Dharwad and Raichur, Karnataka; Jalgaon, Maharashtra; Junagadh, Gujarat; Vriddhachalam, Tamil Nadu) under the All India Coordinated Research Project on Groundnut (AICRP-G) revealed that Vertisols had significantly lower populations of *A. flavus* than Alfisols (AICRP-G 1994, pp. 26-27; AICRP-G 1995; AICRP-G 1996, p. PP7). These results confirmed earlier studies conducted in India (Mehan et al. 1991), and indicated low risks of aflatoxin contamination in Vertisols.

Harvesting, drying, and storage practices vary from region to region and have direct relevance to *A. flavus* invasion and consequent aflatoxin contamination in groundnuts. The harvested plants are usually heaped in the field for at least a few days, when the weather conditions are unfavorable for drying. If rain is anticipated during harvesting, the harvested plants are covered with palm leaves, gunny bags and/or fresh haulm to

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prevent wetting of the pods. Further, it is a common practice to heap the drying pods every evening, and to spread them out the next morning for further drying, to reduce the chances of fungal infection. The crop produce from the rainy season in some southern states dries slowly because of northeast monsoon rains at harvest; this encourages A. *flavus* infection and aflatoxin contamination. Field drying of pods takes 6-12 days (to reach safe seed-moisture level), particularly in case of northeast monsoon rains. Storing of underdried pods usually increases the temperature inside the heap. Slowly dried pods generally show higher percentages of seed infected by *A. flavus,* and higher aflatoxin levels than rapidly dried pods. Poor storage conditions also lead to aflatoxin contamination.

Research on Aflatoxin Management

Preventive methods such as host-plant resistance, and cultural practices are the best methods to reduce aflatoxin contamination. Research in India has shown that seed infection by A *flavus* and aflatoxin contamination increase with increasing maturity of pods, indicating the importance of harvesting the crop at optimum maturity. Studies have indicated that atoxigenic strains are useful in the biological control of preharvest aflatoxin contamination of developing groundnut under greenhouse conditions (Chourasia and Sinha 1994).

Several chemicals and plant products have been found useful in inhibiting A *flavus* invasion and aflatoxin contamination during postharvest processing and storage. The propionic acid (3 µL g^{-1}) treatment of groundnuts, stored at 90% relative humidity, has been found to reduce *A. flavus* infection considerably (Patker et al. 1995).

Genetic resistance to A *flavus* invasion and aflatoxin production is considered one of the cheapest and most viable alternative approaches to combat the groundnut aflatoxin problem in India. Researchers at the National Research Centre for Groundnut (NRCG), Junagadh, have identified several genotypes (GRP 34, ICG 239, Ah 20, NRCGs 698, 8970, 8972, and 8973) as resistant to in vitro seed colonization. Several other genotypes (B 99-1, B 95, ICG 239, Chitra, Spancross, GRP 34, and RS 1) have been found to support low aflatoxin production (Desai et al. 1991, Ghewande et al. 1993). Some genotypes have also been evaluated for their resistance to seed infection (preharvest resistance), and yield at NRCG and ICRISAT. Various lines (ICGVs 88145, 89063, 89065, 89092, 89106, 89112, and 89115, ICG 239, B 95, NRCGs 5506, 8938, and 8939) have shown resistance to seed infection, and have given high yields.

Recently, several organizations have undertaken to improve and stabilize the yields of Hand-Picked-and-Selected (HPS) groundnuts to promote the confectionery industry and exports. The bold-seeded genotypes, GG 11, Koyana, RS 1 (released Indian varieties), B 99-1 (advanced breeding line), and ICG 239 (germplasm line) have been identified as moderately resistant to A *flavus* seed colonization and as low supporters of aflatoxin production (Desai et al. 1991, Ghewande et al. 1993). It is suggested that cultivars such as RS 1, J 11, Chitra, GG 11, and Koyana can be adopted in areas where aflatoxin contamination is a serious problem.

Scientists at the Central Food Technological Research Institute (CFTRI), Mysore, have developed several physical and chemical methods for the decontamination of groundnut oil, cake, and flour. Removal of discolored seeds by hand-picking or by electronic color sorting devices is efficient in reducing aflatoxin content in groundnuts. Exposure of contaminated groundnut oil to bright sunlight completely destroys the toxin. Aflatoxin in oil can also be removed by filtration or by extraction with 10% sodium chloride. Detoxification of groundnut seed and cake can be done using hydrogen peroxide (Shantha 1989).

Technologies for Aflatoxin Management

Based on an understanding of the factors contributing to aflatoxin contamination, various cultural, drying, and storage practices have been identified as effective in preventing or reducing aflatoxin contamination (Mehan 1992, Shantha 1989).

Constraints to the Adoption of Aflatoxin Management Options

The general constraints to the adoption of aflatoxin management options are:

- Lack of awareness and knowledge among farmers, traders, and processors,
- Nonavailability of supplementary irrigation facilities for release of drought stress, and
- Drying problems posed by northeast monsoon rains at harvest time, especially in some southern states.

Present Staffing and Resources Available for Research

Institutes engaged in research on aflatoxin—the National Institute of Nutrition, Hyderabad, CFTRI, Mysore, the Bhabha Atomic Research Centre, Mumbai, and NRCG, Junagadh—have adequate staff and research facilities. The staffing and research facilities at the main centers under AICRP-G, and the state agricultural universities and other universities working on this problem are very limited. There is a need to upgrade their facilities, and provide training to scientists in the use of enzyme-linked immunosorbent assay (ELISA) methods for aflatoxin analysis.

Future Research Plans

- Under the proposed Indian Council of Agricultural Research (ICAR)-ICRISAT collaborative project entitled 'Assessment of aflatoxin contamination of groundnuts in various production systems,' surveys are to be conducted in the major groundnut-growing states such as Andhra Pradesh, Karnataka, Tamil Nadu, Gujarat, and

Maharashtra to assess the extent of aflatoxin contamination and to identify high-, low-, and no-risk areas.

- Under the United Nations Development Programme (UNDP)-funded project on 'Promoting groundnut as food crop for sustained nutritional security,' demonstrations on technologies for the prevention of aflatoxin contamination at the farm level are to be held in Anantapur, Andhra Pradesh.
- Priority will also be given to research on breeding varieties for resistance to preharvest infection, seed colonization, and aflatoxin production with special reference to bold-seeded HPS types.
- Increased emphasis will be laid on the management of aflatoxin contamination through preventive methods (host-plant resistance; cultural, biological, and chemical control practices), monitoring, and detoxification procedures.

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Present Status and Future Prospects of Research on the Groundnut Aflatoxin Problem in Malaysia

Abidin B Hamid[1]

Geographically, Malaysia is located entirely in the equatorial zone. Being in the tropics, the average temperature throughout the year is high (26°C). The diurnal temperature variation range is about 7°C. Humidity is high (about 80%), owing to the high rate of evaporation and the heavy rainfall that occurs almost throughout the year (2500 mm). Under these conditions, stored commodities deteriorate easily and become very susceptible to mold contamination, including mycotoxin-producing species. Mycotoxin contamination can take place at any one of the various points in the food chain, i.e., during production, harvesting, storage, distribution, and processing.

Status of the Aflatoxin Problem

Although groundnut production is not extensive in Malaysia, raw shelled groundnuts are available in almost all the retail outlets in the country. Groundnut is extensively used as an ingredient or a base in a variety of processed foods and dishes.

In a 1981 survey, aflatoxins were found in groundnuts and groundnut products (FTC 1981). Ten of the 17 samples of raw shelled groundnuts were found contaminated with aflatoxin (2-400 µg kg^{-1}). Of these contaminated samples, 47% contained aflatoxin levels of 10-400 µg kg^{-1}. None of the 15 Mengelembu groundnut (boiled and roasted whole pods) samples was contaminated with aflatoxin, probably due to rapid drying and processing technology (48 h after harvest). Eight of the 10 peanut butter samples contained aflatoxin in the range of 4-400 µg kg^{-1}. Locally processed peanut butter samples contained higher levels of aflatoxin than imported ones. All samples of the groundnut product 'rempeyek' (groundnut fritters) were also contaminated (2-400 µg kg^{-1} aflatoxin B_1).

In another study carried out in 1985 (FTC 1985), 96 samples of raw shelled groundnut, randomly collected from retail outlets in most of the major towns throughout peninsular Malaysia, were analyzed for aflatoxins. Of the 96 samples analyzed, 88.5% were aflatoxin positive; 53.1% had a level of more than 40 µg kg^{-1}. In general, samples which appeared moldy always contained high levels of aflatoxin.

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During 1992-95, 403 samples of raw shelled groundnut were randomly collected from retail shops in all the major towns in the states of Selangor (227), Negeri Sembilan (112), and Malacca (64), and analyzed for aflatoxin using a modified mini-column method (Abidin and Mat Isa 1994, Mat Isa and Abidin 1996);

Of the 227 samples from Selangor, 54 (23.8%) were aflatoxin positive, of which only 20 (8.9%) contained levels ranging from 20 to 160 µg kg^{-1}. Most of these samples were from the Hulu Langat district (Abidin and Mat Isa 1994).

Of the 112 samples collected from Negeri Sembilan, 65 (58%) were contaminated with aflatoxin. The majority of these samples (42) contained aflatoxin levels ranging from 5 to 20 µg kg^{-1}. Only 14 samples (12.5%) had aflatoxin levels of 40-80 µg kg^{-1}, and 9 contained 20-40 µg kg^{-1}.

Of the 64 samples collected from Malacca, 43 samples (67.2%) were aflatoxin positive. Eleven samples had aflatoxin levels of 40-160 µg kg^{-1}, and 32 samples 5-40 µg kg^{-1}. Lower percentages of samples from Selangor were found contaminated with aflatoxin than those from Negeri Sembilan and Malacca.

Research on Aflatoxin in Malaysia

A report by Lim in 1964 on the outbreak of a disease which had occurred in 1960 on two pig farms in Malacca was probably the first indication of the occurrence of the aflatoxin problem in Malaysia. The disease which was expressed in gross liver damage, and was said to be associated with feed containing imported groundnut meal (Lim 1964). Later, Lim and Yeap (1966) reported the presence of aflatoxins in various feed ingredients imported into the country, including several types of oil cakes and meals.

Monitoring of aflatoxin in foods including groundnuts and groundnut oil was first carried out by the Institute of Medical Research (IMR) in 1965 (Chong and Beng 1965). The Food Technology Centre (FTC), MARDI, has monitored aflatoxins in agricultural commodities and foods since 1981 (Mat Isa and Abidin 1996).

Research on the biological detoxification of aflatoxins in naturally contaminated copra cake was also carried out. *Rhizopus oryzae* isolated from the substrate was used. Field studies showed that 70-80% of the aflatoxin present was degraded after 5 days of fermentation at ambient temperatures (26-32°C).

Technologies Available for Managing the Aflatoxin Problem

In Malaysia, specific technology for preventing or controlling aflatoxin contamination in agricultural commodities is not available. However, it is generally accepted that the aflatoxin problem can be managed by adopting certain preharvest and postharvest practices.

Preharvest practices include the choice of proper cultivation methods, the correct use of pesticides and irrigation.

Postharvest aflatoxin management emphasizes the segregation of contaminated products, chemical and biological decontamination, and the prevention of further

contamination by using proper harvesting, transportation, processing, and storage methods.

In order to protect the consumer from the risks of aflatoxin contamination in foods, the maximum permissible levels have been regularized and subjected to control by legislative authorities. The purpose of enforcing a maximum permissible or tolerated level is to minimize the risk to human beings.

The Malaysian Food Regulations Act of 1985 fixed the maximum permissible level of aflatoxin or any other mycotoxins in foods and other products at 35 μg kg^{-1}. This Act stipulated that there shall be no importation, preparation, advertisement, or sale of any food which contains aflatoxin in a proportion greater than that specified.

Present Staffing and Resources Available for Research

A small laboratory at the Food Technology Center, MARDI, has been assigned for the analysis of aflatoxins. This laboratory has two research officers and two research assistants. The laboratory is also equipped with a simple thin-layer chromatography (TLC) system to carry out aflatoxin analysis.

Future Research Plans

Aflatoxin monitoring will continue in order to provide a more detailed status of the problem in different areas.

Research efforts will be made to control preharvest contamination. This includes research on the effects of cultural practices and biological control agents. Priority will also be given to research on the biological detoxification of aflatoxin in contaminated commodities.

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Status of the Groundnut Aflatoxin Problem and its Management in the Philippines

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Groundnut production is concentrated in the Cagayan Valley in the northern part of the Philippines. The soil type in areas where groundnut is grown is sandy loam.

Status of the Aflatoxin Problem

The surveys undertaken by the National Post Harvest Institute for Research and Extension (NAPHIRE), and funded by the Australian Centre for International Agricultural Research (ACIAR), between 1991 and 1993, showed that 80% of the 157 groundnut samples tested were contaminated with aflatoxin. Of these samples, 10 were obtained from farmers' fields after harvest, 6 after drying, 25 from farm stores, 12 from commercial stores, and 104 from retail stores. Twenty-four percent of these samples contained aflatoxin above the 20 µg kg^{-1} limit for food that is allowed by the Bureau of Food and Drug in the Philippines. The percentages of contaminated samples (from these sources) ranged from 66.7 to 91.7%. Percentages of samples containing > 20 µg kg^{-1} were higher from commercial storage (58%) than from other sources (10-23%). The maximum levels of aflatoxin found in these samples were: 22 µg kg^{-1} in samples from farms after harvest; 44 µg kg^{-1} in samples taken after drying; 7374 µg kg^{-1} in samples from farm stores; 497 µg kg^{-1} in samples from commercial stores; and 4326 µg kg^{-1} in samples from retail stores.

Samples taken from further down the postproduction chain tended to have higher concentrations of aflatoxin than samples taken during harvest and after drying. More than 50% of the samples collected from commercial storage contained aflatoxin levels greater than 20 µg kg^{-1}. The highest level of aflatoxin determined was 497 µg kg^{-1}. Retail store and farm storage samples showed a relatively lower incidence than samples from commercial storage.

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In another survey, more than 80% of the 114 samples of shelled and unshelled groundnuts showed aflatoxin contamination; 53.5% had aflatoxin levels of >20 µg kg^{-1}. The highest concentration of aflatoxin in unshelled groundnut samples was 7374 µg kg^{-1}, and 3919 µg kg^{-1} in shelled groundnut samples. Aflatoxin levels in processed products (fried groundnuts, sugar-coated groundnuts, and peanut butter) were relatively lower than in raw groundnuts (both unshelled and shelled). The highest concentrations in these processed products ranged from 13.8 to 103 µg kg^{-1}.

