Screening Groundnuts for Resistance to Seed Invasion by *Aspergillus flavus* and to Aflatoxin Production

V.K. Mehan[1]

1528

Abstract

Research in several countries into evaluation of responses of groundnuts to seed colonization and infection by Aspergillus flavus *and or aflatoxin production is reviewed, and progress made in this field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is summarized. Several laboratory and field screening procedures have been developed to screen groundnuts for resistance to* A. flavus *infection and or aflatoxin production. Research on the effects of environmental factors on pod and seed invasion by* A. flavus *has produced information useful in the development of field screening methods. For instance, imposed drought stress has been used to improve large-scale field screening of groundnut genotypes for resistance to preharvest infection of seeds by* A. flavus. *Several genotypes were found resistant to infection, and some of them were also resistant to in vitro seed colonization by* A. flavus *in laboratory inoculation tests. Two genotypes supported only very low levels of aflatoxin B_1 production when seeds were colonized by an aflatoxin-producing strain of* A. flavus.

Résumé

Sélection des arachides résistant à l'invasion par *Aspergillus flavus* et à la production des aflatoxines : *Les recherches menées dans plusieurs pays portant sur l'évaluation des réponses des arachides à la colonisation et à l'invasion par* Aspergillus flavus *et/ou à la production des aflatoxines, sont récapitulées ainsi que les acquis de l'ICRISAT dans ce domaine. Plusieurs méthodes de sélection au laboratoire et au champ ont été mises au point pour étudier cette résistance. Les études sur les effets des facteurs d'environnement sur l'invasion des gousses et des graines ont fourni des informations utiles au développement des méthodes de sélection au champ. Par exemple, la création d'une sécheresse artificielle a permis d'améliorer la sélection au champ à grande échelle de la résistance à l'infection avant la récolte. Plusieurs génotypes se sont montrés résistants, dont certains sont également résistants à la colonisation in vitro par* A. flavus, *dans les tests d'inoculation au laboratoire. Deux génotypes ont présenté de très bas niveaux d'aflatoxine B_1, lorsque les graines ont été colonisées par une souche d'*A. flavus *productrice d'aflatoxine.*

1. Groundnut Pathologist, Legumes Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru PO, Andhra Pradesh 502 324, India.

ICRISAT Conference Paper no. CP 434.

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

Resúmene

La selección de cacahuate resistente a la invasión de sus semillas por *Aspergillus flavus* y a la producción de aflatoxinas: *En este trabajo se examinan las investigaciones realizadas en varios países sobre la evaluación de las respuestas del cacahuate a la colonización e infección de sus semillas por* Aspergillus flavus *y/o la producción de aflatoxinas y se resumen los avances logrados en este rubro por el Instituto Internacional de Investigación sobre Cultivos en los Trópicos Semiáridos (ICRISAT). Varios procedimientos de selección para uso en el laboratorio y en el campo se han desarrollado para la selección de cacahuate resistente a la infección de* Aspergillus flavus *y/o la producción de aflatoxinas. Los estudios realizados sobre los efectos de los factores ambientales en la invasión de las vainas y las semillas por* A. flavus *han producido información útil para el desarrollo de métodos de selección en el campo. Por ejemplo, condiciones de sequía creadas artificialmente han sido utilizadas para mejorar la selección en el campo, a gran escala, de genotipos de cacahuate con resistencia en sus semillas a la infección por* A. flavus, *en el período de precosecha.*

Se identificaron varios genotipos resistentes a la infección mencionada, y algunos de estos resultaron ser también resistentes a la colonización in vitro *por* A. flavus *en sus semillas, en pruebas de inoculación efectuadas dentro del laboratorio. Dos de los genotipos presentaron muy bajos niveles de producción de la aflatoxina B_1 cuando sus semillas fueron colonizados por un cepa aflatoxinógena de* Aspergillus flavus.

Introduction

Aflatoxin contamination of groundnut (*Arachis hypogaea* L.) is a serious problem in most groundnut-producing countries. Invasion of groundnut seed by the aflatoxin-producing fungi *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare, and subsequent contamination with aflatoxins, may occur pre- or postharvest. Preharvest aflatoxin contamination is important in the semi-arid tropics (SAT), particularly under drought conditions, while postharvest contamination is significant under wet and humid conditions (Dickens 1977, Mehan 1987). Aflatoxin contamination can be minimized by adopting some cultural, produce-handling and storage practices (Dickens 1977). These practices have been readily adopted by progressive farmers and those concerned with storage and processing of the produce in developed countries, but unfortunately have not been widely adopted by small farmers in developing countries. The use of cultivars resistant to seed invasion by aflatoxin-producing fungi, or resistant to aflatoxin production (Mixon and Rogers 1973, Mehan and McDonald 1984) would be of value to farmers in both developed and developing countries. This has focused research on identification and utilization of genetic resistance to seed invasion by *A. flavus* and/or aflatoxin production. This paper summarizes progress worldwide in selecting groundnuts resistant to "seed colonization" and "seed infection" by *A. flavus*/*A. parasiticus* and to aflatoxin production, and describes research in this field at ICRISAT. *Aspergillus flavus* is used in this paper in a collective sense for both *A. flavus* and *A. parasiticus*.