Preharvest and Postharvest Aflatoxin Problems

Evidence of preharvest occurrence of aflatoxin in groundnuts can be deduced from data showing the presence of the toxin at harvest. Postharvest aflatoxin contamination is considered a serious problem in the Philippines.

Research on Aflatoxin Management

Research has been undertaken on the effects of some postharvest practices on aflatoxin contamination, and on varietal resistance to *Aspergillus flavus* seed colonization.

Research on aflatoxin management considers the use of chemicals and the biological control of mold growth on susceptible cultivars. Chemical detoxification, using ammonium hydroxide, ammonia, and sodium hydroxide has been found effective in reducing aflatoxin levels in groundnut cake.

Technologies Available for Managing the Aflatoxin Problem

Chemical detoxification of aflatoxin in groundnuts is being employed by commercial-processing companies.

At the farm level, postharvest machines for the primary processing of groundnuts, such as stripping, drying and shelling, are available. These machines are used to avoid delay in the primary processing of groundnuts, and thus maintain quality and possibly reduce aflatoxin production. However, their acceptability to farmers and processors is being studied.

Chemicals to inhibit the aflatoxin-producing fungi and to bind the toxin are available. These chemicals, mainly used to detoxify contaminated feeds, are registered by the Bureau of Animal Industry in the country.

Constraints to the Adoption of Aflatoxin Management Options

Awareness of the aflatoxin problem and its management options is limited. In general, the transfer of information from the researcher to the end user is inadequate.

On the part of the government, there is little incentive to produce afiatoxin-free groundnuts. In addition, funds for research on aflatoxin are meager.

Present Staffing and Resources Available for Research

At present, government agencies are involved in the monitoring of aflatoxins in various commodities. The Bureau of Food and Drug under the Department of Health is responsible for monitoring aflatoxin contamination in processed food commodities including groundnut products in Manila. This agency is equipped with a high pressure liquid chromatography system, a thin-layer chromatography scanner, a gas chromatography system, and other standard laboratory equipment. The Bureau of Animal Industry, under the Department of Agriculture, monitors aflatoxin contamination in animal feeds; its laboratories are equipped with a spectrophotometer, a high performance thin-layer chromatography system, and enzyme-linked immunosorbent assay (ELISA) test kits.

The other institutions engaged in aflatoxin research are the Food and Nutrition Research Institute, NAPHIRE, the National Crop Protection Center, and the University of the Philippines, Los Banos.

Status of the Groundnut Aflatoxin Problem in Thailand

Woothisak Butranu, Montien Somabhi, and Dhanit Sophanodora[1]

Aflatoxin contamination of groundnuts and groundnut products has been studied in Thailand for more than 15 years. About 36% of *Aspergillus flavus* isolates from groundnut in Thailand are reported to be toxigenic (Ketsara 1984). In groundnuts, aflatoxin B_1 has frequently been detected in higher amounts than other aflatoxins. Damaged seeds have been found to contain much higher levels of the toxin than sound seeds. *Aspergillus flavus* populations in soils ranged from 69 to 1044 propagules g^{-1} in the dry season, and from 2302 to 18 060 propagules g^{-1} in the wet season (Arunsri et al. 1995).

Aspergillus flavus Infection in Groundnut

In 1995, surveys were conducted to assess *A. flavus* infection in farmers' groundnuts 7-10 days before harvest, during harvesting, and after drying, but before sale to the markets in the north, northeast, and the central parts of the country (Taksina et al. 1995). In 124 preharvest samples from 24 locations, seed infection was 1-10% in 51, 11-20% in 4, and >20% in 7 samples. In 243 samples from 34 locations taken during harvest, seed infection was 1-10% in 110, 11-20% in 13 and >20% in 28 samples. In 146 postharvest samples from 25 locations, seed infection was 1-10% in 59, 11-20% in 13, and >20% in 19 samples.

Factors Affecting *A. flavus* Infection

Storage conditions. Groundnut pods (8% moisture content) were kept in a fiber sack in the Field Crops Research Center's (FCRC) storage room and in a farmer's storage room in 1995. Samples were taken after 2, 4, and 6 weeks of storage. The experiments were carried out in both wet and dry seasons. The results showed that seed infection in the dry season was less than in the wet season under both the FCRC's and

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the farmer's storage conditions. Seed infection was slightly higher in samples drawn from the farmer's storage room than in those from the FCRC storage room, but no significant differences were found.

Drought stress. Experiments were conducted in field plots during the 1992, 1993, and 1995 dry seasons to compare A *flavus* infection and aflatoxin contamination in groundnut samples under limited water supply and regular irrigation conditions from 65 days after emergence (DAE) up to harvesting (Woothisak et al. 1993, Woothisak et al., unpublished). The results revealed that *A. flavus* infection was 30.9, 27.1, and 2.3% under drought (limited water) conditions in 1992,1993, and 1995, while it was 9.3,15.4, and 1.6% under regular irrigation conditions. Similarly, aflatoxin contamination was higher under drought conditions than under regular irrigation conditions (166.8 versus 3.6 µg kg^{-1} in 1992, and 24.5 versus 3.1 µg kg^{-1} in 1993).

A. flavus Infection in Plant Parts

During 1992-94, studies were carried out in both wet and dry seasons in farmers' fields in two locations. The cultivar Khon Kaen 60-1 was sown, and plant sampling was done every 2 weeks up to 12 weeks after emergence (WAE). The results showed that A, *flavus* invaded every plant part from 2 WAE onwards and consequently up to crop harvesting. Stems, leaves, and petioles showed *A. flavus* infection from week 2 up to week 12 (1.1-7.3, 0.3-10.3, 0.3-7.5%). Incidence of A *flavus* infection on pegs, nubs (very young pods), and pods was found after 6, 8, and 10 WAE (0.4-5.8, 0.1-6.5, and 2.9-7.7%). With the exception of stems and pods, levels of infection in leaves, petioles, pegs, and nubs in the dry seasons was higher than in the wet seasons.

Aflatoxin Contamination

Storage periods and aflatoxin contamination. Groundnut pods of three cultivars (Khon Kaen 60-1, Lampang, and Tainan 9) were sampled after 0, 2, 4, 6, and 8 weeks of storage under room conditions in both wet and dry seasons during 1993. The results showed that aflatoxin was not detected in any seed samples at the time of storage. Tainan 9 had the lowest amount of aflatoxin (mainly B_1*:* 569.1 µg kg^{-1}). Khon Kaen 60-1 and Lampang had higher levels (950.5 and 1077.7 µg kg^{-1}). In all three cultivars, the toxin levels increased after 2 weeks of storage but declined after 6 and 8 weeks.

Aflatoxin production. Seven groundnut lines/cultivars from the Kasetsart University, Bangkok, and the North Carolina State University, Raleigh, USA, were examined for their ability to support aflatoxin production (Aree and Orapin 1989). Dry seed inoculation was done after 50, 65, 80 and 95 days of storage in the dry season of 1988. Two lines (PI 337394 F and PI 337409) showed the maximum resistance to aflatoxin production (1236-3054 µg kg^{-1}). The cultivars Asp 220 and Asp 229 also

supported low toxin production (4243-5341 µg kg^{-1}). The cultivars Tainan 9, Asp 533, and Asp 243 were highly susceptible to aflatoxin production; the average levels of aflatoxin ranged from 11005 to 14273 µg kg^{-1}. In all lines, aflatoxin production increased from 65 to 95 days.

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Present Status and Future Prospects of Research on the Groundnut Aflatoxin Problem in Vietnam

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Groundnut is an important food and cash crop in Vietnam with a high export potential. At present, Vietnam exports more than 100 000 t of shelled groundnuts annually. Groundnut products are also used as various foods and feeds. Recent surveys of foods have indicated that aflatoxin contamination is a serious problem in groundnut and maize. Therefore, various research institutions have begun to pay increased attention to this important problem. Vietnam considers the aflatoxin problem in groundnut to be of great importance, particularly in view of Vietnam's expanding trade in this commodity, and the increasing use of groundnut cake as animal feed.

Current Research on the Aflatoxin Problem in Groundnut

Since 1990, several outbreaks of aflatoxicosis in poultry and dairy animals have been reported from both northern and southern Vietnam; these are attributed to high levels of aflatoxins (200-5000 µg kg^{-1}) found in groundnut cake used as animal feed (Hao 1992 and 1995).

In northern Vietnam, 65% of the 200 samples of groundnut cake obtained from the Nghe An, Thanh Hoa, and Ha Bac provinces were found contaminated with aflatoxins (10-3500 µg kg^{-1}; mean 500) (Hao 1992 and 1995). Groundnut cake samples from the Nghe An province had much higher levels of aflatoxin than those from other provinces. All the four major aflatoxins were detected in some samples, indicating the presence of *Aspergillus parasiticus* infection. However, 85% of the contaminated samples contained only aflatoxin B_1. In southern Vietnam, aflatoxin

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levels have been found to be higher in groundnut cake prepared from rainy-season groundnuts (mean aflatoxin 1140 µg kg^{-1}) than in cake from the dry-season irrigated crop (mean aflatoxin 525 µg kg^{-1}).

Researchers at the National Institute of Nutrition (NIN), Hanoi, have monitored aflatoxins in various foods and feeds. Between 1990 and 1995,199 samples of groundnuts and groundnut products (peanut candy, peanut sauce, oil) were analyzed; 35 (18%) of which were found contaminated. Aflatoxin levels ranged from 2 to 511 µg kg-^{1}. Two of the 15 infant foods (containing soybean flour) tested also showed a range of 15-31 µg kg^{-1} aflatoxin (Quang et al. 1996). In 1992, the Ministry of Health, Vietnam, established limits for aflatoxins in food and feed (total aflatoxin or B_1*:* 5-20 µg kg^{-1} in food, 10-20 µg kg^{-1} in feed; aflatoxin M_1*:* 0.05-0.5 µg L^{-1} in milk). NIN has now increased emphasis on the surveillance and monitoring of foodstuffs and animal feeds for the control of aflatoxins, and is keen to initiate research on the development of appropriate food-processing technologies to reduce or eliminate aflatoxins in food products. At several of the research centers of NIN in Vietnam, priority is now given to the training of farmers and processors in improved crop management, food quality, and storage for the prevention of aflatoxin-producing fungi, and aflatoxin contamination.

Researchers at the Postharvest Technology Institute (PTI), Hanoi, have found *Aspergillus flavus* infection in 70-100% of the kernels of various groundnut samples collected from northern Vietnam. Substantial levels of aflatoxin were detected in 50 samples of groundnuts (40-200 µg kg^{-1}) and groundnut cake (40-500 µg kg^{-1}; mean 144 µg kg^{-1}) from markets in Ha Bac and Hanoi. Some samples of refined groundnut oil were also found contaminated with aflatoxin (5-7 µg kg^{-1}).

The National Institute of Plant Protection (NIPP), Hanoi, in collaboration with ICRISAT, has recently conducted surveys to assess soil populations of aflatoxin-producing fungi, and the extent of fungal infection and aflatoxin contamination in farmers' fields (particularly in rice-based production systems) and markets in northern Vietnam. Moderate to high populations of *A. flavus* (1000-6000 propagules g^{-1} soil) have been found to be prevalent in sandy and sandy loam soils in the major groundnut-producing provinces of Ha Tinh and Nghe An; such inoculum levels can lead to preharvest A *flavus* infection, and subsequent aflatoxin contamination. *Aspergillus flavus* infection levels were low to moderate (2-18%) in samples from farmers, while they were high (12-49%) in market samples. Seven of the 10 samples from markets in Ha Bac showed ≥ 40% *A. flavus* infection. In Ha Bac, wet and humid conditions during the harvest of the spring-season crop can lead to drying problems and contribute to *A. flavus* infection and subsequent aflatoxin contamination. High levels of aflatoxin (100-500 µg kg^{-1}) found in various market samples are indicative of a serious postharvest aflatoxin problem in the Ha Bac province (Mehan et al., unpublished).

Over 98% of the isolates obtained from soils and seed from various groundnut-growing areas belong to A *flavus.* Of the 50 purified isolates tested, 35 (70%) were found to produce aflatoxin B_1.

In the preliminary in vitro tests conducted at the NIPP with 41 groundnut genotypes (including Vietnamese cultivars and ICRISAT breeding lines), most of the

genotypes (92.7%) were susceptible or highly susceptible to A *flavus* seed colonization. Three cultivars (BK 1, SB 2, and Sen Nghe An) showed low levels of seed colonization (10-13%). Several ICRISAT breeding lines (ICGV 87391, 87054, 87139, 86143, 87884, 86055, 87074, 87981) were highly susceptible. Many of these lines were not specifically bred for resistance to A *flavus.*

Recently, researchers at the Oil Plant Institute (OPI), in collaboration with ICRISAT, assessed *A. flavus* infection and aflatoxin contamination in 147 samples of groundnuts from farmers and markets/oil mills in the major groundnut-growing areas of southern Vietnam. Results indicated that preharvest aflatoxin contamination is not likely to be a serious problem in adequately irrigated groundnuts in southern Vietnam. However, groundnuts raised under residual moisture or limited irrigation conditions can be contaminated under conducive environmental conditions in the winter-spring season (Mehan et al, unpublished). A few samples from farmers' fields that received 10 irrigations showed moderate levels of *A. flavus* infection. Soilborne diseases such as stem/pod rot and bacterial wilt, prevalent in many parts of southern Vietnam, are likely to encourage *A. flavus* infection in the field.

Research on Aflatoxin Management

In Vietnam, research on aflatoxin management is very limited. Some research on the detoxification of contaminated feed through ammonia and propionic acid has been conducted at the National Institute of Veterinary Research (NIVR), Hanoi. Recently, efforts have been made to enhance awareness of the aflatoxin problem and its management among farmers, traders, and extension and research staff in southern Vietnam. A brochure on 'Aflatoxin Contamination Problems in Groundnuts and Groundnut Products' has been prepared by Dr V K Mehan (ICRISAT) and Dr Phan Lieu (OPI). This was translated into Vietnamese and distributed to many farmers, extension workers, and groundnut-industry personnel in southern Vietnam. Vietnam, in collaboration with ICRISAT, now has similar plans for enhancing aflatoxin awareness in northern Vietnam during June-July 1996.