Resistance to Seed Invasion and Colonization by *Aspergillus flavus*

Mixon and Rogers (1973) first suggested that use of groundnut cultivars resistant to seed invasion and colonization by *A. flavus* could be an effective means of preventing aflatoxin

contamination. The existence of seed resistance was a logical assumption, considering that seeds with damaged testae are more easily and rapidly invaded by the fungus than are seeds with intact testae, and colored testae conferred greater resistance to invasion by *A. flavus* than white or variegated testae (Carter 1970,1973).

Mixon and Rogers (1973) developed a laboratory inoculation method for screening groundnut genotypes for resistance to *A. flavus* invasion and colonization of rehydrated, mature, sound, stored seeds. They selected two valencia-type genotypes, PI 337394F and PI 337409, that showed a high level of resistance to in vitro seed colonization by *A. flavus*. Six more breeding lines (GFA 1, GFA 2, AR 1, AR 2, AR 3, and AR 4) were later reported resistant (Mixon 1986). The mean seed colonization levels in the resistant genotypes tested over several years ranged from 8-13% (Mixon 1986).

Several other researchers have used Mixon and Rogers' method, or modifications of it, to screen groundnuts for resistance to seed colonization by aflatoxin-producing strains of *A. flavus* (LaPrade et al. 1973, Bartz et al. 1978, Zambettakis et al. 1981, Mehan and McDonald 1980, Tsai and Yeh 1985, Pua and Medalla 1986).

At ICRISAT we have used a modification of the method to screen 850 germplasm accessions for their reaction to seed invasion and colonization by *A. flavus*. The tests were carried out on und, mature seeds from intact pods that were dried and stored for about 1 month. Seeds (20-g mples) were surface sterilized with a 0.1% aqueous solution of mercuric chloride, rinsed in sterile deionized water, hydrated to 20% moisture content, and surface inoculated with a conidial suspension of an aflatoxigenic strain of *A. flavus* (AF 8-3-2A), and then incubated at 25°C for 8 days under 98 ± 2% relative humidity. The percentages of seeds of different genotypes with sporulating colonies of *A. flavus* ranged from 6 to 100%. Genotypes with 15% or fewer seeds colonized were regarded as resistant (Mehan and McDonald 1980). Resistance of the three genotypes, PI 337394F, PI 337409, and UF 71513, was confirmed, and six new sources of resistance (Ah 7223, J 11, U 4-47-7, Var. 27, Faizpur, and Monir 240-30) were identified. In various tests on seed from rainy-season groundnut crops produced on the ICRISAT Center farm from 1980 to 1986 these genotypes consistently had low percentages of seed colonized (8-14%). Resistance in three of them (PI 337394F, PI 337409, and J 11) has also been confirmed by other workers (Wynne 1983, Zambettakis et al. 1981, Kisyombe et al. 1985). A comprehensive list of genotypes reported from different countries to have resistance to seed colonization by *A. flavus* is given in Table 1.

It was observed that absolute percentage incidence of seeds colonized by *A. flavus* varied considerably for specific genotypes within trials in the same season, and between seasons. Effects of environment (climate, location, soil type) and postharvest drying procedures on in vitro seed colonization were examined. In various genotypes tested, seeds from the postrainy-season rigated crops had significantly higher colonization than seeds from rainy-season crops (Mehan et al. 1983). This may be due to fluctuation in soil moisture during pod development and very rapid drying under the hot, dry conditions during harvest of the postrainy-season crop. Several workers (Dickens and Pattee 1973, Glueck et al. 1977, Woodward 1973) have reported that rapid drying weakens the seed testa, and testa damage decreases resistance to fungal penetration.

In all reported cases of rehydrated, cured, sound, mature seed resisting invasion and colonization by *A. flavus* the protective role of the seed testa has been emphasized (Dieckert and Dieckert 1977, Mixon and Rogers 1975, Mehan et al. 1983), the resistance depending upon the seed testa being intact. The resistance to seed colonization may be of value if groundnuts dried in the field or in storage are wetted, or absorb moisture from the atmosphere. The resistance may be of less value for decorticated seed that may have suffered damage to the testa in processing. It is significant that in spite of considerable differences in seed colonization levels caused by variation

Table 1. Groundnut genotypes reported resistant to seed invasion and colonization by *Aspergillus flavus* in laboratory inoculation tests.

Genotypes	Origin	Reference(s)
PI 337394F, PI 337409	Argentina Argentina	Mixon and Rogers (1973), Mehan et al. (1981), Zambettakis et al. (1981)
UF 71513	USA	Bartz et al. (1978), Mehan et al. (1981)
J 11	India	Mehan et al. (1981), Wynne (1983), Kisyombe et al. (1985)
Ah 7223, Var. 27, Faizpur, Monir 240-30	Nigeria Cuba India ?	Mehan and McDonald (1984!
55-437, 73-30	Senegal Senegal	Zambettakis et al. (1981)
U4-47-7	Uganda	Mehan et al. (1986 b)
GFA 1, GFA 2, AR 1, AR 2, AR 3, AR 4	USA USA USA USA USA USA	Mixon (1986)
Basse, C116(R), M395 C184, F-7, GE 652, Ah 6487 Maria-B, Roxo (Sal.), NC 449, NC 482 PI 196621, PI 196626 RMP 12, Sp. 218, Sp. 424	Gambia India ? ? ? USA ? Burkina Faso ?	Tsai and Yeh (1985)
ACC 63 CES 48-30, Celebes UPL PN 4	? ? Indonesia Philippines	Pua and Medalla (1986)

in environmental and crop handling methods the resistance in certain genotypes holds good (Mixon 1981, 1986).

In the last 15 years there has been much research into genetic resistance to *A. flavus* colonization of rehydrated, mature, sound, stored seed. This has possibly been stimulated by the aflatoxin problem being regarded largely as a postharvest phenomenon. This is no longer valid, as significant invasion by *A. flavus* of intact groundnut pods, and subsequent aflatoxin contamination, is known to occur before harvest (Davidson et al. 1983, Blaney 1985, Mehan et al. 1986b), and identification and possible use of seed testa resistance is definitely regarded as worthwhile.

Resistance to Pod Infection by *Aspergillus flavus*

The groundnut shell has logically been considered as a barrier to penetration by *A. flavus*, as seeds from pods with damaged shells are more frequently contaminated with aflatoxin than those from intact pods (McDonald and Harkness 1967).

Zambettakis (1975) reported that two cultivars, Darou IV and Shulamit, had lower levels of pod infection by *A. flavus* than other cultivars field tested in Senegal. Varietal differences in pod infection were confirmed in subsequent studies, and the differences in resistance appeared to be linked to varietal differences in pod shell structure (Zambettakis et al. 1981). They also reported a significant correlation between natural pod infection and seed infection by *A. flavus* in various genotypes tested in Senegal from 1976 to 1979 (Zambettakis et al. 1981). Pod and seed infection was estimated as sporulating colonies of *A. flavus* on surfaces of dried pods and seeds (examined under a binocular stereoscope in the laboratory). The percentages of seeds with colonies of *A. flavus* observed on their surfaces were consistently lower than those of pods with colonies showing on their surfaces, indicating that the shell acts as a barrier to fungal invasion of seeds. However, internal infections of seeds with *A. flavus* may be present without visible external growth of the fungus.

nsidering the concept of the existence of pod shell resistance to *A. flavus*, two groups of workers in the USA, Kushalappa et al. (1979) and Mixon (1980) examined the effects of pod noculation with *A. flavus* on shell infection and subsequent seed infection in various genotypes n the laboratory. They concluded that resistance to pod infection was highly variable, and appeared to be caused by the presence of antagonistic microflora. At ICRISAT we found that in some genotypes seeds were colonized or infected by the test fungus in pods which did not show colonies of *A. flavus* on their surfaces, while in others seeds were not colonized or infected in pods which showed one or more colonies of *A. flavus* . Colonies of several commonly occurring fungi in groundnut shells such as *Macrophomina phaseolina*, *Fusarium* spp, and *Aspergillus niger* were found on surfaces of both *A. flavus*-inoculated and noninoculated, intact, rehydrated, mature, stored pods of genotypes used in these studies (Mehan, McDonald, and Lalitha, unpublished data). Although the laboratory pod-inoculation method was not pursued, we have used pod inoculation to field test genotypes for resistance to seed infection and subsequent aflatoxin contamination. This aspect is further discussed in the section on resistance to natural seed infection to *A. flavus* in the field.

Resistance to *A. flavus* Seed Infection/Aflatoxin Contamination in the Field

ecent years, realization of the importance of preharvest *A. flavus* infection and aflatoxin contamination stimulated considerable research into possible genetic resistance in groundnuts to *A. flavus* seed infection in the field (Blankenship et al. 1985, Davidson et al. 1983, Kisyombe et al. 1985, Mehan and McDonald 1984, Mehan et al. 1986b). A few studies (Mixon 1980, 1983, 1986) indicated that the genotypes PI 337394F, PI 337409, GFA 1 and GFA 2, resistant to in vitro seed colonization by *A. flavus* (IVSCAF-resistant), showed considerably lower levels of natural seed infection with *A. flavus* and of aflatoxin contamination than the susceptible (IVSCAF-susceptible) genotypes, Florunner and PI 331326. In these studies, observations on natural seed infection were made primarily to determine the "initial" levels of *A. flavus* infection that could interfere with the seed inoculation tests for resistance in the laboratory. The natural seed infection was estimated from sporulating colonies of *A. flavus* on rehydrated seeds that had not been inoculated.