Vietnam also plans to initiate research on aflatoxin management in cooperation with ICRISAT scientists and prepare a detailed brochure on aflatoxin management options at all levels—production, marketing, and processing.

Present Staffing and Resources Available for Research at Various Institutions

Research on the groundnut aflatoxin problem is being mainly conducted by NIPP, PTI, NIN, and the Department of Plant Protection, Hanoi. OPI has recently initiated research on aflatoxin contamination problems in groundnut. Aflatoxin surveys have been carried out jointly by Vietnamese and ICRISAT scientists. At NIPP, two scientists and one research assistant are currently working on several aspects of the groundnut aflatoxin problem. Research on this problem has been given high priority. NIPP

and OPI have developed strong collaborative research plans with ICRISAT. NIPP has effective linkages for cooperative research with the Department of Plant Protection (DPP) which has very good laboratory facilities for aflatoxin research. The mycotoxin laboratories of the PTI, NIN, and NIVR have good facilities and the equipment required for research on mycotoxins. NIVR mainly conducts research on aflatoxin problems in animal feeds, and on the detoxification of contaminated groundnut cake.

At OPI, one scientist and one research assistant are engaged in aflatoxin research, particularly on aflatoxin monitoring and analytical methods. Two researchers at PTI are also conducting aflatoxin research. The Vietnam Agricultural Science Institute (VASI) has recently initiated research on varietal resistance to the toxigenic fungus.

Funding for aflatoxin research is very limited.

Major Constraints to the Adoption of Aflatoxin Management Options

The major constraints are:

- Lack of knowledge and awareness of the aflatoxin problem and its management options,
- Lack of funding to initiate research on aflatoxin management, and
- Difficulties faced by farmers in adopting recommended practices to alleviate drought stress, disease and pest attacks, particularly in resource-poor conditions of rainfed groundnut production systems in northern Vietnam.

Future Plans

In Vietnam, high priority will be given to research on the groundnut aflatoxin problem. The following aspects will receive attention in the near future:

- Systematic surveys will be conducted to assess aflatoxin contamination levels in groundnuts grown under various production systems, particularly in rainfed crops in the northern and central regions. This is necessary in order to highlight the risks of aflatoxin contamination and to identify the regions and cropping systems where the problem is serious.
- High priority will be given to enhancing awareness of the aflatoxin problem and its management aspects among groundnut farmers, traders, processors, and personnel concerned with storage.
- Emphasis will be given to the training of Vietnamese researchers in analytical methods to enhance their capabilities to conduct effective research on the aflatoxin problem.
- Priority will be given to upgrading facilities for aflatoxin research in the major research institutions.
- Development of appropriate measures to reduce *A. flavus* infection and aflatoxin contamination at various levels will be given importance.

- Collaborative research efforts between Vietnamese institutions, ICRISAT, and Cereals and Legumes Asia Network (CLAN) member countries will be further strengthened.

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Methods for Aflatoxin Analysis, and Risks of Aflatoxin Contamination

Analytical and Immunochemical Methods for the Analysis of Aflatoxins in Groundnuts and Groundnut Products

V K Mehan[1]

Many analytical and immunochemical methods are available for the estimation of aflatoxins in agricultural commodities. Analytical methods for aflatoxin analysis using a thin-layer chromatography (TLC) system were developed in the 1960s, and some of them are still being widely used. In the 1970s and early 1980s, various high performance liquid chromatography (HPLC)-based methods were developed and, being more sensitive than TLC methods, were used whenever a high degree of accuracy was required. Rapidity of analysis is sometimes more important than absolute accuracy, and several minicolumn methods have been developed with this in mind. With the availability of monoclonal and polyclonal antibodies against aflatoxins, various simple, sensitive and specific enzyme-linked immunosorbent assays (ELISAs) have been developed for aflatoxin analysis. ELISA-based aflatoxin kits, using monoclonal antibodies, are commercially available. In this paper, various analytical and immunochemical methods for the analysis of aflatoxins in groundnuts and groundnut products are critically reviewed, and future trends in aflatoxin analysis are discussed.

Analytical Methods

Numerous physicochemical methods have been developed for the analysis of aflatoxins in agricultural commodities. Many of them are minor modifications of basic methods, adapted to specific commodities or problems. These methods differ in the solvents used to extract the toxins, and in techniques for estimating the intensity of fluorescence. However, all analytical methods for aflatoxins basically involve the same steps - sampling, extraction, clean-up, separation, and quantification. Observations by early researchers (Sargeant et al. 1961) that aflatoxins are much more soluble in chloroform than in hydrophilic solvents, and that isolated toxins fluoresce brightly

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under long wave ultraviolet (UV) light have provided the basis for most extraction/ separation and quantification procedures. In the majority of the methods, one of the following solvent systems is used for extracting aflatoxins: methanol:water (55:45, v/v), acetone:water (85:15), acetonitrile:water (90:10), and chloroform:water (250:25). These methods are based on the principle that aqueous solvents penetrate hydrophilic tissues and effectively extract the toxins.

TLC-based Methods

Thin-layer chromatography (TLC) has been the most widely used quantification method since the early 1960s (Coomes et al. 1964, 1965). Various analytical procedures use TLC plates coated with silica gel (Kieselgel G), and solvent systems such as chloroform:methanol and chloroform:acetone for TLC development (Pons et al. 1966, Pons and Goldblatt 1969). The addition of water to these solvent systems can improve the resolution of aflatoxins. The water:acetonexhloroform (1.5:12:88, v/v) system has been reported to give the best resolution (Stubblefield et al. 1969). Developed plates are examined under UV light (long wave, 365 µm), and aflatoxin concentrations are estimated by visual comparison of the fluorescence intensities of the spots in sample extracts with those of appropriate aflatoxin standards chromatographed on the same plate. This method is sensitive and concentrations as low as 3-4 x 10^{-4} µg of aflatoxin can be detected (Coomes et al. 1965).

However, visual estimations present problems in accuracy and precision (Pons et al. 1966); coefficients of variations in this method commonly range from 20 to 30%. Compared to visual estimations, fluorodensitometric measurements of aflatoxins directly on TLC plates are more accurate and precise (Pons and Goldblatt 1968). Densitometers are commercially available, but analysts in many laboratories continue to compare the fluorescent spots visually as high costs often preclude the use of such equipment.

The Official Methods of Analysis (AOAC 1980) recommends several TLC-based procedures for aflatoxin estimation in groundnuts and groundnut products. Most of the commonly used methods are based on TLC detection and quantification procedures that have been evaluated extensively in collaborative studies. TLC methods for aflatoxin analysis have been subjected to an extensive evaluation by the Smalley Check Sample Program, conducted by the American Oil Chemists' Society (McKinney 1981) and by the International Mycotoxin Check Sample Program, conducted by the International Agency for Research on Cancer (Friesen et al. 1980, Friesen and Garren 1982). These studies have demonstrated the lack of precision associated with commonly used TLC procedures, as the coefficients of variation differed widely (30-122%). The TLC quantification step has been reported to be the major source of error (Whitaker and Dickens 1981, Coker 1984). The use of TLC methods to quantify aflatoxin levels has been reviewed by Schuller et al. (1976) and Stoloff (1982).

The four most popular TLC-based methods, the Contamination Branch (CB), the Best Foods (BF), the European Economic Community (EEC), and Pons' methods, are compared in Table 1. The CB method is the most effective, and is often used as a

Table 1. TLC-based analytical methods for analysis of aflatoxins in groundnuts and/or groundnut products.

Step	Method			
	Contamination Branch (CB)	Best Foods (BF)	Pons'	European Economic Community (EEC)
Sample size Groundnuts or groundnut meal	50 g	100 g	50 g	50 g
Peanut butter	-	50 g	-	-
Extraction solvent	Chloroform:water (250 mL:25 mL) + diatomaceous earth (25 g)	Methanol:water (55:45,v/v;500 mL) + hexane (200 mL) Methanol:water (55:45,v/v;250 mL) + hexane (100 mL)	Acetone:water (85:15, v/v) (250 mL)	Chloroform:water (250 mL:25 mL) + diatomaceous earth (25 g)
Blending/shaking	Shake (30 min)	Blend (1 min)	Shake (30 min)	Shake (30 min)
Work-up/ Clean-up	Column clean-up 200 mL chloroform 150 mL hexane 150 mL diethyl ether elution with 150 mL chloroform:methanol (97:3, v/v)	Chloroform (25 mL)	Lead acetate solution (20 mL)+80 mL water	Column clean-up 200 mL Chloroform 100 mL hexane 100 mL diethyl ether elution with 150 mL chloroform:methanol (97:3, v/v)
TLC development solvent	Chloroform:acetone (90:10, v/v)	Chloroform:acetone (90:10, v/v)	Chloroform:acetone (90:10,v/v)	Chloroform:acetone (90:10, v/v)
TLC quantification	Comparison of standards	Comparison of standards	Comparison of standards	Comparison of standards

standard of excellence against which new methods are judged. However, it has two major disadvantages: (1) it is very expensive as it uses large amounts of solvents, and (2) the major solvent used is chloroform, which is hazardous. The BF method uses a methanol:water (55:45) extraction solvent, and provides a rapid assay for aflatoxins in groundnuts and groundnut products (groundnut cake/meal, peanut butter). The solvent system of acetone-water in Pons' method is advantageous since neutral and polar lipids are insoluble in an acetoner:water solvent, and efficient defatting and aflatoxin extraction occur simultaneously. All the four methods use the chloroform:acetone (90:10) solvent system for TLC development.

Combined with visual estimation, TLC is a reasonably cheap quantification method, and can be used for routine applications.

Confirmatory Tests for Aflatoxins

Some compounds which behave like aflatoxins may appear on TLC plates. To eliminate such false positives, the identity of the toxin in positive samples needs to be confirmed. Confirmatory tests can be performed directly on a TLC plate; these tests are based on the formation of a derivative that has different properties (e.g., color of fluorescence and polarity) from the presumptive toxin. Both aflatoxin standard and suspected sample extracts are subjected to the same derivatization reaction. In positive samples, the derivative should be identical to the derivative from the standard toxin. Confirmatory tests developed by Przybylski (1975) and Verhulsdonk et al. (1977) have been officially adopted by AOAC. In both methods, aflatoxin B_1 is derivatized under acidic conditions on the TLC plate into its hemiacetal aflatoxin B_{2a}, which has a blue fluorescence at a lower Rf than B_1. In Przybylski's method, this is achieved by superimposing trifluoroacetic acid (TFA) directly onto the extract spot before plate development. After reaction, the plate is developed and examined under UV light for the presence of the blue fluorescent spot of B_{2a}, which can be recognized with the help of the B_1 standard spotted on the same plate, which was treated by the same procedure. For additional confirmation, sulfuric acid (50%) is sprayed on another part of the plate where unreacted aliquots of sample extract and B_1 standard were developed. The sulfuric acid changes the fluorescence from blue to yellow. This test only confirms the *absence* of aflatoxin, i.e., spots which do not turn yellow are not aflatoxin, whereas various materials other than aflatoxin may give yellow spots.

Davis et al. (1981) have reported another confirmatory test using the fluorescence of the iodine derivative of aflatoxin B_1.

High Performance Thin-Layer Chromatography (HPTLC)

The lack of precision associated with conventional TLC procedures can occur as a result of the introduction of errors at various stages—sample extract application, plate development, or plate interpretation. High performance thin-layer chroma-

tography (HPTLC) procedures improve precision in three ways: (1) by automating the sample application and plate interpretation steps, (2) by improving the uniformity of the adsorbent layer, and (3) by developing the plate under controlled conditions.

HPTLC is a comparatively new procedure which has been comprehensively reviewed by Coker (1984). HPTLC uses an automated sample applicator, a scanner, and a computing integrator. It is important that the spots are accurately positioned if an automated densitometer/scanner is used. This technique can be successfully used in conjunction with a computing integrator to complete the automation of the plate interpretation step.

Sample volumes of <1 µL can be applied to HPTLC plates as against 10-20 µL required for conventional TLC. The consequent reduction in spot size, to <1 mm, facilitates the application of numerous spots. Sample and standard spots can be applied using an automated spotting technique to ensure that each spot is accurately positioned. Concentrations of aflatoxin as low as 5 pg can be detected using HPTLC. Researchers at the Natural Resources Institute, UK, have detected 30 pg of aflatoxin B_1 in groundnuts using HPTLC (Coker 1984).

The appearance of HPTLC procedures may result in increased interest in TLC as an efficient quantification technique for aflatoxins, ideal for the analysis of a large number of samples.

High Performance Liquid Chromatography (HPLC) Methods

Highly automated HPLC systems offer very precise, selective, and sensitive quantification techniques for aflatoxin analysis. HPLC methods have been developed using both normal phase (liquid: solid; polar stationary phase) and reverse phase (liquid:liquid; polar mobile phase) systems in conjunction with UV absorption and fluorescence detection. In the normal phase, the stationary phase is solid and more polar than the mobile phase. In the reverse phase, the normal phase is reversed—the stationary phase is liquid and less polar than the mobile phase. Reverse-phase HPLC separations of aflatoxins are more widely used than normal-phase separations.

The recent emphasis on reverse phase separations of aflatoxins, coupled with the efficiency and convenience of post-column derivatization with iodine, reflects diminished interest in the silica-gel packed flow-cell technique (Coker and Jones 1988). Gilbert and Shepherd (1985) have used both normal and reverse phase HPLC in a survey of aflatoxins in groundnuts and peanut butter. Sample extraction and clean-up were performed using a modified version of the CB method. The sample was extracted with chloroform:water, the crude extract separated by centrifugation, and further cleaned-up on a silica gel column. The final peanut butter extracts were dissolved in benzene-acetonitrile (98:2, v/v). Normal phase HPLC was used for the assay of peanut butter using a 5 µm silica gel column and a nonquenching mobile phase of benzene-acetonitrile-formic acid, 90% (83:12:15). Edible nuts and confectionery products were assayed by the reverse phase method, using post-column iodination. A

detection limit of 0.5 μg kg^{-1} for each aflatoxin was reported. Hurst et al. (1984) have used reverse phase HPLC in combination with TFA derivatization to determine aflatoxin levels in raw groundnuts. The HPLC methods developed by De Vries and Chang (1982) and Tarter et al. (1984) also use TFA derivatization, and compare favorably with other methods. Using reverse phase HPLC with derivative formation, aflatoxins B_1, B_2, G_1, and G_2 can be detected at concentrations as low as 5 pg.