Davidson et al. (1983) could not show significant differences in *A. flavus* infection or in aflatoxin contamination of seed of two cultivars, Sunbelt Runner (reported to be resistant to *A. flavus* colonization of seeds) and Florunner (susceptible to seed colonization) at harvest. Blankenship et al. (1985) reported that four genotypes (A 72118 (GFA 1), A 7404 (AR 3), UF 77316 and UF 791041) resistant to seed colonization, and the cultivar Florunner grown under late-season drought stress were all highly susceptible to aflatoxin contamination. Other workers (Zambettakis et al. 1981) have reported several IVSCAF-resistant genotypes as having field resistance to *A. flavus* infection in Senegal, significant correlations being found between seed colonization in the laboratory and field infection. Kisyombe et al. (1985) demonstrated a correlation between field resistance to *A. flavus* seed infection and in vitro seed colonization in only one of 14 genotypes tested.

At ICRISAT,we give high priority to screening of groundnuts for resistance to seed infection by *A. flavus* in the field. We estimate levels of natural infection, i.e., infection resulting from invasion of pod and seed in the ground prior to harvest, or during postharvest drying. Genotypes that have received similar treatment in the field are compared for natural seed infection by *A. flavus* at either of these two stages. Seeds from intact, mature pods, are surface sterilized in 0.1% aqueous mercuric chloride solution for 3 min, rinsed in sterile distilled water, then incubated on Czapek Dox Rose Bengal Streptomycin Agar at 25°C for 5-7 days. Fungi grow from the seeds are recorded.

We tested various genotypes (IVSCAF-resistant, -susceptible, and -highly susceptible) for natural seed infection by *A. flavus* in replicated field trials at ICRISAT Center, from 1979 to 1982. In all four rainy seasons, the IVSCAF-resistant genotypes PI 337394F, PI 337409, and J-11, had significantly lower percentages of seed infected with *A. flavus* than the IVSCAF-susceptible or highly susceptible genotypes both at normal harvest (at optimum maturity) and late harvest (10 days after maturity) (Mehan et al. 1986b).

We also evaluated six IVSCAF-resistant (PI 337394F, UF 71513, J 11, Ah 7223, Var. 27, and U 4-47-7) and five IVSCAF-susceptible (TMV 2, Gangapuri, EC 76446(292), NC Ac 17090, and F1-5 × NC Ac 17090) genotypes for resistance to field infection of seed by *A. flavus*, and for aflatoxin contamination, in four drought-prone sites in Andhra Pradesh, India. All IVSCAF-resistant genotypes except Var. 27 had significantly lower percentages of seed infected (0.8-1.5%) than IVSCAF-susceptible genotypes (4.2-19.1%) over environments (sites and seasons). Resistance to field infection of seed by *A. flavus* in five of the six IVSCAF-resistant genotypes was stable across environments (Mehan et al. 1987). The IVSCAF-resistant genotypes, Ah 7223, J 11, U 4-47-7, and UF 71513, had significantly lower levels of aflatoxin B_1 (5-9 $\mu g\ kg^{-1}$ seed) than the IVSCAF-susceptible genotypes (39-151 $\mu g\ kg^{-1}$ seed).

We confirmed resistance to preharvest *A. flavus* seed infection in five of the six IVSCAF-resistant genotypes grown under imposed drought stress during pod maturation (30 days before harvest) in the 1984/85 and 1985/86 postrainy seasons (ICRISAT 1987).

Of 37 IVSCAF-resistant genotypes (Table 1), only 10 (PI 337394F, PI 337409, UF 71513, Ah 7223, J 11, Var. 27, 55-437, 73-30, Monir 240-30, and RMP 12) have been tested for resistance to seed infection by *A. flavus* in field trials (Kisyombe et al. 1985, Mehan et al. 1986, Zambettakis et al. 1981). Only three genotypes, PI 337409, PI 337394F, and J 11, have been evaluated in more than one country. J 11 was found resistant to *A. flavus* seed infection in North Carolina, USA (Kisyombe et al. 1985) and in India (Mehan et al. 1987). PI 337409 showed resistance in Senegal (Zambettakis et al. 1981) and in India, but was susceptible in the USA (Kisyombe et al. 1985).

Zambettakis et al. (1981) reported highly significant correlations between seed colonization in the laboratory and field infection of seed by *A. flavus* in 101 genotypes tested in several field trials in Senegal. It should not be assumed that all IVSCAF-resistant genotypes will have

sistance to seed infection by *A. flavus* in the field, or that all IVSCAF-susceptible genotypes ill show susceptibility to field infection by the fungus. For example, the IVSCAF-resistant :notypes Var. 27, Monir 24-30, and RMP 12 showed similar susceptibility to *A. flavus* ıfection in the field to that of the IVSCAF-susceptible genotypes TMV 2 and F1-5 × NC Ac 7090. Similar findings have been reported by Kisyombe et al. (1985). On the other hand, some VSCAF-susceptible genotypes such as Lampang (Kisyombe et al. 1985) and Exotic 6 (Mehan, '.K., unpublished data) have been found to have low levels of seed infection by *A. flavus* in the eld.

Methods for Screening Groundnuts for Resistance to *A. flavus* Infection ınd Aflatoxin Contamination

ɔome distinctive problems are encountered when screening groundnuts for resistance to 4. *flavus* and/or aflatoxin production under natural field conditions. Only intact pods can be ısed as damage of any kind is likely to override resistances. *A. flavus* is a weak pathogen and its ıbility to invade intact pods and seeds is strongly influenced by environmental conditions. Little ›wn of the comparative pathogenicity of different strains of the fungus, and their capacity to produce aflatoxin. Some environments are conducive to *A. flavus* infection of groundnuts, and extra attention is required to ensure uniform levels of infection for effective resistance screening. For environments where levels of *A. flavus* and aflatoxin contamination of susceptible cultivars are usually low, it is necessary to modify the environment to ensure high levels of infection/ contamination.

Screening trials should be on a light sandy soil, preferably with high populations of *A. flavus* in the soil mycoflora. A test site in a drought-prone area where late-season drought stress is common would be most effective as it provides a congenial environment for the fungus. Otherwise, the screening might have to be carried out on early or late-sown crops, or on irrigated dry-season crops where control of soil moisture during late stages of pod development can be assured.

At ICRISAT, we grow an irrigated dry-season crop and it is relatively simple to impose drought stress when required, it is thus possible to screen large numbers of genotypes for field resistance.

We screened over 500 genotypes for resistance to field infection of seed by *A. flavus* in the 1984, 1985, and 1986 rainy seasons, when severe to moderate drought stress occurred during pod maturation. Levels of *A. flavus* infection ranged from less than 2 to 38% (ICRISAT 1987). In the 1985/86 postrainy season, we used imposed drought stress (95–125 DAS) to field screen 432 onal genotypes for resistance to field infection of seed by *A. flavus*. Levels of seed infection ranged from 1.7 to 47% (ICRISAT 1987).

As drought stress during pod maturation predisposes groundnuts to *A. flavus* invasion it was thought that drought-tolerant cultivars might be resistant to preharvest infection by the fungus. However, several drought-tolerant genotypes tested to date (e.g., NC Ac 17090, Gangapuri, Manfredi × M13) are quite susceptible. Most genotypes found tolerant to end-of-season drought are of the valencia type, many of which appear to have weak pod shell structures. It is of interest that the drought-tolerant spanish cultivar C55-437 shows relatively low levels of seed infection at harvest. More research is needed to answer the important question: can the drought-tolerance of a cultivar reduce stress on pod and seeds and so reduce the chances of invasion by *A. flavus* in the soil? The resistance of the groundnut pod to *A. flavus* invasion appears to be associated with

certain structural and biochemical characters of the pod and seed, and there is a possibility that genotypes may have differential effects upon the populations of *A. flavus* in the geocarposphere. It would be interesting to determine if cultivars of different botanical types and pod characters show substantial differences in their reactions to *A. flavus*.

We also used a line-source sprinkler irrigation system that imposes a water deficit gradient to further evaluate some 40 genotypes for their reaction to *A. flavus* (ICRISAT 1987). A significant, positive, linear relationship was found between water deficits and *A. flavus* seed infection in all genotypes. Genotypic differences for infection were clearly evident over a considerable range of water deficits (62–99%), indicating the value of this method for screening genotypes for their reaction to *A. flavus* over a wide range of water deficits (drought intensities). Simultaneous screening for *A. flavus* seed infection and for drought tolerance is particularly useful as drought stress strongly influences seed infection by *A. flavus*. A pod zone soil temperature gradient is associated with the water deficit gradient, and this is important when considering aflatoxin production. Temperatures between 25° C and 31° C are reported to favor aflatoxin production in groundnuts subjected to drought stress during pod development and maturation (Blankenship et al. 1984, Cole et al. 1985). The position on the stress gradient (water deficit and temperature gradients) can be chosen in the field from which material is collected for resistance screening for *A. flavus* infection or aflatoxin production. High levels of *A. flavus* seed infection can be obtained under severe water-deficit conditions accompanied by high maximum temperatures (38–41° C) in the pod-zone soil, conditions that favor *A. flavus* growth and fungal invasion by suppressing other microbial competitors.

A useful evaluation of genotypes for resistance to aflatoxin contamination can be obtained by comparing the aflatoxin contents of seeds across genotypes. Resistance to *A. flavus* seed infection may be used as an index of possible resistance to aflatoxin contamination, but not all strains have a similar aflatoxin-producing ability. The combination of *A. flavus* strain and host genotype can influence aflatoxin production. However, for all practical screening purposes field resistance to the fungus is important in conferring resistance to aflatoxin contamination.

Genotypes can also be evaluated for resistance to *A. flavus* infection and aflatoxin contamination under artificial inoculation conditions by applying inoculum of an aflatoxin-producing strain of *A. flavus* to the soil around developing pods (20 to 30 days before harvest) to produce uniform, high levels of infection and aflatoxin contamination. Care should be taken to avoid injury to the pegs and pods while adding inoculum. We have used this technique to evaluate selected genotypes (IVSCAF-resistant and IVSCAF-susceptible genotypes) for their reaction to *A. flavus* infection and aflatoxin contamination in the field. Four IVSCAF-resistant genotypes (Ah 7223, J 11, PI 337394F, and UF 71513) had significantly lower levels of infection and aflatoxin contamination than the IVSCAF-susceptible genotypes we tested (Mehan and McDonald 1984).