Davis and Diener (1980) developed a reverse phase method with fluorescence detection of an iodine derivative of aflatoxin B_1. This led to the development of post-column derivatization methods (Shepherd and Gilbert 1984). A comprehensive review of HPLC-based methods has been published by Coker and Jones (1988).

No collaborative studies of these HPLC methods have been reported. A few check sample programs involved the use of HPLC methods for the estimation of aflatoxins in peanut butter and de-oiled groundnut meal (Friesen et al. 1980, Friesen and Garren 1982). The HPLC methods involve complex extraction and clean-up steps; they are time-consuming and expensive.

Minicolumn Methods

Some quality control procedures require that the presence of aflatoxin in a batch of a particular commodity be reported as rapidly as possible. In such situations, a rapid qualitative assessment is possible with the help of the minicolumn procedure. Compared to TLC methods, minicolumn procedures are rapid, less expensive, and simple. These methods can provide only qualitative assessments, but cannot accurately distinguish and quantify individual aflatoxins.

The first rapid screening procedure using a minicolumn for aflatoxin detection in groundnuts was reported by Holaday (1968). Since then, several improved procedures have been developed (Davis et al. 1981, Holaday 1976, Holaday and Lansden 1975, Romer 1975).

The heart of the minicolumn technique is a chromatography column built in a glass or plastic tube, typically with a 3-6 mm inside diameter and 20 cm long. Extracts are prepared by conventional methods, but concentration is achieved by liquid/liquid transfer to a relatively small volume of benzene or chloroform (Romer 1975). The original minicolumn (Holaday 1968) used only silica gel as the adsorbent, and depended on capillary action. When the column was examined under long wave UV light, a blue fluorescent band was considered as evidence for the presence of aflatoxin.

Further developments in minicolumn techniques (i.e., passing a precise volume of extract through the column rather than dipping the column into the extract, the use of different adsorbents, and the use of the final layer as an adsorbent trap for aflatoxins) have resulted in detection limits comparable to those of more elaborate methods. The minicolumn commonly used contains florisil, overlaid with silica gel and/or alumina, and is developed with solvents normally used to elute aflatoxins from silica gel or alumina (Holaday and Lansden 1975, Romer 1975).

The methods developed by Davis et al. (1981), Holaday (1976), Holaday and Lansden (1975) are rapid screening procedures for detecting aflatoxins in groundnuts.

Only a few collaborative studies have been conducted to evaluate the efficiency of these methods (Shotwell and Holaday 1981). The Holaday-Velasco method has been adopted as an official 'first action' method (AOAC 1980). The minicolumn method of Romer (1975) has also been adopted by the AOAC for aflatoxins in groundnuts, groundnut products, and various other commodities. In this method, aflatoxins are extracted with acetone:water (85:15) and interferences are removed by adding cupric carbonate and ferric chloride gel. The aflatoxins are then extracted from the aqueous phase with chloroform, and the chloroform extract is washed with a basic aqueous solution to effect further purification. The chloroform extract is applied to the top of a minicolumn containing successive layers of neutral alumina (top), silica gel, and florisil (bottom), with calcium sulfate drier at both ends. The column is developed with chloroform:acetone (9:1) trapping the aflatoxins as a tight band at the top of the florisil layer, where they can be detected by their blue fluorescence under UV light. The fluorescence can be measured directly by inserting the developed minicolumn into the fluorotoxinmeter which may be calibrated to give a direct read-out of the total aflatoxins (in $\mu g\ kg^{-1}$) in the sample. A fluorotoxinmeter is essentially a spectrophotometer that can accommodate a Romer-type minicolumn. It is a rapid, reasonably priced quantification system that merits greater use. However, individual aflatoxin components cannot be quantified using this equipment.

Reference columns are prepared using chloroform extracts from a noncontaminated sample spiked with a suitable ratio and level of aflatoxins. Packed sample and reference minicolumns are commercially available (source: Myco-Lab Co., P O Box 321, St. Louis, MO 63017, USA).

Romer's method can also be used to detect aflatoxins in groundnut oil. One of the advantages of Romer's method is that the remaining chloroform extract is sufficiently clean to be used for a TLC presumptive test in case the screening test proves to be positive. However, this method has been found to extract lower amounts of aflatoxin from groundnuts compared with the CB and BF methods (Mehan et al. 1985). Coker (1984) has discussed factors that can affect the chromatographic behavior of the aflatoxin band in the minicolumn, particularly when analyzing groundnuts.

Madhyastha and Bhat (1984) have reported a minicolumn confirmation method for aflatoxins; the identity of aflatoxins could be confirmed by applying 20% H_2S0_4, 20% HC1, or TFA in 20% $HN0_3$.

Immunochemical Methods

In search of simpler and more specific methods, a number of investigators have explored the possibility of using an immune response, with quantification of the reaction by competitive binding of either radio-labeled aflatoxin or enzyme-linked aflatoxin (El-Nakib et al. 1981, Pestka et al. 1980, Pestka et al. 1981). In the last decade, there has been rapid progress in the development of various ELISA systems for the determination of aflatoxins, using polyclonal and monoclonal antibodies (Tables 2 and 3).

Some polyclonal antibodies for aflatoxin B_1 (Pestka et al. 1980, Morgan et al. 1986) give very high cross-reactivity (100-125%) to B_2 and moderate cross-reactivity

Table 2. Direct ELISA methods for analysis of aflatoxin B_1 in groundnuts and groundnut products.

Solvent extraction	Incubation time (h)	Detection limits (µg kg^{-1})	Commodity	Reference
Methanol:water: hexane (55:45:10)	Ia+0.5s[1]	3-5	Peanut butter	El-Nakib et al. (1981)
Chloroform:water	Ib+Ia+0.25s	1	Peanut butter	Ueno (1985)
Methanolrwater: hexane (55:45:10)	0.5b+Ia+0.25s	5-10	Groundnuts, peanut butter	Ram et al. (1986)
Methanolrwater: dimethylformamide (70:29:1)	0.5a+0.17s	5-10	Corn, peanut butter, mixed feeds	Chu et al. (1987)
Methanol:water (55:45)	2a+0.5s	5-10	Groundnuts	Anjaiah et al. (1989)
Methanol:water (55:45)	0.5b+2a+0.35s	1	Groundnuts	Candlish et al. (1985)
Methanol:water (60:40)	Ib+0.5a+0.5s	1	Peanut butter	Kawamura et al. (1988)
Methanol:water:KCl (70:30; 0.5%)	0.5b+Ia+0.3s	5	Groundnuts	Ramakrishna and Mehan (1993)

1. The letters following the incubation time indicate different steps of incubation; a = first antigen-antibody incubation; b = blocking step; s = substrate incubation time.

Table 3. Indirect ELISA methods for analysis of aflatoxin B_1 in groundnuts and groundnut products.

Solvent extraction	Incubation time (h)	Detection limits (µg kg^{-1})	Commodity	Reference
Methanohwater: hexane (55:45:10)	0.5b+0.8a+ 0.88aa+0.6s[1]	5	Peanut butter	Fan and Chu (1984)
Acetonitrile: water (50:50)	3a+2a3+0.25s	0.25	Peanut butter	Morgan et al. (1986)
Methanol:water (60:40)	Ib+Ia+ Iaa+0.5s	1	Peanut butter	Kawsmura et al. (1988)
Methanol:water:KCl (70:30;0.5%)	0.5b+Ia+Iaa+0.35s	5	Groundnuts	Ramakrishna and Mehan (1993)

1. The letters following the incubation time indicate different steps of incubation; a = first antigen-antibody incubation; aa = second antibody incubation time; b = blocking step; s = substrate incubation time.

(around 30%) to aflatoxin G_1. Most of the monoclonal antibodies produced against aflatoxin are highly specific for aflatoxin B_1 and are partially cross-reactive with aflatoxin G_1 (Kawamura et al. 1988). However, the monoclonal antibodies reported by Candlish et al. (1985) were highly specific to B_1 and showed low cross-reactivity with B_2, G_1, and G_2. On the other hand, the monoclonal antibody reported by Hefle and Chu (1990) has very high cross-reactivity with all four aflatoxins.

In screening samples for the presence of aflatoxins, it is important to consider the cross-reactivity of the antibody. The accuracy of the immunoassay of aflatoxins in naturally contaminated samples is affected by the specificity of the antibody used and by the presence of structurally related analogs of the mycotoxin in the sample, that may react with the antibody. Recently, Zhang and Chu (1989) and Hefle and Chu (1990) produced polyclonal and monoclonal antibodies, respectively, that show good cross-reactivity with the most important naturally occurring aflatoxins, particularly B_1 and G_1.

It is emphasized that monoclonal antibodies are not always very specific. It is important to select an antibody that has high specificity for the mycotoxin of interest. Some countries regulate the total levels of aflatoxins rather than just B_1. Hence, using an antibody which reacts equally well with all four major aflatoxins would be more appropriate than using one which reacts only with B_1. Recent efforts have been directed at producing antibodies with good cross-reactivity with all major aflatoxins (Zhang and Chu 1989, Hefle and Chu 1990).

Enzyme-linked Immunosorbent Assays (ELISA)

Two types of ELISA have been used for the analysis of aflatoxins: (1) direct ELISA, and (2) indirect ELISA. Both types are heterogeneous competitive assays. Direct competitive ELISA involves the use of an aflatoxin-enzyme conjugate, while indirect competitive ELISA involves the use of a protein-aflatoxin conjugate and a secondary antibody such as goat anti-rabbit IgG to which an enzyme has been conjugated. Although horseradish peroxidase (HRP) is the most commonly used enzyme for conjugation, other enzymes such as alkaline phosphatase have also been used (Chu 1984, Anjaiah et al. 1989).

Direct Competitive ELISA

In this assay, a specific antibody is first coated to a solid phase such as a microliter plate (Chu 1984, Chu et al. 1987). The sample extract or standard toxin is generally incubated 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 specific substrate solution. The resulting color is then measured spectrophotometrically or by visual comparison with standards. Since this assay is based on competition for antibody binding sites, free toxin concentration is inversely related to antibody-bound enzyme conjugate.

Several direct competitive ELISA procedures have been reported for the analysis of aflatoxins in groundnuts and groundnut products (Table 2). Some of the ELISA procedures took a rather long time to complete, and gave large coefficients of variation within each sample because the sample matrix often interfered with the assays (Chu et al. 1987). This problem could be overcome by dilution of the sample to a range which does not affect the assay (Chu et al. 1987, Chu 1989), or by using a control sample extract as diluent. Chu et al. (1987) developed a simple ELISA protocol that takes about an hour for analysis of aflatoxin B_1 in groundnut and maize. In this method, samples are extracted with 70% methanol in water containing 1% dimethylformamide, diluted with assay buffer (sodium phosphate buffer, pH 7.2, 0.01 mol L^{-1} with 0.15 mol L^{-1} of NaCl) to a final concentration of 3.5% methanol, and then subjected to ELISA. High recovery (95.4%) of aflatoxin B_1 added to peanut butter has been reported with this method (Chu et al. 1987).

Various direct ELISA systems are summarized in Table 2. Most ELISA procedures employ simple extraction step using aqueous methanol. Diluting the sample extract (at least 1:10) ensures high sensitivity and avoids interference from the sample matrix. The sensitivity of ELISA can be improved when a clean-up step (e.g., extraction with hexane) is included (Chu et al. 1989). A similar ELISA protocol with longer incubation time for the analysis of aflatoxin B_1 in peanut butter was described by Ram et al. (1986), who included a defatting procedure.

A few collaborative studies have been conducted to check the efficiency of some of the ELISA procedures (Park et al. 1989, Trucksess et al. 1989).

Indirect ELISA

A few indirect ELISA procedures have been reported for the analysis of aflatoxin in agricultural commodities (Table 3). Morgan et al. (1986) used an indirect ELISA for the analysis of aflatoxin in peanut butter, using aqueous acetonitrile (50%) as the extraction solvent. In this procedure, aflatoxin-protein conjugate (KLH-aflatoxin B_1) is coated onto the microtiter plate. Sample or standard aflatoxin is added to the wells followed by an aliquot of anti-aflatoxin antibody. The amount of antibody bound to the plate is detected by the addition of goat anti-rabbit IgG conjugated to alkaline phosphatase (ALP) followed by reaction with p-nitrophenyl phosphate to give a colored product. The toxin is determined by comparing with a standard curve from known toxin concentrations. This procedure takes a long time (about 5.5 h). Kawamura et al. (1988) developed an indirect ELISA that takes about 3.5 h. This assay utilizes a monoclonal antibody and aqueous methanol as the extraction solvent; the detection limit is 1 µg kg^{-1}. Ramakrishna and Mehan (1993) reported both direct and indirect competitive ELISAs for aflatoxin B_1 in groundnuts. In these assays, methanoi-water-KCl (70+30 v/v, 0.5%) extracts of groundnuts were diluted to 1:10 with PBS-Tween buffer and then assayed. Both ELISAs detected aflatoxin B_1 as low as 20 pg $well^{-1}$.

The sensitivity of indirect ELISA is comparable to that of direct ELISA. Because only small amounts of antibody are required for indirect ELISA, this method is used

not only for toxin analysis, but also to monitor the antibody titers of hybridoma culture fluids for the screening of monoclonal antibody-producing cells.

Of the two types of ELISA, direct ELISA is usually preferred for aflatoxin since it utilizes a single conjugated protein, requires one less incubation step and one less washing step, and shows less variability than indirect ELISA.

Commercial ELISA Kits

Several companies in the UK, France, Japan, and USA have produced ELISA-based aflatoxin assay kits on a commercial scale for routine use in analytical laboratories. Commercially available ELISA kits for quantitative analysis of aflatoxin(s) in groundnuts are summarized in Table 4. The 'Biokits' uses indirect competitive ELISA, while all other kits use direct competitive ELISA. The Biokits assays all four major aflatoxins, the Aflasure kit aflatoxins B_1 and B_2, and the Quantitox kit aflatoxin B_1 alone.

Some of the ELISA procedures have been designed as rapid screening methods, suitable when aflatoxin levels are > 20 µg kg^{-1} (e.g., Agri-Screen test for aflatoxin B_1, kit available from Neogen Corporation, 620 Lesher Place, Lansing, Michigan 48912-1509, USA). These methods are designed for use in situations where quick, simple, and relatively low-cost analysis is required. Another approach is to immobilize

Table 4. Commercially available ELISA kits for analysis of aflatoxin(s) in groundnuts.

	ELISA				
Character	Quantitox[1] (UK)	Aflasure[2] (UK)	Biokits[3] (UK)	Transia[4] (France)	Afla-Check[5] (Japan)
Specificity	B,	B_1, B_2	B_1, B_2, G_1, G_2	G_1, C_2	B_1
Detection (absorption)	450 nm	450 nm	414 nm	410 nm	492 nm
Quantification (µg kg^{-1})	2-30	2-200	2-200	1-30	2-40
Extraction solvent	Methanol: water (55:45)	Aceto-nitrile: water (60:40)	Aceto-nitrile: water (50:50)	Methanol: water (80:20)	Methanol: water (55:45)

1. May and Baker Diagnostics Ltd., 187 George Street, Glasgow G1 1YT, UK.
2. Cambridge Life Sciences Plc, Milton Road, Cambridge CB4, 4GN, UK.
3. Thames Genelink Ltd., Deeside, Clwyd CH5 2NT, UK.
4. TRANSIA, 8 rue Saint Jean de Dieu, 69007 Lyon, France.
5. UBE Industries Ltd., 12-32 Akasaka 1-Chome, Minato-ku, Tokyo, 107, Japan.

the antibody on a paper disk mounted on a plastic card (Immunoassay Quick-Card Test) (Cole et al. 1987). This assay is similar in principle to direct ELISA. Sample extract (a few drops) is first applied to the test spot (paper disk) on the plastic card, then aflatoxin-enzyme conjugate is applied, and finally the substrate solution is added. The absence of color at the spot indicates the presence of aflatoxin; a negative control spot, where a bright blue color appears, indicates the absence of aflatoxin. Although this is a simple way to screen for aflatoxins, it is not suitable for the quantitative estimation of aflatoxins.

The high cost of commercially available ELISA kits may limit their use in developing countries. The major application for ELISA procedures at present appears to be screening for aflatoxin below a predetermined level. The kits are used primarily to test export/import consignments of agricultural products. These kits are also suitable for use in aflatoxin monitoring and surveillance programs. More development is required before ELISA techniques can be adopted for critical quantification. Methods need to be developed that will determine aflatoxins individually, rather than only collectively.

Initially, there were expectations that antibodies could be produced with very high specificity so that most of the extract clean-up could be avoided. This has so far been achieved only in very few cases. However, in many cases, serum titers are high enough to detect aflatoxin at very low levels.

Conclusions and Future Trends

An ideal assay procedure for aflatoxins should be simple, rapid, accurate, sensitive, selective, and cost-effective (in terms of equipment and consumables). While no assay procedure satisfies all these criteria, an appropriate procedure can be chosen depending on the specific objective (e.g., routine analysis or research). Conventional analytical methods have been well standardized, while immunochemical methods such as ELISA are making rapid progress. Immunoassay techniques are highly selective and sensitive and, therefore, very little sample clean-up is required. They are associated with a high sample throughput and should, therefore, be useful during aflatoxin surveys. As immunoassays grow more popular, conventional methods are being increasingly replaced by these faster methods. The commercial success of ELISA kits shows that the value of immunoassays for aflatoxins is being more widely recognized.

Since all immunoassays are based on the interactions of analytes with specific antibodies, the availability of antibodies is a key factor in determining the feasibility of immunoassays for aflatoxins. Specific polyclonal antibodies can be easily and cost-effectively produced using conventional methods (immunization of a mycotoxin-protein conjugate in rabbits). In contrast, the production of monoclonal antibodies is very costly, time-consuming, and requires sophisticated facilities. Highly specific monoclonal antibodies are commercially available, and can be used in ELISA procedures. ICRISAT has produced specific polyclonal and monoclonal antibodies and limited quantities can be supplied to NARS researchers (requests should be sent to the Director, Crop Protection Division).

In the 1960s and 1970s, international research centers and advanced institutions supplied researchers in developing countries with aflatoxin standards. This greatly boosted NARS aflatoxin research and surveillance programs. Similar efforts are now required; NARS researchers need specific antibodies for use in ELISA procedures and ELISA reader facilities that are now available only in advanced laboratories. If these two critical needs are met, it is anticipated that ELISA procedures will be developed and used much more widely in developing countries.

The advent of microprocessor-controlled automation will revolutionize the analysis of aflatoxins. Much emphasis on automation of the quantification step is anticipated. But, regardless of the method selected, effective sampling and sample preparation are imperative to ensure the accuracy of results.

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Risks of Aflatoxin Contamination of Groundnut in Vietnam: a Preliminary Study

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Aflatoxin contamination of groundnut occurs on an extensive scale in the tropics. It is an important food quality reducer. The main cause of aflatoxin contamination is the susceptibility of the groundnut germplasm currently in use by farmers. There is also an abundance of the aflatoxin-producing fungus, *Aspergillus flavus,* particularly in sandy and sandy loam soils which are suitable for groundnut production.

Biotic Factors and Aflatoxin Contamination

Aflatoxin contamination of groundnut becomes a serious problem in the presence of drought stress, pod-damaging insect pests, and soilborne diseases. In areas where groundnut is grown year after year in the same field, aflatoxin contamination is observed to be high.

In the presence of one or all of the biotic factors enumerated above for the occurrence of aflatoxin in groundnut in the field, the spread, intensity, and the extent of the damage caused to the quality of groundnut kernels is climate-driven. A detailed study of the weather parameters favoring aflatoxin contamination was initiated: (1) to highlight key climatic parameters that control contamination at various phenological stages of growth, and (2) to analyze the different agroecologies of Vietnam, in which the groundnut crop is grown, for aflatoxin risks.

Weather x Aflatoxin Contamination

The weather parameters that affect aflatoxin contamination are:

- Drought during seed-formation and maturation stages of crop growth, particularly in the top 10 cm of soil,
- Release of a long stretch of dry-soil conditions, when the crop is at maturation stage, by rain,

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- Occurrence of wet weather during field drying of the crop after harvest, and
- Climate of the storage space, which has an important bearing on the prevalence of aflatoxin contamination in seed, especially if the crop has escaped A *flavus* infection/aflatoxin contamination in the field. High humidity of the storage area, coupled with high temperatures or seepage of water into the store rooms, increases the chances of aflatoxin contamination.

The hot spots for aflatoxin contamination in groundnut production systems are generally drought-prone sandy soils, in which the groundnut crop is grown year after year, and the humid conditions under which the crop produce is stored.

Groundnut Production in Different Agroecologies in Vietnam

Vietnam has been divided into seven agroecological zones (Table 1). In the northern parts of the country (AEZs I-IV), three main seasons are recognized. A cool dry season when average daily temperatures are <20°C. It extends from Oct to Mar in the Northeast highlands ecoregion. This is followed by an intermediate season with a temperature regime ranging between 20 and 28° C. The warm season with a temperature regime exceeding 28°C is observed in AEZs II and IV; it extends during the summer season from May/Jun to Aug.

Of the three AEZs in southern Vietnam, a cool temperature regime (<20°C) is found only in the Southwest highlands ecoregion, otherwise these ecoregions are characterized by an intermediate thermal regime (20-28° C) for most of the year except for the rainy months.

The soil-moisture regime, particularly during drought stress, is favorable for at least 230 to 290 days across three of the AEZs. The length of the unfavorable soil-moisture regime, when moisture deficits occur, is about 75 days in AEZs I and II (the Northeast highlands and Northern midlands ecozones). In all the other ecozones, it ranges between 105 and 135 days.

Rainfall and Soil Moisture in Some Selected Locations

Groundnut is grown in all the seven AEZs of Vietnam (Fig. 1). In northern Vietnam, groundnut is grown mainly in the spring season (Feb-Jun), while in southern Vietnam it is mainly grown in the winter-spring season (Nov-Feb). One location with long-term climatic data was selected in each of the agroecological zones to quantify the probabilities of weekly rainfall for rainfall/potential evaporation (PE) ≥ 0.33, mean weekly rainfall, and soil-moisture variations in weekly steps. The Markov Chain model was used to calculate initial (W) and conditional (W/W) probabilities of rainfall. The 'standard meteorological weeks' scheme (established by the World Meteorological Organization) was employed. Soil moisture was simulated from weekly rainfall and PE records. The soil-moisture balance was estimated by the WATBAL:

Table 1. Agroclimatic characteristics of seven ecoregions of Vietnam[1].

		Thermal regime						Moisture regime[3]			
		Cool season (< 20°C)		Intermediate season (20° – 28°C)		Warm season (> 28°C)		Favorable		Unfavorable	
Agro-ecozone[2]		Period	Days	Period	Days	Period	Days	Period	Days	Period	Days
I	Northeast highlands	Oct–Mar	135±15	Apr–Sep	230±15	–	–	Mar–Nov	290±30	Dec–Feb	95±15
II	Northern midlands	Dec–Mar	105±15	Apr–May Sep–Nov	170±30	Jun–Aug	90±15	Mar–Nov	290±30	Dec–Feb	75±15
III	Northwest midlands	Dec–Mar	105±15	Apr–Nov	260±15	—	–	Apr–Sep	230±30	Oct–Mar	135±15
IV	North central midlands	Dec–Mar	60±30	Mar–Apr Sep–Nov	185±15	May–Aug	120±15	May–Nov	230±30	Dec–Mar	105±15
V	South central midlands	–	–	Oct–Apr	215±15	May–Sep	150±15	Apr–Nov	245±30	Dec–Mar	120±30
VI	South western highlands	Dec–Jan	30±30	Jul–Nov	235±15	–	–	Apr–Nov	260±30	Dec–Mar	105±15
VII	Southern lowlands	–	–	Jun–Mar	320±15	Apr–May	45±15	May–Nov	230±30	Dec–Apr	135±15

1. This table is a revision of the ecoregionalization of Vietnam reported by Dao The Tuam (1982).
2. In all the agroecozones, high-, mid-, and low-lands coexist. Generalized climatic tendency has been described as an average condition.
3. Moisture regime is based on a ratio of precipitation (P) and evaporation (E). If P/E is ≤ 0.5 the moisture regime is termed unfavorable for potential crop production, otherwise favorable.

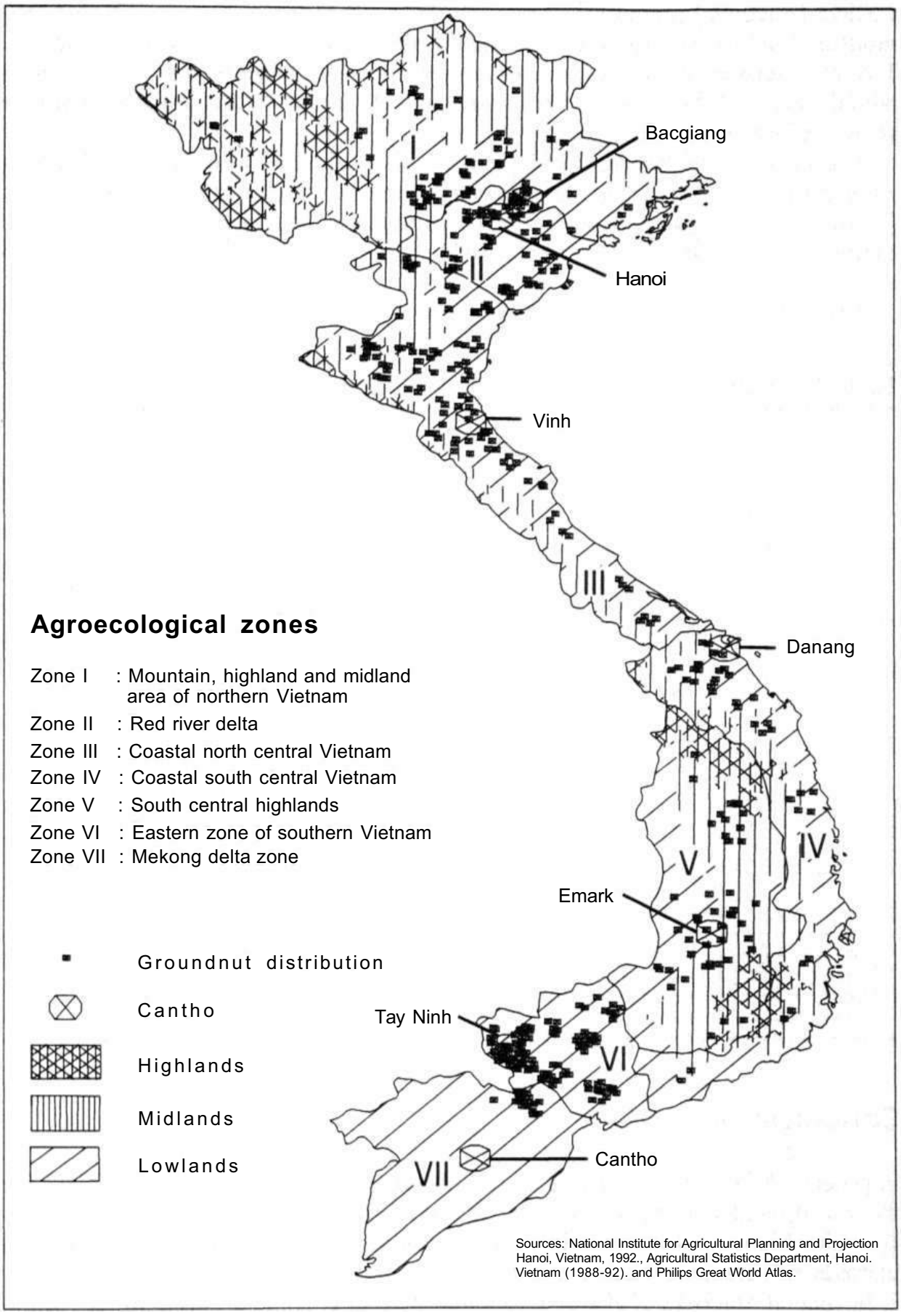

Figure 1. Groundnut distribution in the agroecological zones (AEZs) of Vietnam.

CSIRO (Australia) method; 120 mm was assumed as the maximum available soil-moisture holding capacity of the groundnut root profile across all the agro-ecozones. Two soil-moisture variation scenarios are given for each location: one for the lowest rainfall year, and the other for the highest rainfall year in the data available. Data are shown in Figures 2 to 8.