Resistance to Aflatoxin Production

Rao and Tulpule (1967) reported varietal resistance in groundnut to aflatoxin production. The genotype US 26 (PI 246388) did not support aflatoxin production when seeds were colonized by aflatoxigenic strains of *A. flavus* in laboratory inoculation tests. Kulkarni et al. (1967) reported that the cultivar Asiriya Mwitunde supported very low levels of aflatoxin production under field conditions. These findings were not confirmed by other workers, but did stimulate research on possible varietal resistance (Doupnik 1969, Aujla et al. 1978, Doupnik and Bell 1969, Nagarajan and Bhat 1973, Tulpule et al. 1977). Most of these researchers used autoclaved seeds of

groundnut inoculated with aflatoxigenic strains of *A. flavus* to test genotypes for their ability to support aflatoxin production.

At ICRISAT, we developed a laboratory inoculation method to screen groundnuts for resistance to aflatoxin production (Mehan and McDonald 1980). The method is similar to that used for the seed colonization test. Intact mature seeds are surface sterilized in a 0.1% aqueous solution of mercuric chloride, rinsed in sterile distilled water, and hydrated to 20% moisture. The seeds (20-g samples) are then placed in 9-cm diameter petri dishes, their testae scarified with a sterile needle, and inoculated with 1 mL of a conidial suspension (4×10^6) conidia mL^{-1} of an 8-day-old culture of an aflatoxin-producing strain (AF 8-3-2A) of *A. flavus*. After incubation at 25°C for 10 days the seeds are tested for aflatoxin content. We have tested 502 genotypes for their ability to support aflatoxin B_1 production (Mehan et al. 1986a), and found significant differences in rate and total accumulation of aflatoxin. Levels of aflatoxin B_1 produced in different genotypes ranged from below 10 to 195 $\mu g\ g^{-1}$ seed. We identified two genotypes, U 4-7-5 and VRR 245, that supported production of very low levels of aflatoxin B_1 (7–10 $\mu g\ g^{-1}$ seed). There were indications that aflatoxin production levels were slightly lower in seed (of some genotypes tested) from rainy-season crops than in seed from postrainy-season crops, indicating possible environmental effects. Comparisons of the chemical constituents (such as phytate, zinc, boron) of seed of different genotypes grown in different environments may indicate possible mechanisms of resistance to aflatoxin production.

We tested 30 more genotypes with oil contents that ranged from 33.7 to 48.4% for their ability to support aflatoxin production. No correlation was found between oil content and capacity to support aflatoxin production.

We also tested 16 wild *Arachis* species (9 in section *Arachis*, 3 in section *Erectoides*, 2 in section *Rhizomatosae*, and one each in sections *Extranervosae* and *Triseminale*). All supported production of aflatoxin B_1 (34–110 $\mu g\ g^{-1}$ seed).

Some genotypes resistant to seed colonization by aflatoxigenic fungi are good substrates for aflatoxin production, while others that are susceptible to fungal colonization do not support high levels of aflatoxin production. For example, the IVSCAF-resistant genotypes, PI 337394F, PI 337409, J 11, and UF 71513 support high levels of aflatoxin B_1 production, while some IVSCAF-susceptible genotypes (U 4-7-5 and VRR 245) support only low levels of aflatoxin B_1 production. No correlation was observed between fungal growth (estimated visually or based on ergosterol contents of colonized seeds of J 11, U 4-7-5, and VRR 245) and aflatoxin production. Similar findings have been reported by Priyadarshini and Tulpule (1978) with regard to fungal growth (based on chitin content) and aflatoxin production in several varieties of groundnut and maize.

How Can Genetic Resistance be Applied to Aflatoxin Management?

The ideal solution would be to identify or breed a groundnut cultivar immune to invasion by *A. flavus*, or one that would not support aflatoxin production. But this is not likely to be achieved, at least in the near future, and it is more logical to aim for cultivars with a high degree of resistance to *A. flavus* invasion before and after harvest, that support only low levels of aflatoxin production. *Aspergillus flavus* resistance should be incorporated into both oil and confectionery groundnut cultivars adapted to particular agroecological regions. Such cultivars could be grown using cultural and crop-handling procedures that were found useful in reducing *A. flavus* invasion. Cultivars resistant to fungal invasion in the soil would be particularly desirable for the semi-arid regions where preharvest aflatoxin contamination is a serious

problem. The good level of resistance in the commercial cultivars J 11 and C 55-437 could be useful in minimizing aflatoxin contamination in some environments.

Resistance to *A. flavus* infection is also important in order to maintain seed quality as the fungus also causes seed rots and aflaroot seedling disease. Cultivars with resistance to *A. flavus* invasion are also likely to have resistance to seed invasion by other soilborne pathogens that reduce produce quality and cause seed and seedling diseases.

References

Aujla, S.S., Chohan, J.S., and **Mehan, V.K.** 1978. The screening of peanut varieties for the accumulation of aflatoxin and their relative reaction to the toxigenic isolate of *Aspergillus flavus* Link ex Fries. Journal of Research, Punjab Agricultural University 15:400-403.