The relative risks of aflatoxin contamination in the different AEZs of Vietnam were assessed by comparing the amount of available soil moisture at three stages: (1) from establishment through to the reproductive phase of the crop, (2) from harvest to field drying of the crop, and (3) storage of the crop produce at the farm level.

The first approximation results obtained are given in Table 2.

Table 2. Potential incidence of aflatoxin in groundnut in Vietnam.

		Rating[1] of risks of aflatoxin contamination at 3 stages		
	Agro-ecoregion	Maturity[2]	Field drying	Storage[3]
I	Mountain highland and midland areas of northern Vietnam	2	4	8
II	Red river delta of northern Vietnam	2	4	8
III	Coastal north central area of Vietnam	2	2	7
IV	Coastal south central area of Vietnam	2	1	6
V	South central highlands	2	2	9
VI	Eastern zone of southern Vietnam	2	1	6
VII	Mekong delta zone	2	3	3

1. Rated on a scale of 1-9, where 1 = lowest, 9 = highest. This is based on frequency of drought stress and relative humidity of the air in storage rooms.
2. Maturity = Maturation stage of growth and development.
3. Storage means on-farm storage.

Conclusions

In general, AEZ VII represented by the Mekong delta zone presents conditions for the lowest incidence of aflatoxin in groundnut. Zones II and IV also present relatively low risks. Medium aflatoxin-risk areas are located in AEZs III and V, while the high aflatoxin-risk areas are AEZs I and II.

In general, the risks of aflatoxin contamination of groundnut during storage at the farm level are high throughout Vietnam.

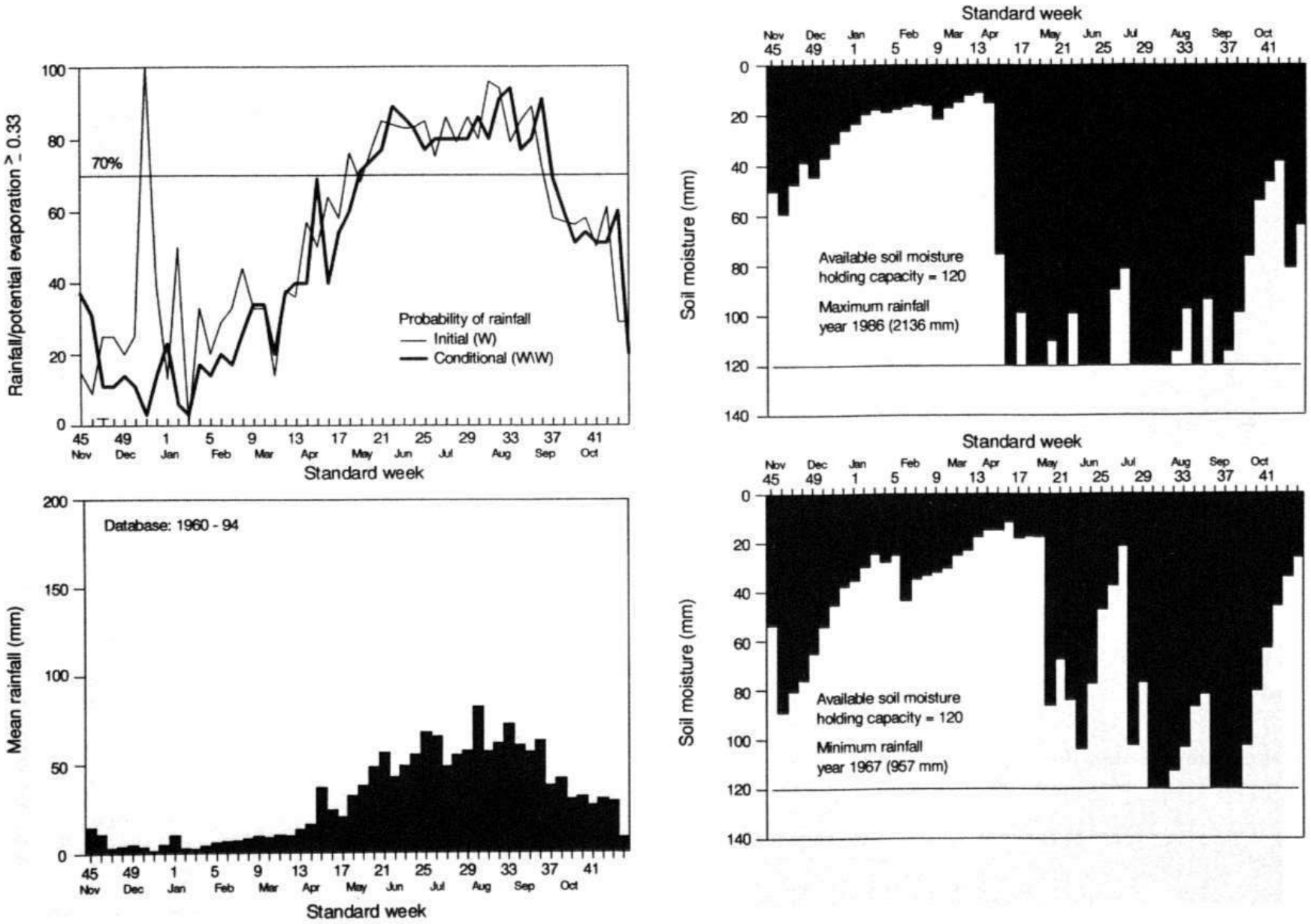

Figure 2. Rainfall and soil moisture in Bacgiang (21°16'N, 106°11'E; AEZ I).

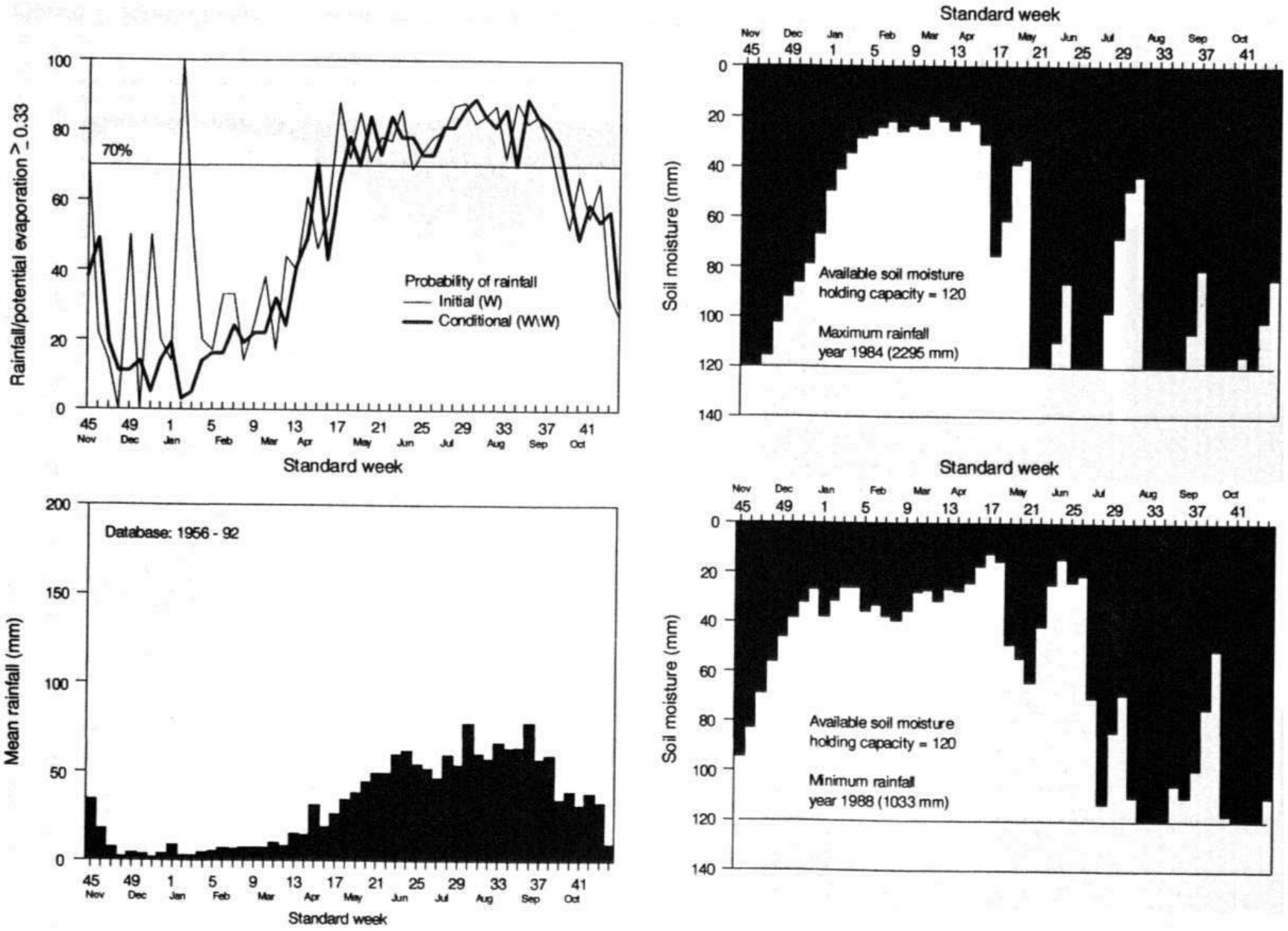

Figure 3. Rainfall and soil moisture in Hanoi (21°05'N, 105°55'E; AEZ II).

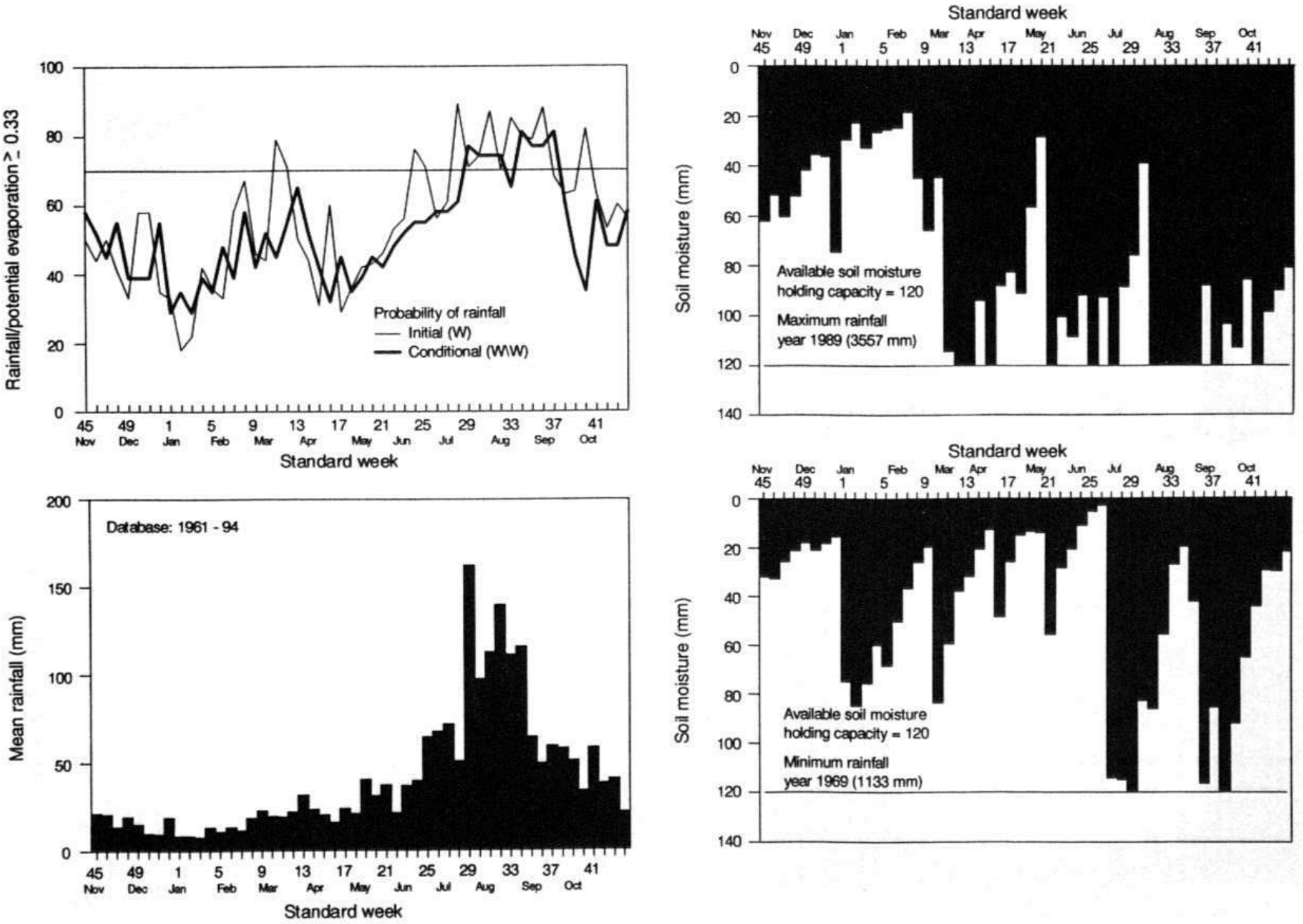

Figure 4. Rainfall and soil moisture in Vinh (18°45'N, 105°38'E; AEZ III).

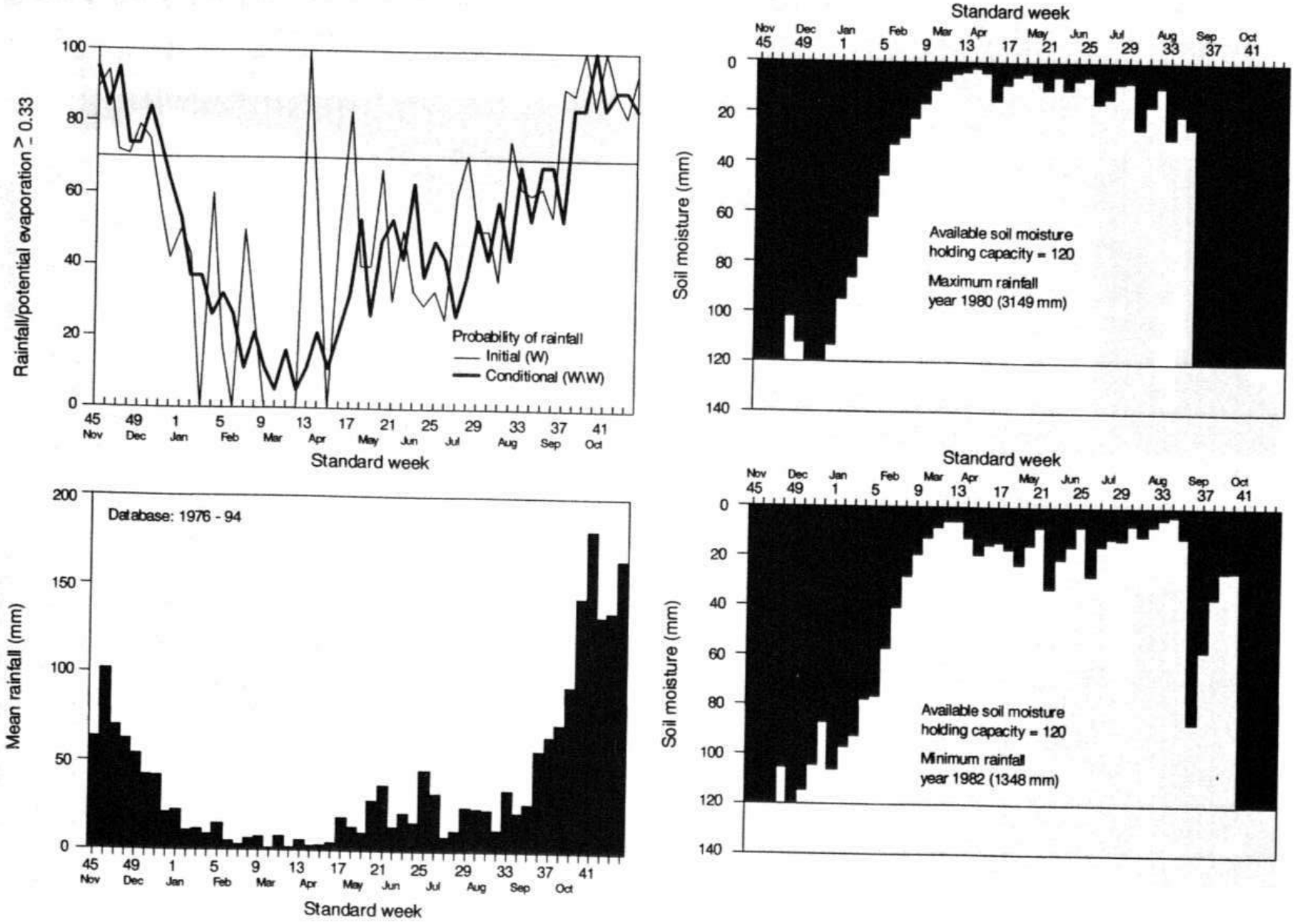

Figure 5. Rainfall and soil moisture in Danang (16°5'N, 108°10'E; AEZ IV).

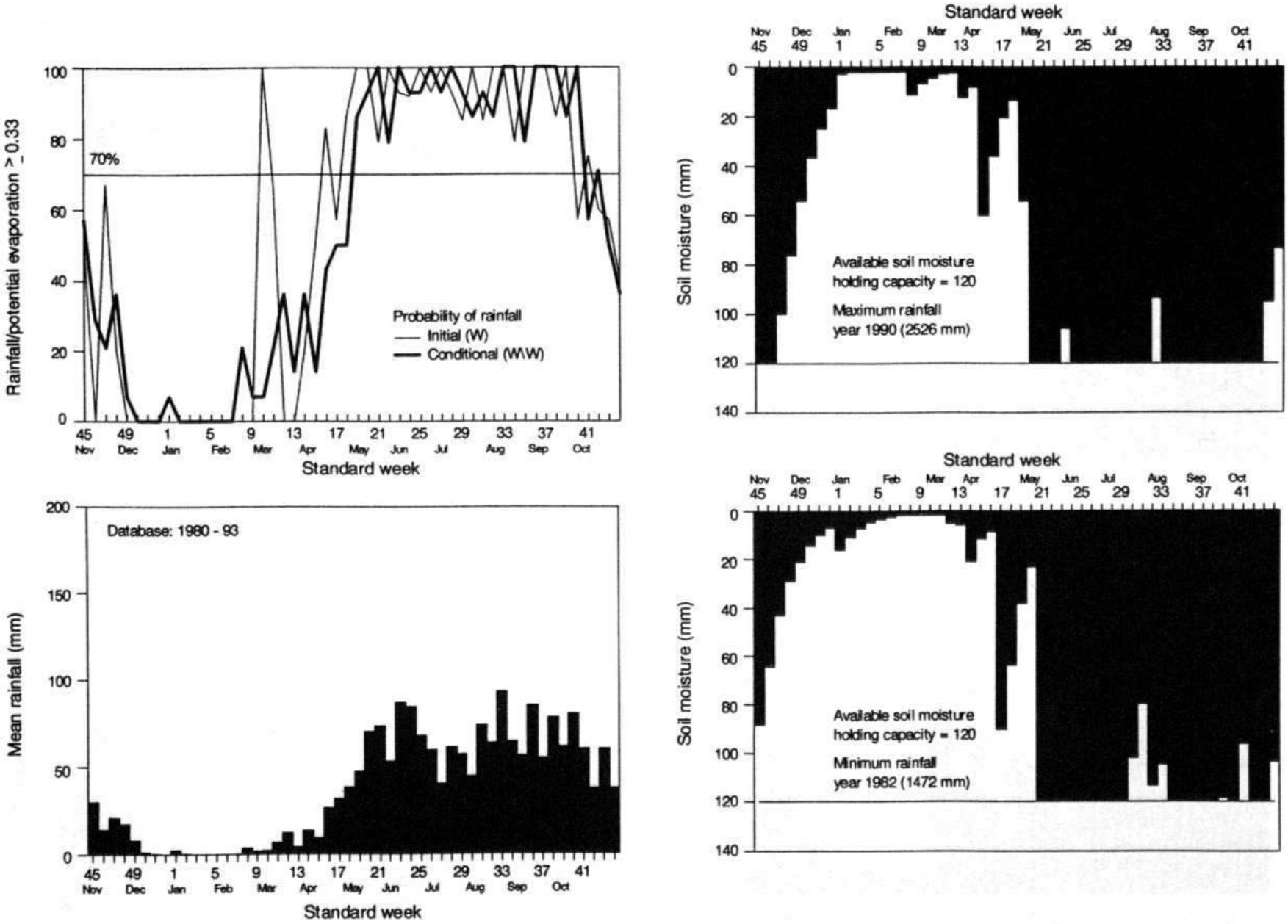

Figure 6. Rainfall and soil moisture in Emark (12°26'N, 108°13'E; AEZ V).

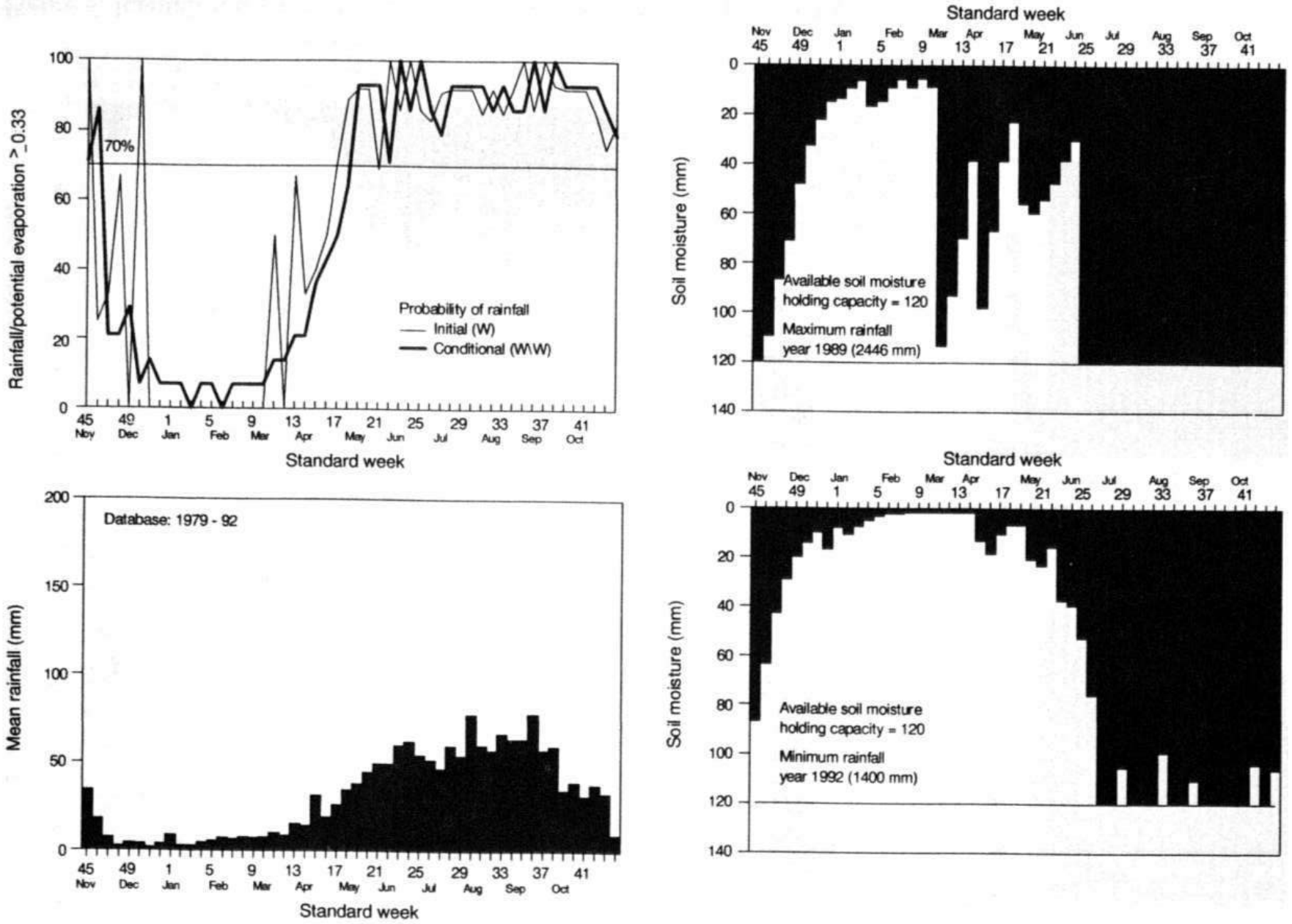

Figure 7. Rainfall and soil moisture in Tay Ninh (11°18'N, 106°04'E; AEZ VI).

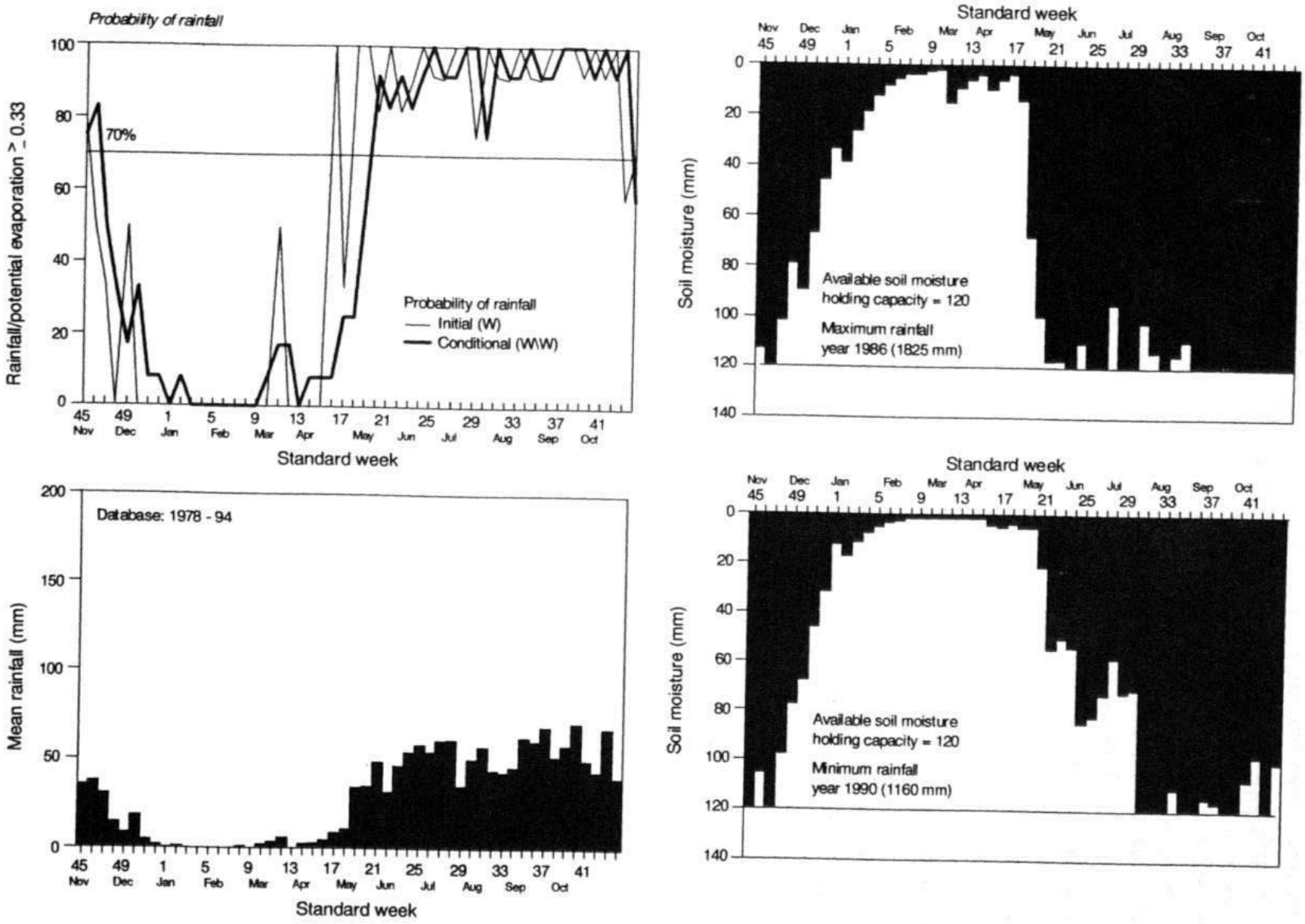

Figure 8. Rainfall and soil moisture in Cantho (10°2'N, 105°46'E; AEZ VII).