Bartz, Z.A., Norden, A.J., LaPrade, J.C., and **Demuynk, T.J.** 1978. Seed tolerance in peanut (*Arachis hypogaea* L.) to members of the *Aspergillus flavus* group of fungi. Peanut Science 5:53-56.

Blaney, B.J. 1985. Mycotoxins in crops grown in different climatic regions of Queensland. Pages 97-108 *in* Trichothecenes and other mycotoxins (Lacey, J. ed.). Chichester, UK: John Wiley and Sons.

Blankenship, P.D., Cole, R.J., Sanders, T.H., and **Hill, R.A.** 1984. Effect of geocarposphere temperature on pre-harv colonization of drought-stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85:69-74.

Blankenship, P.D., Cole, R.J., and **Sanders, T.H.** 1985. Comparative susceptibility of four experimental peanut lines and the cultivar Florunner to preharvest aflatoxin contamination. Peanut Science 12:70-72.

Carter, J.B.H. 1970. Studies on the growth of *Aspergillus flavus* on groundnut kernels. PhD thesis, University of Reading, Reading, London, UK. 170 pp.

Carter, J.B.H. 1973. The influence of the testa damage and seed dressing on the emergence of groundnut (*Arachis hypogaea*). Annals of Applied Biology 74:315-323.

Cole, R.J., Sanders, T.H., Hill, R.A., and **Blankenship, P.D.** 1985. Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress. Mycopathologia 91:41-46.

Davidson, J.I., Jr., Hill, R.A., Cole, R.J., Mixon, A.C., and **Henning, R.J.** 1983. Field performance of two peanut cultivars relative to aflatoxin contamination. Peanut Science 10:43-47.

Dickens, J.W. 1977. Aflatoxin, occurrence and control during growth, harvest and storage of peanuts. Pages 99-105 *in* Mycotoxins in human and animal health (Rodricks, J.V., Hesseltine, C.W., and Mehlman, M.A., eds.). Park Forest South, Illinois, USA: Pathotox Publishers.

Dickens, J. W., and **Pattee, J.E.** 1973. Peanut curing and postharvest physiology. Pages 509-522 *in* Peanuts: culture and uses. Stillwater, Oklahoma, USA: American Peanut Research and Education Association.

Dieckert, M.C., and **Dieckert, J.W.** 1977. Genetically determined structural parameters of the seed coat affecting th colonization of peanut seeds by aflatoxin-producing *Aspergilli.* Annales de Technologie Agricole 26:353-366.

Doupnik, B., Jr. 1969. Aflatoxins produced on peanut varieties previously reported to inhibit production. Phytopathology 59:1554.

Doupnik, B., Jr., and **Bell, D.K.** 1969. Screening peanut breeding lines for resistance to aflatoxin accumulation. Journal of the American Peanut Research and Education Association 1:80-82.

Glueck, J.A., Clark, L.E., and **Smith, O.D.** 1977. Testa comparisons of four peanut cultivars. Crop Science 17:777-782.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1987. Annual report 1986. Patancheru, A.P. 502 324, India: ICRISAT.

Kisyombe, C.T., Beute, M.K., and **Payne, G.A.** 1985. Field evaluation of peanut genotypes for resistance to infection by *Aspergillus parasiticus.* Peanut Science 12:12-17.

Kulkarni, L.G., Sharief, Y., and **Sarma, V.S.** 1967. 'Asiriya Mwitundé groundnut gives good results in Hyderabad. Indian Farming 17:11-12.

Kushalappa, A.C., Bartz, J.A., and **Norden, A.J.** 1979. Susceptibility of pods of different peanut genotypes to *Aspergillus flavus* group fungi. Phytopathology 69:159-162.

LaPrade, J.C., Bartz, J.A., Norden, A.J., and **Demuynk, T.J.** 1973. Correlation of peanut seed coat surface wax accumulations with tolerance to colonization by *Aspergillus flavus*. Journal of the American Peanut Research and Education Association 5:89-94.

McDonald, D., and **Harkness, C.** 1967. Aflatoxin in the groundnut crop at harvest in northern Nigeria. Tropical Science 9(3):148-161.

Mehan, V.K. 1987. The aflatoxin contamination problem in groundnut: control with emphasis on host plant resistance. Pages 63-92 *in* Proceedings of the First Regional Groundnut Plant Protection Group Meeting, 15-21 Feb 1987, Harare, Zimbabwe. Lilongwe, Malawi: International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Regional Groundnut Program for Southern Africa. (Limited distribution.)

Mehan, V.K., and **McDonald, D.** 1980. Screening for resistance to *Aspergillus flavus* invasion and aflatoxin production in groundnuts. ICRISAT Groundnut Improvement Program Occasional Paper no.2. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 15 pp. (Limited distribution.)

Mehan, V.K., and **McDonald, D.** 1983. Aflatoxin production in groundnut cultivars resistant and susceptible to seed invasion by *Aspergillus flavus*. Pages 221-228 *in* Proceedings of the International Symposium on Mycotoxins, 6-8 Sep Cairo, Egypt. Cairo, Egypt: National Research Centre.