Future Research

This is a preliminary study. It is predominantly driven by climatic factors and, therefore, presents only a first approximation of aflatoxin contamination risks in groundnut during its production cycle and storage under on-farm conditions. It is suggested that well-designed experiments should be conducted across the seven AEZs in Vietnam. Some well-characterized benchmark sites should be chosen. All data pertaining to soil, climate, crops, and diseases should be collected. Farmers' produce and market arrivals of groundnut should be checked for A *flavus* and aflatoxin levels at regular intervals, using reliable analytical techniques.Complete analysis of the climatic data and abundance of aflatoxin-producing fungus should be carried out. This is a priority research area for Vietnam, as higher levels of groundnut production have been targeted for the coming years.

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Genetic Enhancement of Resistance

Aflatoxin Contamination of Groundnut: Prospects for a Genetic Solution through Conventional Breeding

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Aflatoxin contamination of groundnut is a serious problem in most groundnut-producing countries. The aflatoxin-producing fungi, *Aspergillus flavus and A. parasiticus,* can invade groundnut seed in the field before harvest, during postharvest drying and curing, and in storage. The semi-arid tropical environment is conducive to preharvest contamination when the crop experiences drought before harvest, whereas in wet and humid areas, postharvest contamination is more prevalent. Aflatoxin contamination can be minimized by adopting some cultural, produce-handling, and storage practices. However, these practices have not been widely adopted by small farmers in developing countries which contribute about 60% of the world's groundnut production. Cultivars resistant to seed invasion by aflatoxin-producing fungi or to aflatoxin production would be of great value to farmers in both developed and developing countries. Therefore, breeding for resistance to aflatoxin-producing fungi and/or aflatoxin production can play a significant role in preventing aflatoxin contamination in groundnut, consequent economic losses, and health hazards.

The alleviation of aflatoxin contamination through genetic manipulation has been attempted since the mid 1970s. In spite of the significant progress achieved to date, these efforts have not resulted in complete freedom from aflatoxin contamination. The current status and future prospects of genetic solutions to the aflatoxin contamination problem are briefly discussed in this paper.

Current Status of Genetic Resistance

In groundnut, depending on the site at which it operates, resistance to aflatoxin-producing fungi may be of three types—resistance to pod infection (pod wall), to

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seed invasion and colonization (seed coat), and to aflatoxin production (cotyledons). The fungi have to penetrate the pod wall and the seed coat to reach the cotyledons from which they derive their sustenance. Resistance to pod infection is attributed to pod-shell structure, while resistance to seed invasion and colonization is physical, and has been correlated with thickness, density of palisade cell layers, absence of fissures and cavities, and presence of wax layers. There are conflicting reports regarding the role of fungistatic phenolic compounds in imparting resistance to seed colonization.

All the three types of resistance sources have been reported (Mehan 1989). These include Shulamit and Darou IV for resistance to pod infection, PI 337394 F, PI 337409, GFA 1, GFA 2, UF 71513, Ah 7223, J 11, U 4-47-7, Var 27, Faizpur, and Monir 240-30 for resistance to in vitro seed colonization by A *flavus* (IVSCAF), and U 4-7-5 and VRR 245 for resistance to aflatoxin production. The importance of preharvest aflatoxin contamination was realized only in the late 1980s, and some of the IVSCAF-resistant genotypes (PI 337394 F, PI 337409, GFA 1, GFA 2, J 11, UF 71513, Ah 7223) were reported to have considerably lower natural seed infection by *A. flavus* than various IVSCAF-susceptible genotypes (Mehan 1989).

The value of a resistance source depends upon the level and stability of its resistance. Resistance to pod infection has been reported to be highly variable and of a low level. Similarly, IVSCAF-resistance is not absolute and even the best sources show up to 15% seed colonization; only a few lines (J 11, PI 337394 F, and PI 337409) have shown stable resistance. For aflatoxin contamination, resistance levels are not very high (Anderson et al. 1995).

Relationships between Types of Resistance

There are conflicting reports on the relationship between IVSCAF-resistance and resistance to natural seed infection, and aflatoxin contamination in the field. In the breeding lines developed and evaluated at IAC, no correlation (-0.07) was observed between IVSCAF and seed infection in the field, indicating two independent genetic mechanisms. The high correlation observed in an earlier study (Mehan et al. 1987) might have been due to the inclusion of some selected germplasm lines; whereas the absence of correlation observed in breeding lines developed at IAC might have resulted from the recombination of genes controlling these mechanisms. The studies conducted, in the 1980s, in the USA and at IAC showed low levels of aflatoxin contamination in IVSCAF-resistant genotypes. However, the genotypes which were earlier reported to be resistant to IVSCAF or preharvest aflatoxin contamination contained high levels of aflatoxin, when subjected to an extended period of heat and drought stress, and none of them was more resistant than the susceptible cultivar Florunner in the USA (Anderson et al. 1995). Highly significant genotype (G) x environment (E) interaction effects for aflatoxin contamination were observed in this study. The exact information on the relationship between different resistance mechanisms, their interactions, and possible contributions in reducing aflatoxin contamination has not been clearly established.

Genetics of Resistance

There are only three published reports on the inheritance of resistance, which give estimates of broad sense heritability and combining ability. The broad sense heritability estimates ranged from 55 to 79% for seed colonization, from 27 to 87% for seed infection, and from 20 to 47% for aflatoxin production. These studies were conducted in the USA (Mixon 1979, Utomo et al. 1990) and India (Upadhyaya and Nigam, unpublished). A report from the USA indicates that there is no significant correlation among the three types of resistance, indicating that they are controlled by different genes (Utomo et al. 1990). In a diallel study, significant reciprocal effects were noticed in some crosses indicating maternal influence on testa structure (Rao et al. 1989).

The genetics of resistance mechanisms has not been clearly established. The allelic relationship among various sources for each resistance trait needs to be elucidated to enable breeders to pyramid the non-allelic genes for each resistance mechanism.

Current Status of Resistance Breeding

Breeding efforts for resistance to pod infection have not received any attention. Further, it was assumed that if shell thickness was related to resistance, resistance breeding would result in low shelling percentages. In the past, seed colonization resistance received the maximum attention due to the ease of screening procedures. Of late, natural seed infection and aflatoxin production have received increasing attention, although screening for resistance to aflatoxin production is expensive. A much cheaper ELISA-based methodology was recently developed at ICRISAT.

Research on breeding for resistance to aflatoxin contamination is in progress in India, Senegal, Thailand, and the USA. The groups at Tifton, USA, and IAC, India, have successfully transferred IVSCAF-resistance to different genetic backgrounds. The group at Tifton produced six breeding lines GFA-1, -2, AR-1, -2, -3, and -4 (Mixon 1983a and 1983b). GFA-1 and -2 (both runner market types), whose yields were equal to or better than that of Florunner, had equal or less than average seed colonization than the resistant control genotype (PI 337409). The yield potentials of AR-1, -2, -3, and -4 are too low for their practical use as commercial cultivars.

In India, resistance breeding activities are mainly conducted at IAC and the National Research Center for Groundnut (NRCG). At IAC, research on breeding for resistance to aflatoxin contamination started in 1976. Several hundred breeding lines have since been tested for yield and IVSCAF-resistance, and several lines with IVSCAF-resistance and high yield have been identified. Four hundred and seventy-two lines were evaluated for preharvest seed infection and yield. Some of them have seed infection and colonization equal to or less than the best resistant control cultivar, J 11, and high-yield potential across seasons/years and locations. Of these, ICGV 88145 and ICGV 89104 have been released as improved germplasm lines (Rao et al. 1995). Recently, four such lines (ICGVs 91278, 91279, 91283, and 91284) were evaluated for yield and other agronomic traits in national programs in Thailand and

Vietnam, and they have performed very well. Three lines (ICGVs 87084, 87094, and 87110), bred at IAC for resistance to seed infection, were also found to be resistant in Niger, Senegal, and Burkina Faso in West Africa (Waliyar et al. 1994).

In Thailand and Senegal, PI 337394F, PI 337409, UF 71513, and J 11 are commonly used as resistant donors. The lines AR-1, -2, -3, and -4 are also being used in Thailand as sources of resistance; 55-437 has been used in Senegal.

In the breeding scheme at IAC, the selection for resistance traits is delayed until later generations. However, it would be desirable to screen segregating generations and select only resistant plants/progenies. This would require modification of screening techniques currently being used to make them more suitable at the single plant level.

Future Prospects of Breeding for Aflatoxin Resistance

Although researchers have not been able to locate germplasm lines which show complete resistance to fungi at the pod-wall, seed-coat, and cotyledon levels, it was expected that the levels of resistance could be improved further by pyramiding resistance genes, from different and diverse sources. It was also thought that by combining the three different kinds of resistance in one genetic background, the problem of aflatoxin contamination could be overcome to a large extent. Unfortunately, the progress made so far in conventional breeding has not been able to meet these expectations. The recourse to biotechnology, through modification of the aflatoxin biosynthesis pathway or the use of variants of hydrolytic enzymes (chitinases and glucanases), to provide transgenic protection to groundnut against infection by aflatoxin-producing fungi may help in obtaining groundnuts free from aflatoxin.

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Summary of Discussions and Recommendations

Summary of Discussions and Recommendations

The Working Group discussed various issues related to the management of aflatoxin contamination, priorities for future research, and collaborative research activities among Working Group members. The following are specific recommendations from the Group:

Enhancing Awareness

It was considered necessary to create and enhance awareness regarding aflatoxin contamination and its implications for human and animal health. Programs for enhancing awareness should involve farmers, traders, processors, and consumers. These programs should also explicitly involve administrators and policymakers, nongovernmental agencies, medical and nutrition scientists, and public-health agents. Farmers should receive information on pre- and post-harvest factors that increase aflatoxin contamination in groundnuts and groundnut products, and aflatoxin management options. The Group recommended that detailed brochures (in the local languages) be prepared in each country to provide information on aflatoxin management options at the various levels of production, storage, processing, and trade in groundnuts and groundnut products. As importing countries set lower permissible levels of aflatoxin in groundnuts, contaminated produce tends to be locally consumed, thus increasing the health risks of the human and animal populations in groundnut-producing countries.

Surveys

Systematic surveys were considered important for documenting and assessing the risk of aflatoxin contamination in various agroecological zones, and identifying high-, low-, or no-risk areas. The need to prepare and distribute proper survey procedures and protocols to staff involved in aflatoxin research was stressed. The involvement of socioeconomists in developing survey procedures and questionnaires was emphasized. The Group also recommended the organization of training programs on survey and analytical methodologies. Thin-layer chromatography (TLC) and enzyme-linked immunosorbent assay (ELISA) methods were considered cost-effective and reliable for surveys on aflatoxin contamination.

Agronomic Management

The need for testing available agronomic management practices, furthering research pertaining to the preharvest aflatoxin problem, and managing aflatoxin contamination

during storage was strongly stressed. Cultural management should include components related to pest and disease management, supplementary irrigation, soil amendments (fertilizer, manure, lime, gypsum, etc.), adjusting sowing time, crop rotations, choice of cultivars, and drying and storage methods.

Breeding for Resistance

Although a long-term endeavor, breeding for resistance was considered important. In the absence of adequate knowledge of the mechanisms of the inheritance of resistance to *A. flavus* seed infection and aflatoxin production, breeding was considered a complex exercise. The use of results from research in biotechnology was recommended as and when genes conferring resistance to A. *flavus*/aflatoxin production were identified. Meanwhile, priority is to be given to the evaluation of local cultivars in hot-spot locations for resistance to A *flavus* infection and aflatoxin contamination. Resistant varieties should be multiplied and popularized in risk-prone areas. Breeding lines developed at ICRISAT and elsewhere are to be shared with other member countries of the Working Group.

Technical Coordination

The participants endorsed the formation of the Asia Working Group on Groundnut Aflatoxin Management. Dr V K Mehan, ICRISAT, was nominated Technical Coordinator. The Cereals and Legumes Asia Network (CLAN) was requested to provide the necessary logistic and administrative support to the Working Group.

The Technical Coordinator was requested to:

- Prepare protocols for sampling procedures and survey methods,
- Recommend analytical methods for the analysis of aflatoxins and resistance screening procedures, and
- Develop a project on aflatoxin surveillance and management, and seek funding from donor agencies.

In-Country Coordination

One participant from each country, except for Vietnam, was nominated to liaise and coordinate research on groundnut aflatoxin management. Each of them is to confirm his/her role as a National Coordinator (NC), subject to approval by his/her parent organization. An alternative scientist is to be nominated from each country to take care of coordination, if a NC is transferred or retires. The Vice Minister, MARD, Vietnam, is to nominate one scientist as the NC for Vietnam. The Group endorsed the proposal that other countries be invited to join the Working Group. Links should be established with the West Africa Working Group on Aflatoxin for exchange of information and technology in order to have a global perspective.

Work Plans

The following areas were identified for collaborative research among Working Group members:

- **Agronomic management options**

  - Soil-moisture conservation
  - Adjustment of sowing dates
  - Improved drying and storage methods

  India, Malaysia, the Philippines, Thailand, and Vietnam will be involved in these studies.

- **Breeding for resistance**

  ICRISAT, China and India will concentrate on research on breeding for resistance to A *flavus* infection and aflatoxin production.

- **Identification of resistance**

  All countries are to undertake research on identification of resistance to *A. flavus* seed infection among local varieties.

- **Exchange of germplasm and breeding lines**

  ICRISAT is to facilitate the exchange of germplasm and breeding lines among member countries.

- **Use of biotechnology and molecular markers**

  Advanced research institutions will be requested to undertake research on the use of biotechnology and molecular markers to develop aflatoxin resistant varieties.

- **Publication of** a **news sheet**

  The Technical Coordinator is to coordinate the publication of a news sheet for the exchange of information among Working Group members.

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About ICRISAT

The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the world's population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

ICRISAT's mandate crops are sorghum, pearl millet, finger millet, chickpea, pigeonpea, and groundnut; these six crops are vital to life for the ever-increasing populations of the semi-arid tropics. ICRISAT's mission is to conduct research which can lead to enhanced sustainable production of these crops and to improved management of the limited natural resources of the SAT. ICRISAT communicates information on technologies as they are developed through workshops, networks, training, library services, and publishing.

ICRISAT was established in 1972. It is one of 16 nonprofit, research and training centers funded through the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an informal association of approximately 50 public and private sector donors; it is co-sponsored by the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Programme (UNDP), the United Nations Environment Programme (UNEP), and the World Bank.

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