Mehan, V.K., and **McDonald, D.** 1984. Research on the aflatoxin problem in groundnut at ICRISAT. Plant and Soil 79:255-260.

Mehan, V.K., McDonald, D., and **Lalitha, B.** 1983. Effect of season, location and field-drying treatment on *in vitro* seed colonization of groundnut genotypes by *Aspergillus flavus*. Oléagineux 38(10):553-558.

Mehan, V.K., McDonald, D., Nigam, S.N., and **Lalitha, B.** 1981. Groundnut cultivars with seed resistant to invasion by *Aspergillus flavus*. Oléagineux 36(10):501-507.

Mehan, V.K., McDonald, D., and **Rajagopalan, K.** 1987. Resistance of peanut genotypes to seed infection by *Aspergillus flavus* in field trials in India. Peanut Science 14:17-21.

Mehan, V.K., McDonald, D., and **Ramakrishna, N.** 1986a. Varietal resistance in peanut to aflatoxin production. Peanut Science 13(1):7-10.

Mehan, V.K., McDonald, D., Ramakrishna, N., and **Williams, J.H.** 1986b. Effects of genotype and date of harvest on infection of peanut seed by *Aspergillus flavus* and subsequent contamination with aflatoxin. Peanut Science 13(2):46-50.

Mixon, A.C. 1980. Comparison of pod and seed screening methods on *Aspergillus* spp infection of peanut genotypes. Peanut Science 7:1-3.

Mixon, A.C. 1981. Reducing aflatoxin contamination in peanut genotypes by selection and breeding. Journal of the American Oil Chemists' Society 58:961A-966A.

Mixon, A.C. 1983. Peanut germplasm lines AR-1, AR-2, AR-3, and AR-4. Crop Science 23:1021.

Mixon, A.C. 1986. Reducing *Aspergillus* species infection of peanut seed using resistant genotypes. Journal of Environmental Quality 15(2):101-103.

Mixon, A.C., and **Rogers, K.M.** 1973. Peanut accessions resistant to seed infection by *Aspergillus flavus*. Agronomy Journal 65:560-562.

Mixon, A.C., and **Rogers, K.M.** 1975. Factors affecting *Aspergillus flavus* Link ex Fr. colonization of resistant and susceptible genotypes of *Arachis hypogaea* L. Peanut Science 2:18-22.

Nagarajan, V., and **Bhat, R.V.** 1973. Aflatoxin production in peanut varieties by *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. Applied Microbiology 25:319-321.

Priyadarshini, E., and **Tulpule, P.G.** 1978. Relationship between fungal growth and aflatoxin production in varieties of maize and groundnut. Journal of Agricultural and Food Chemistry 26:249-252.

Pua, A.R., and Medalla, E.C. 1986. Screening for resistance to *Aspergillus flavus* invasion in peanut. *In* Seventeenth Anniversary and Annual Convention of the Pest Control Council of the Philippines, 8-10 May 1986, Iloila City, Philippines. (Abstract).

Rao, K.S., and Tulpule, P.G. 1967. Varietal differences of groundnut in the production of aflatoxin. Nature 214:738-739.

Tsai, A.H., and Yeh, C.C. 1985. Studies on aflatoxin contamination and screening for disease resistance in groundnuts. Journal of Agricultural Research of China 34:79-86.

Tulpule, P.G., Bhat, R.V., and Nagarajan, V. 1977. Variations in aflatoxin production due to fungal isolates and crop genotypes and their scope in prevention of aflatoxin production. Archives de l'Institut Pasteur de Tunis 54(3-4):487-493.

Discussion

J.I. Pitt. Do you have a carefully standardized procedure for raising the moisture to 20%? What is the extent of variation?

V.K. Mehan. The seeds are immersed in water for a duration that varies with cultivar, e.g., 8-9 min for the spanish types; this being determined by weighing. Variation from seed to seed is less than 1%.

T. Shantha. Is there any variety which is susceptible to fungal colonization but does not support aflatoxin production?

V.K. Mehan. This is a good question. We have found genotypes which are poor substrates for aflatoxin production, but none of them has marked resistance to fungal colonization. When testing seeds for ability to support aflatoxin production, we scarify the testa to remove resistance to fungal colonization and we inoculate with a highly toxigenic strain of *A. flavus*. Production of aflatoxin in the substrate is obviously dependent upon the fungal growth and this can be estimated using the chitin or ergosterol determination techniques.

K.K. Shresta. Although *A. flavus* is said to be a weak pathogen, it causes aflaroot disease and reduces crop yield. How can we control this disease?

V.K. Mehan. Incidence of aflaroot disease can be reduced by sowing clean seed. This can be ensured by careful attention to harvesting, drying, and storing of sowing materials from the previous season. Use of suitable seed-protectant fungicides can also help.

R.E. Pettit. In 1986, a severe outbreak of aflaroot disease totally destroyed a farmer's crop in South Texas. This outbreak was due to sowing of seed heavily infected with *A. flavus*.